EXERCISE AS DISEASE-MODIFYING STRATEGY FOR PARKINSON'S: A MULTIDIMENSIONAL ASSESSMENT OF ACUTE AND LONG-TERM INTERVENTIONS

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by

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Abstract

Parkinson's Disease (PD) is a complex and variable neurodegenerative condition. Due to its progressive nature and lack of effective treatments, a range of motor and non-motor symptoms develop and, usually, lead to disability and disengagement with active lifestyles. Exercise interventions have the potential of improving and sustaining physical and cognitive function in PD, as well as stimulating functional and structural neuroplasticity. Published research suggests that multi-modal (MM) exercise, that also includes cognitive tasks, may be more beneficial than single modalities in improving physical and/or cognitive function. However, there have been contrasting results between studies, owing to differences in study design (mode, timing, amount, and intensity of the exercise) and analytical methods used to measure biomarkers, which makes it difficult to generate conclusions and definitive exercise guidelines for people with PD (PwP). As a result of this, the overall objectives of this thesis were to propose acute and long-term interventions that are beneficial for PwP and can be implemented in real-world settings (at home or in the community), investigate associated functional and cognitive outcomes concurrently, and assess potential mechanisms underlying the neuroprotective effects of exercise interventions (MM, aerobic and combined exercise with cognitive tasks) completed in real-world or clinical environments.

The first study (presented in Chapter 3) evaluates neurotrophins levels (i.e., BDNF and pro-BDNF) as candidate biomarkers for PwP in several sample types (plasma, serum, and saliva) with the aim of improving current inconsistent methodologies that compromise the reliability and validity of these measurements. Optimisation trials of ELISA assays were completed and revealed that the use of an appropriate combination of reagent diluent for each sample type and analyte is key to improve assay performance and measurement accuracy. The samples collected for the studies presented in Chapters 4 and 6 were subsequently analysed following the methodological steps reported in this study. Future work should include these methodological considerations and previous studies not reporting these details must be interpreted with caution.

The second study (Chapter 4) presents the long-term implementation of a weekly community-based MM exercise programme for PwP and shows that exercise attendees improve and maintain function for up to 1, 2 or 3 years. Compared to non-active PwP, PD exercisers improve their mobility, lower extremity strength, cognition and BDNF levels, slowing down PD progression.

Subsequently, focus groups were conducted in the studies presented in chapters 5 and 7 to gain in-depth understanding of participants' views about the MM exercise class and its change towards an online delivery due to the coronavirus 2019 (COVID-19) pandemic. Participant's discussions about the feasibility, practicality, and perceptions of the online class for PD, were gathered to develop guidelines for the online delivery of exercise for PwP.

Finally, using an experimental laboratory setting, chapter 6 further explores the neuroprotective effects of acute bouts of aerobic exercise (alone or combined with cognitively challenging tasks) on cognitive function. This pilot study provides preliminary evidence of the beneficial effects that, both, a second bout of cycling 24h after the first session and cycling combined with cognitive tasks have on cognitive function.

Taken together, both the optimisation and lab-based experimental studies provide directions for future research, such as methodological steps to ensure accurate BDNF and pro-BDNF measurements and exercise interventions that are suggested to elicit cognitive benefits. Furthermore, this thesis provides evidence that a community-based MM exercise programme is able to improve and maintain physical and cognitive functions in PwP. This intervention offered an evidence-based exercise class that had been running for over 4 years and, following COVID-19 pandemic, transitioned from the community towards an online-based setting where it has been successfully running for the last 2 years and is ongoing. Accordingly, this thesis also provides novel insights into the online delivery of MM exercise for PwP and presents guidelines for an appropriate setting-up and delivery of online exercise programmes for health-care professionals and researchers working with PwP.

Declaration

No part of this thesis has been submitted in support of an application for any degree or other qualification of the University of Kent, or any other University or Institution of learning.

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Covid-19 Mitigation Statement

On the 17th of March 2020, following UK Government guidance, the University of Kent released a statement saying that all laboratory testing, clinic work, and community exercise classes had to cease immediately. Furthermore, face-to-face contact with individuals outside of one's household was not permitted. Consequently, any data collection for the ongoing research studies had to immediately stop. Therefore, all the studies included in this thesis were affected by the global COVID-19 pandemic and its restrictions.

The closure of facilities prevented further recruitment for the studies 2 and 4 outlined in Chapters 4 and 6, respectively, resulting in smaller than required sample size, particularly for study 4. Please refer to the 'Sample Size and Statistical Analyses' section of Chapter 4 and 6 for a detailed description of how the global pandemic impacted both studies.

In summary, due to all social meetings, including exercising groups and group assessments, being prohibited from March 2020, the intervention presented in study 2 (Chapter 4) had to be immediately interrupted and no further assessments could be carried out. Unfortunately, this meant that not all participants were able to participate in all the required study assessments to complete the full period of 1, 2 or 3 years. Furthermore, when COVID-19 pandemic struck, the intervention presented in study 4 (Chapter 6), also had to suddenly stop, which meant that all the data that had been collected so far had to be discarded. Seven months later, adhering to all the restrictions that were put in place, out of the initial 20 participants recruited before March 2020, only 6 participants were able to complete the study. In order to be able to complete this limited and extremely time restricted data collection, the student had to request an extension that was approved by the University of Kent.

On the other hand, since the community-based exercise class had to unexpectedly stop, the mode of delivery was adjusted to the situation: from face-to-face to online delivery. Taking advantage of the situation, it was decided to evaluate this change of platform and the student organised focus groups with class participants with the aim to develop guidelines for other practitioners working with online delivery of exercise aimed at PwP.

Scientific Output

Publications

Chapter 5 – Study 3:

Fullerton, C., Ferrusola-Pastrana, A., Davison, G. & Meadows, S. (2022) 'Exercise is part of my whole medication regime' – people with Parkinson's and their partners' participation experiences with a community-based group exercise class. Qualitative Research in Sport, Exercise and Health. In review.

Conference Communications

Chapter 4 – Study 2:

Ferrusola-Pastrana, A., Meadows, S., Davison, G. & Fullerton, C. (2019). Can exercise preserve motor and non-motor function in Parkinson's? 24th Annual Congress of the European College of Sport Science, Prague, Czech Republic. July 2019. Poster Presentation.

Ferrusola-Pastrana, A., Meadows, S., Davison, G., Fullerton, C. & Waters, A. (2019). Can exercise preserve motor and non-motor function in Parkinson's? 5th World Parkinson's Congress, Kyoto, Japan. June 2019. Poster Presentation. KAR id:74219, DOI: 10.3233/JPD-199900

Ferrusola-Pastrana, A., Meadows, S., Davison, G. & Fullerton, C. (2020). Multi-Modal Exercise as a Disease Modifying Strategy for Parkinson's. 25th Annual Congress of the European College of Sport Science, online. October 2020. Oral Presentation.

Ferrusola-Pastrana, A., Meadows, S., Davison, G. & Fullerton, C. (2021). The Therapeutic Effects of Exercise for People with Parkinson's. World Parkinson's Day Lecture, Research & Exercise, April 2021. Online Oral Presentation.

Chapter 6 – Study 4:

Ferrusola-Pastrana, A., Meadows, S., Davison, G. & Fullerton, C. (2021). The Therapeutic Effects of Exercise for People with Parkinson's. World Parkinson's Day Lecture, Research & Exercise, April 2021. Online Oral Presentation.

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Abbreviations

1-STS	1-Minute Sit-to-stand
4-PL	Four-Parameter Logistic
6-OHDA	6-Hydroxydopamine
6MWT	6-Minute Walk Test
ACSM	American College of Sports Medicine
ADLs	Activities of Daily Living
AIC	Akaike Information Criteria
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
ATS	American Thoracic Society
AZ	Alzheimer's Disease
BBB	Blood Brain Barrier
BD	Bipolar Disorder
BDNF	Brain-Derived Neurotrophic Factor
$BDNF_{MET}$	Met Variant of Val66Met Polymorphism
$BDNF_{VAL}$	Val/Val genotype
BESTest	Balance Evaluation Systems Test
BG	Basal Ganglia
BMI	Body Mass Index
BP	Blood Pressure
BR	Brain Reserve
BRUMS	Brunel Mood Scale Questionnaire
BSA	Bovine Serum Albumin
CBD	Cortico-Basal Degeneration
CBPR	Community-Based Participatory Research
CR	Cognitive Reserve
CDT	Clock Drawing Test
CNS	Central Nervous System
COMT	Catechol-O-Methyl Transferase
COVID-19	Coronavirus Disease 2019
CSF	Cerebrospinal Fluid
CT	C-Terminal Region
CV	Coefficient of Variation
CVD	Cardiovascular Disease
D2-SPECT	Dopamine D2 Receptors SPECT
DAT	Dopamine-Transporter
DBS	Deep Brain Stimulation

DLB	Dementia with Lewy Bodies
ECG	Electrocardiogram
EDO	Early Disease Onset
ELISA	Enzyme-Linked Immunosorbent Assay
Emmean	Estimated Marginal Means
ENS	Enteric Nervous System
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FoG	Freezing of Gait
FPP	Finger Prick Plasma
GABA	γ-Aminobutyric Acid
GDNF	Glial Cell-Derived Neurotrophic Factor
GPi	Globus Pallidus Pars Interna
GS	Goat Serum
GS	Grip Strength
GSD	Geometric Standard Deviation
GWAS	Genome-Wide Association Studies
HIIT	high-intensity interval training
HOA	Healthy Older Adults
HR	Heart Rate
HRP	Horseradish-Peroxidase
HRR	Heart Rate Reserve
HRV	Heart Rate Variability
IPAQ	International Physical Activity Questionnaire
L-DOPA	Levodopa
LB	Lewy Bodies
LED	Levodopa Equivalent Dose
LEDD	Levodopa Equivalent Daily Dose
Ln	Natural Log
LP	Lewy Pathology
LRTs	Likelihood Ratio Tests
LSVT	Lee Silverman Voice Treatment
LTM	Long-Term Memory
MAO-B	Monoamine Oxidase B
MCI	Mild Cognitive Impairment
MCID	Minimal Clinically Important Difference
MDC	Minimal Detectable Change
MDD	Major Depressive Disorder
MDS-PD	International Parkinson and Movement Disorder Society

MET	Metabolic Equivalent
Met	Methionine
Mg	Magnesium
MICT	moderate-intensity continuous training
MID	Minimal Important Difference
ML	Maximum Likelihood
MM	Multi-Modal
MM-EX	Multi-Modal Exercising Group
MMP	Mini-Mental Parkinson
MMSE	Mini-Mental State Examination
MoCA	Montreal Cognitive Assessment
MPP^+	N-Methyl-4-Phenylpyridinium Ions
MPTP	1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine
MR	Motor Reserve
MRI	Magnetic Resonance Imaging
MSA	Multiple System Atrophy
MSNs	Medium Spiny Neurons
na-PD	Non-Active People with PD
ND	Non-Tremor Dominant
NGF	Nerve Growth Factor
NHS	National Health Service
NICE	National Institute of Clinical Evidence
NTFs	Neurotrophic Factors
OD	Optical Density
OLST	One-legged Stance test
OPQOL	Older People's Quality of Life Questionnaire
OPQOL-brief	Brief Older People's Quality of Life questionnaire
$p75^{NTR}$	Pan-Neurotrophin Receptor
PAR-Q	Physical Activity Readiness Questionnaire
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PD	Parkinson's Disease
PDQ-39	39-Item Parkinson's Disease Questionnaire
PET	Positron Emission Tomography
PGC-1α	Peroxisome proliferator-activated receptor-γ coactivator 1α
PNS	Peripheral Nervous System
POMS	Profile of Mood States
PP-P	Platelet-Poor Plasma
PR-P	Platelet-Rich Plasma

PSP	Progressive Supranuclear Palsy
PwP	People with Parkinson's
QoL	Quality of Life
RBD	Rapid Eye Movement Sleep Behaviour Disorder
RCI	Reliable Change Index
RDP	Rapid Disease Progression
rhAmp	RNase H2 Enzyme-Based Amplification
RHR	Resting Heart Rate
RPE	Rating of Perceived Exertion
RPM	Revolutions per Minute
rTMS	Repetitive Transcranial Magnetic Stimulation
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SCT	Social Cognitive Theory
SD	Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
Se	Selenium
SE_b	Standard Error
SEM	Standard Error of the Mean
sIgA	Secretory Immunoglobulin A
SN	Substantia Nigra
SNP	Single Nucleotide Polymorphisms
SN_{PC}	Subtantia Nigra Pars Compacta
SN_{PR}	Subtantia Nigra Pars Reticulata
SPECT	Single-Positron Emission CT
Sr	Strontium
sRPE	Session Rating Of Perceived Exertion
SRT	Single Reaction Time
SSES REAG	School of Sport and Exercise Sciences' Research Ethics Advisory Group
TD	Tremor Dominant
tDCS	Transcranial Direct Current Stimulation
TH	Tyrosine Hydroxylase
TIMP-1	Tissue Inhibitors of Metalloproteinases
TMB	Tetramethylbenzidine
TMT	Trail Making Test
tPA	Tissue Plasminogen Activator
TrkB	Tropomyosin-Related Kinase Receptors B
TUG	Timed Up and Go
UKPDSBB	United Kingdom PD Society Brain Bank
UPDRS	Unified Parkinson Disease Rating Scale

Val	Valine
VEGF	Vascular Endothelial-Derived Growth Factor
VO_2max	Maximal Oxygen Consumption
WC	Waist Circumference
WCST	Wisconsin Card Sorting Test

Chapter 1. Literature Review

1.1 Description of Parkinson's Characteristics

Parkinson's disease (PD) is a progressive neurodegenerative condition that causes significant motor, non-motor and cognitive deficits. Although symptoms suggestive of PD had been found in Egyptian papyrus, Sanskrit texts and other documents from ancient times, it was first thoroughly detailed and distinguished from other similar conditions by Dr James Parkinson more than 200 years ago (Parkinson, 2002). Parkinson, defined the condition that he named as 'The Shaking Palsy' as "*Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured*." (2002, p. 223). With a main focus on motor symptoms, Parkinson concentrated primarily on tremor and gait disturbances, and it was not until the early twentieth century that non-motor symptoms including cognitive problems were accepted as part of this condition. Currently, it has become clearer that non-motor symptoms can appear before motor symptoms and be equally or even more disabling than the motor symptomatology, especially when their significance is assessed based on Quality of Life (QoL) surveys (Chaudhuri, Healy, & Schapira, 2006).

After providing an overview about the epidemiology, risk factors, subtypes, diagnosis and symptomatology of PD, a narrative synthesis approach has been carried out to review the current state of the scientific knowledge relating to available interventions for PwP. More specifically, the overall aim of this literature review is to focus on the potential protective and attenuating effects that non-pharmacological interventions, particularly exercise, may have for people with PD (PwP) and better understand which underlying mechanisms might be responsible for these beneficial effects. Moreover, the current knowledge on exercise prescription, proposed exercise guidelines and appropriate clinical exercise testing for PwP is also reviewed. The search strategy followed for this narrative literature review has been carried out according to the recommendations of international guidelines for narrative reviews (Ferrari, 2015). Therefore, a literature search was conducted using the databases PubMed and Google Scholar, and used several terms and their combination, such as: 'Parkinson's Disease', 'exercise', 'multi-modal', 'single-mode', 'aerobic', 'strength', 'goal-based', 'community-based', 'cognitive function', 'physical function', 'cognitive training', 'mechanism', 'neuroplasticity', 'neuroprotective', and 'brain-derived neurotrophic factor', amongst others. Moreover, additional references were identified by a manual search among some of the references that had been reviewed. No date restrictions were placed on the search and all types of quantitative and qualitative study designs were included (e.g., randomised control trials (RCT), case reports, cohort and observational studies, focus groups, interviews, etc.).

1.1.1 PD's Epidemiology

PD is the second most common neurodegenerative disorder after Alzheimer Disease (AZ) and its prevalence is increasing at a faster rate than other neurodegenerative diseases, affecting 1% of the population over the age of 60 and 4.3% of people over the age of 85 (Chaudhuri & Titova, 2019; Keus et al., 2014). In total, an estimated 10 million people worldwide live with PD and 5% of people with Parkinson's (PwP) were diagnosed when they were younger than 40 years old (Van Den Eeden et al., 2003). Furthermore, Parkinson's prevalence in industrialised countries is 0.3% of the entire population (Chaudhuri & Titova, 2019; de Lau & Breteler, 2006; Keus et al., 2014; Nussbaum & Ellis, 2003), whilst in less industrialised countries such as Nigeria, Libya, Tunisia, Ethiopia and Togo, the prevalence rates are 0.01%, 0.0314%, 0.043%, 0.007% and 0.02%, respectively (Okubadejo, Bower, Rocca, & Maraganore, 2006). Therefore, overall, prevalence rates of PD in Africa are lower than in their industrialised counterparts, for instance Europe and North America (Dorsey et al., 2018; Okubadejo et al., 2006). Nevertheless, it has to be considered that most of the studies included in Okubadejo and colleagues' systematic review used a World Health Organization (WHO, 1981) screening instrument and protocol that was not specific for PD and that did not differentiate secondary causes of Parkinsonism from PD. Hence, further epidemiologic studies using PD-specific screening instruments in Africa and less industrialised countries are needed. Additionally, as a condition, PD presents unequal age-adjusted gender and racial impact, being more common in men (the male:female ratio has been largely unchanged since 1990 and is still estimated to be 1.4 [between 1.1 and 2.3]) and presenting higher incidence rates amongst Hispanics and Whites (Dorsey et al., 2018; Pringsheim, Jette, Frolkis, & Steeves, 2014; Van Den Eeden et al., 2003; Willis, Evanoff, Lian, Criswell, & Racette, 2010). In addition to the gender differences in PD diagnosis, it has been suggested that women present a more benign phenotype than men. That is, compared to men, women: are older at symptom onset, more often present a tremor dominant form of PD (associated with a slower disease progression) and milder degeneration (Haaxma et al., 2007). However, some part of racial and gender rates differences could be due to research methodological issues, differences in host or environmental exposures between the studied populations, genetic susceptibility to PD or the presence of genetic risk factors that have yet to be fully uncovered for idiopathic cases of PD (Pringsheim et al., 2014; Dorsey et al., 2018; Van Den Eeden et al., 2003). Thus, more appropriate and detailed epidemiological studies are still needed in order to calculate reliable estimates of incidence and prevalence rates and identify both risk and protective factors that should be considered when calculating epidemiological estimates. For instance, Rossi et al (2018) calculated the relative risks of PD for former smokers and relative smokers, taking into account the impact of declining smoking rates. Whilst relying on their hypothesis that smoking is neuroprotective for PD, their results indicate that in cases of a causal-relation of tobacco non-use and an increased risk of PD, former projections of the expected PD prevalence in 2040 might be underestimated by at least 10%. Although the nature of smoking and PD incidence association is still poorly understood, identifying risk and protective factors together with their rate of change over time is important and is necessary for a more accurate depiction of epidemiological estimates and the future PD burden.

Additionally, marked demographic shifts with increased longevity in both developed and economically developing countries, have an impact in increasing PD's prevalence with age, due to larger proportions of the population living longer and, thus, allowing more time for the visible features of PD to appear. Therefore, an improvement in the key unmet needs, such as early-diagnosis techniques, neuroprotective trials and successful disease-modifying treatment strategies are crucial to: reduce the burden that PD imposes on sufferers, families, carers and healthcare professionals, avoid potential unsustainable demands on limited healthcare resources in the future for an aging population, and reduce the overall cost of PD, which has been deemed as one of the most expensive and prevalent neurological disorders (Gustavsson et al., 2011). To date, a large number of pharmacological neuroprotective trials have failed due to the disease complexity; nonetheless, during the recent decades, diagnosis techniques and the development of potential treatments and strategies with the aim to cover the current unmet needs of PD have made impactful and significant progress, which is encouraging.

1.1.2 Genetic Factors' influence on PD

Although PD aetiology is still poorly understood, there is considerable evidence suggesting that PD is a multifactorial disease that results from a complex interaction of environmental and genetic factors, with several molecular pathways acting together to induce the degeneration of dopaminergic neurons (Sarkar, Raymick, & Imam, 2016). These altered molecular pathways include mitochondrial dysfunction, oxidative stress, protein aggregation, impaired autophagy, and neuroinflammation (Simon, Tanner, & Brundin, 2020). In addition to the degeneration of dopaminergic neurons, another neuropathological hallmark of PD is the appearance of insoluble neuronal cytoplasmic inclusions, known as Lewy bodies (LB), which are composed primarily of abnormal aggregates of misfolded proteins, mainly populated by α-synuclein (Spillantini et al., 1997). The presence and spread of α-synuclein misfolding is known as Lewy Pathology (LP).

α-synuclein is a protein, principally expressed in the brain at presynaptic terminals, that takes part in multiple cellular processes (Emamzadeh, 2016); however, its precise physiological function remains unknown and mutations in its encoding gene (i.e., SNCA) have been identified in cases of autosomal dominant PD or sporadic PD (Hoffman-Zacharska et al., 2013; Polymeropoulos et al., 1997; Singleton et al., 2003). Mutations in the SNCA gene are rare overall but were the first mutations reported to cause autosomal-dominant PD. In addition to these, some of the most common mutations that have been linked to familial and sporadic forms of PD are mutations that have been isolated from the genes: parkin (PARK2), UCH-L1 (PARK5), PINK1 (PARK6), DJ-1 (PARK7), LRRK2 (PARK8), and ATP13A2 (PARK9) (Klein & Westenberger, 2012; Li, Tan, & Yu, 2014).

Initially, since most of the PD patients presented an idiopathic disease-type, it was thought unlikely that there could be a genetic underlying cause. This view changed due to studies performed in twins and families, which showed that genetics could be involved in some forms of Parkinsonism

(Duvoisin & Johnson, 1992; Tanner et al., 1999). For instance, it was observed that monozygotic twins with an early onset of disease (i.e., <50 years old) have a much higher rate of concordance than dizygotic twins with similar early-onset disease (Tanner et al., 1999). Although Tanner et al. (1999) showed that the genetic component for patients diagnosed with typical PD after the age of 50 was highly unlikely, the opposite was observed for pairs of twins diagnosed before the age of 51. This suggests that genetic factors are important with younger-onset disease (i.e., when it begins at, or before, the age of 50). In addition, studies performed on families affected by PD provided further evidence that genetics did play a role by showing that there was an increased risk of developing PD amongst first-degree relatives of PwP (Marder et al., 1996; Payami, Larsen, Bernard, & Nutt, 1994). Following these reports, the understanding of PD's genetics was further strengthened by studies that identified missense mutations to the encoding α-synuclein gene in families with early-onset, Lewy-body-positive autosomal dominant PD (Polymeropoulos et al., 1997).

More recently, genome-wide association studies (GWAS) have been used as a genetic approach to associate specific genetic variations with Parkinson's. By scanning the genome of different PD populations, this methodology has allowed the identification of susceptibility loci involved in typical PD forms (Foo et al., 2020; Satake et al., 2009; Simón-Sánchez et al., 2009). Meaning that presenting a specific allele (i.e., variant form of a gene) at a susceptibility locus, increases the risk but is neither necessary nor sufficient for the expression of the disease (Greenberg, 1993). The identification of susceptibility loci in PD, and rare pathogenic genetic variants related with familiar forms, has supported the idea that PD is associated with multiple genes and pathways. Additionally, GWAS comparing different PD populations, have shown population differences, which further strengthens the view that PD is highly heterogeneous, and that population-specific differences could contribute to the genetic heterogeneity of PD (Foo et al., 2020; Satake et al., 2009; Simón-Sánchez et al., 2009). Overall, epidemiological studies have identified that only about 10% of PD cases are hereditary and linked to genetic risk factors, such as the monogenic mutations that have been mentioned (Tysnes & Storstein, 2017). Although this figure is small, it is clear that genetics play a very important role in PD. On one hand, as discussed, there are numerous genetic risk factors that can increase the risk to develop PD, and, on the other hand, whilst rare, genetic forms of PD are highly important to shed some light and gain a better understanding of the pathophysiology of idiopathic PD.

It has also been suggested that gene expression changes, or post-translational modifications of specific proteins of the family of neurotrophic factors (NTFs), could dictate the disease progression (Hernandez-Baltazar et al., 2018). Those include: glial cell-derived neurotrophic factor (GDNF), neurotrophin nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), important secretory proteins for the survival, protection, maintenance, reorganisation and regeneration of specific neuronal populations and connections in the adult brain (known as synapses) (Allen, Watson, Shoemark, Barua, & Patel, 2013).

1.1.3 Role of Neurotrophic Factors in PD

In recent years, there has been an increasing interest in NTFs. They are abundant in the CNS and play a crucial role in the establishment, modulation, and elimination of synapses; process termed as synaptic plasticity (Pöyhönen, Er, Domanskyi, & Airavaara, 2019). Currently, it is well understood that mammalian brains can be remarkably plastic and NTFs, such as neuron-derived BDNF, have been shown to contribute significantly to the dynamic modulation of synaptic density (Causing et al., 1997). The more synaptic density a neural network has, the greater the connectivity of the neurons that integrate the network and the greater their ability to store and process information, thus, increasing the information flow (i.e., spike rate, which is the frequency at which neurons elicit action potentials) (Brewer et al., 2009). Imaging techniques have been able to show that synaptic changes, including a loss of synaptic density, occur due to aging but also, and more severely, in neurodegenerative diseases such as PD (Matuskey et al., 2020). Therefore, the importance of potentiating neuroprotective mechanisms is indisputable. Relevantly, both BDNF and GDNF are important for dopaminergic neuron survival, BDNF and NGF promote cholinergic neuron survival, and BDNF is also important in the survival and function of serotonergic, hippocampal and cortical neurons (Alderson, Alterman, Barde, & Lindsay, 1990; Allen et al., 2013; Kordower et al., 2000; Mattson, Maudsley, & Martin, 2004). Therefore, NTFs are of interest for their neuroprotective capabilities. Altogether, their properties and a better understanding of their potential as therapeutic neurorestorative and neuroprotective agents, make NTFs especially relevant for neurodegenerative disorders, such as PD, where it has been suggested that deficits of these factors may lead to an increased neuronal death and symptoms progression.

A growing body of literature have reported that decreased levels of BDNF have been associated with PD (Chauhan, Siegel, & Lee, 2001; Howells et al., 2000; Mogi et al., 1999; Parain et al., 1999; Rahmani et al., 2019; Scalzo, Kümmer, Bretas, Cardoso, & Teixeira, 2010). However, Rocha et al. reported somewhat contradictory results in a study involving Parkinson's patients. They observed that circulating levels of NTFs did not differ between PwP and aged-matched healthy controls (Rocha et al., 2018). The methodological approach taken by Rocha and colleagues (2018) was to measure plasma circulating levels, as opposed to using post-mortem brain tissues samples to measure BDNF. Measuring circulating BDNF levels from peripheral blood (i.e., whole blood, plasma or serum), can be used as proxy for the measurement of central BDNF levels, which are difficult to analyse and quantify. The relevance of measuring circulating BDNF levels has been supported by animal studies showing that BDNF can cross the blood brain barrier (BBB), and central BDNF levels are closely associated to peripheral BDNF levels (Klein et al., 2011; Pan, Banks, Fasold, Bluth, & Kastin, 1998). However, it is imperative to highlight that plasma BDNF levels are significantly influenced by the centrifugation strategy, and physical activity levels of participants; both topics will be covered in a later methodological section (Gejl et al., 2019; Gilder, Ramsbottom, Currie, Sheridan, & Nevill, 2014). The lack of information about these important variables in Rocha's et al (2018) study could explain the absence of impaired neurotrophic support observed in their results. Thus, it is important

to note that methodological, pre-analytical and analytical conditions can strongly influence NTFs measurements, particularly BDNF levels, and affect the results and reproducibility of these studies (Polacchini et al., 2015; Tsuchimine, Sugawara, Ishioka, & Yasui-Furukori, 2014). Moreover, BDNF expression predominantly takes place in the brain, and skeletal muscle (Matthews et al., 2009), however, platelets upon activation can also express high amounts of BDNF (Fujimura et al., 2002). Since platelets cannot cross the BBB, brain BDNF levels may not be reflected by the amount of BDNF carried in platelets, but rather by the amount of free-BDNF present in plasma, which is a minor free fraction in plasma (Fujimura et al., 2002). Consequently, BDNF measurements can differ depending on the type of sample that is being analysed: serum, plasma, or platelet-poor plasma. Some research reports fail in providing this information, which might account for the variability within research study findings.

In regards to PD research, BDNF is a pleiotropic neurotrophin that raises interest due to two documented roles on mechanisms that are hampered in PD: 1) BDNF fosters the survival and function of dopaminergic neurons, and 2) it influences the structure and function of the principal target of those dopaminergic neurons, striatal medium spiny neurons (MSNs), inducing neuroplasticity (Hyman et al., 1991; Mercado, Collier, Sortwell, & Steece-Collier, 2017). In PD, dopaminergic neurons and their projections degenerate, leading to both motor and non-motor symptoms. Henceforth, neuroprotective and neurorestorative agents such as BDNF may present therapeutic implications to mitigate PD pathology.

1.1.4 Environmental Factors influence on PD

Apart from the cases where genetic risk factors have been identified (i.e., 10% of PD cases), the remaining 90% are sporadic and result from complex interactions between environmental and genetic factors, with an underlying molecular pathogenesis that involves multiple pathways and mechanisms; and it is their synergistic interaction that leads to the development of nigrostriatal damage (Sarkar et al., 2016). In the attempt to explain some proportion of the percentage of sporadic PD cases, several reports have shown that some environmental risk factors can elevate the risk of PD (Noyce, Lees, & Schrag, 2016). Following animal studies that have previously linked pesticides to dopaminergic cell death and Parkinsonism, over 20 studies have now related the use of pesticides with PD in humans, suggesting that some pesticides and proxies, such as maneb and paraquat (a chemical that is also used to create animal models of PD), might be causal agents of PD particularly when exposure takes place at a young age (Costello, Cockburn, Bronstein, Zhang, & Ritz, 2009). Other environmental factors with strong significant evidence demonstrating increased PD risk are: having a farming occupation, being part of rural populations and drinking well water (Noyce et al., 2012). Moreover, a recent epidemiological study reported a significant association between PD mortality rates and selenium (Se), strontium (Sr) and magnesium (Mg) concentrations in top soil (Sun, 2018), showing that high soil Se and Mg concentrations reduced that estimate whilst high soil concentrations of Sr correlated with high PD mortality rates. These elements are abundantly present in our environment,

and an inadequate supply can result in different deficiencies or diseases because some (e.g., Mg) are essential nutrients required for various biochemical and physiological functions (Vormann, 2003). Moreover, the soil elemental composition can be affected by the use of pesticides, organic solvents and air pollution, and can place our health at risk (Abrahams, 2002; De Miranda & Greenamyre, 2020). The relation between these environmental risk factors and Parkinson's incidence, has been strengthened by observations of emissions changes. After the 1990s, there has been a significant reduction in air pollution, use of pesticides and specific industrial solvents (e.g., trichloroethylene) that, in some countries, such as the Netherlands, has coincided with a substantial decrease in incidence of PD (Darweesh, Koudstaal, Stricker, Hofman, & Arfan Ikram, 2016). Thus, this suggests that, to an extent, PD can be affected by human-made risk factors and could, therefore, be controlled and managed.

In addition to the exposure to toxic chemicals, insults such as head injury might also be associated with a greater risk of presenting PD later in life (Jafari, Etminan, Aminzadeh, & Samii, 2013). However, this association has not been observed in large sample sized studies (Kenborg et al., 2015). There is widely conflicted literature on head injury and PD, and, although twin studies tried to overcome these problems, many variables could potentially confound this association (e.g., different lifestyles, environment and genetic factors) (Goldman et al., 2006). Overall, factors including severity, chronicity, and individual susceptibility may influence the ultimate clinical outcome, which could lead an individual to develop PD after experiencing head injury.

1.1.5 Where does PD start? – Description of Current Hypothesis

Anatomical sites, such as the gastrointestinal tract (i.e., gut) and the olfactory system, can be exposed to the external environment. During the 20th century, PD was mainly identified as a brain-disorder characterised by a progressive degeneration of dopaminergic neurons. In 1919, Constantin Trétiakoff was the first to demonstrate that there was a substantial loss of pigmented nigral cells in brains from PwP who were analysed post-mortem. He also showed the presence of inclusion bodies in the remaining neurons which he named as "corps de Lewy" (i.e., LB), in honour of Friederich H. Lewy, who first described them in 1912 (Geibl, Henrich, & Oertel, 2019). However, it is currently known that PD is not simply a brain-disorder but a multicentric condition, where the gut and olfactory bulb also seem to play a critical role in its pathophysiology (Borghammer & Van Den Berge, 2019). Both anatomical sites could represent a route of entry for some of the recognised environmental factors to initiate the pathological process, thus setting the stage for the 'dual-hit' hypothesis which involves olfactory structures and the 'gut-brain'-axis (Braak et al. 2003; Hawkes, Del Tredici and Braak 2007). In 2003, Braak and colleagues identified that the PD-brain, with LB affecting susceptible neuronal pathways, undergoes sequential changes as the disease progresses (Braak et al. 2003). They proposed a staging scheme based on the sequence of neuropathological changes that they observed, suggesting that neuronal damage does not occur in a random way but, rather, develops following a predetermined sequence of pathological events.

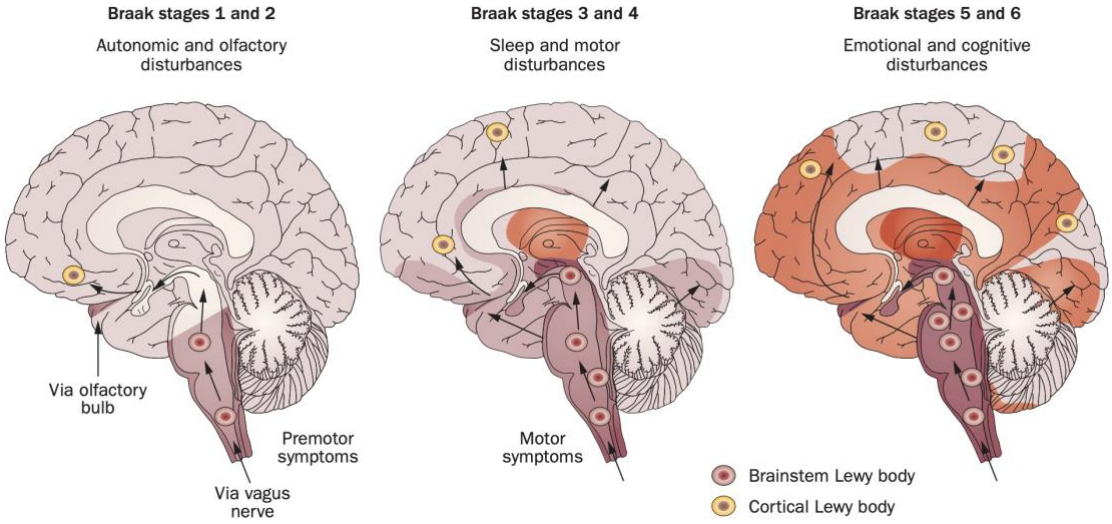

Figure 1.1 Illustration of the Braak staging system of PD pathology, which suggests that α-synuclein pathology starts in the olfactory bulb and brainstem regions (Braak stages 1 and 2) and, subsequently, the Lewy pathology spreads into the midbrain and the basal forebrain (Braak stages 3 and 4) before finally reaching cortical regions (Braak stages 5 and 6). Originally from (Halliday, Lees, & Stern, 2011) and modified and published by (Doty, 2012). Reproduced with permission from Springer Nature.

Braak et al. (2003) divided PD's-related pathology evolution in the 6 stages presented in **Figure 1.1**, where stage 1 included the areas affected at the beginning of the disease (Braak et al. 2003). At stage 1, lesions initially occur in the dorsal motor nucleus of the glossopharyngeal and vagal nerves (lower brainstem) and anterior olfactory nucleus. Thereafter, the disease sequentially progresses following an ascending course towards nuclei of the midbrain and forebrain and, finally, in the later stages, affecting the neocortex (see **Figure 1.1**). The vagal nerve innervates and connects the enteric nervous system (ENS) – a division of the peripheral nervous system (PNS)– with the central nervous system (CNS), and LB have been observed in the ENS (Braak, Ghebremedhin, Rüb, Bratzke, & Del Tredici, 2004).

The precise starting point of Parkinson's pathology remains unknown; however, Braak's studies, together with animal and human studies that investigated α-synuclein and LB pathology-process (via truncal vagotomy and a-synuclein deficiency models), have led a range of researchers to postulate that, in PD, a putative agent may enter in the periphery (e.g., through the nose, mouth, stomach or gut) and induce the α-synuclein pathology, which can spread in a prion-like fashion via retrograde axonal transport to the brain (Braak et al., 2004; Kim et al., 2019; Liu et al., 2017; Svensson et al., 2015). Additionally, it has also been suggested that changes in the composition of the gut microbiome could affect gastrointestinal motor function and provoke low-grade T cell-driven inflammation of the gut wall that could impact neuroinflammation and α-synuclein misfolding (Berstad & Berstad, 2017; Sampson et al., 2016). Taken together, this body of research provides substantial evidence suggesting that the pathological changes in PD could start in the periphery. Nevertheless, this hypothesis for PD's aetiology does not account for all patients and is still

controversial; therefore, it requires further investigation (Jellinger, 2019; Lionnet et al., 2017). In an alternative explanation, it has been suggested that PD's heterogeneity could be explained better by classifying PD patients into a) PNS-first and b) CNS-first subtypes (Borghammer & Van Den Berge, 2019). In this context, a better understanding of the brain-gut-microbiome interactions could bring a new insight into the pathophysiology of the disease.

1.1.6 Parkinson's Subtypes

It is well recognized that, in both early and late stages of the disease, PD patients present high clinical heterogeneity (Hoehn & Yahr, 1967). The reason for this, may be linked to the pathological variability that has been observed in PD. Although Braak and collegues in 2003 identified sequential neuropathological changes that occur as the disease develops, PD can progress very differently between individuals (Jellinger 2019; Braak et al. 2003). In cases of early PD onset and long disease duration, the Braak staging system seems to consistently describe the progression of the pathology. However, it has also been reported that, in some cases, LP might not follow a linear progression and, instead, skip certain specific brain regions (Halliday, Hely, Reid, & Morris, 2008; van de Berg et al., 2012). Additionally, age and mode of onset, different expression of the cardinal signs and symptoms, rate of progression, degree of functional impairment and different individual response to therapeutic treatments, characterise the marked diversity that exists in PD (Hoehn & Yahr, 1967). Currently, it is well recognised that there are different clinical phenotypes in PD, which can also be identified at early stages of the disease (Lewis et al., 2005). Lewis and colleagues, in 2005, described four different PD clinical subtypes based on the four clinical phenotypes presented in **Table 1.1**.

Table 1.1 Parkinson's Disease Subtypes based on four different clinical phenotypes. The table is based on Jellinger, 2019; Lewis et al., 2005; Selikhova et al., 2009; van de Berg et al., 2012.

Clinical Phenotype Subtypes	**Description**
Early disease onset (EDO) subtype: Below the age of 55 at disease onset.	EDO subtype usually presents a slow rate of disease progression, with mild motor symptoms, without cognitive impairment, and lower depression ratings. Cortical LP similar to TD and RDP subtypes.
Tremor dominant (TD) subtype: Aged 55 or over at disease onset, presenting resting tremor as sole initial symptom or with dominance over bradykinesia and rigidity.	TD subtype typically demonstrates a slow rate of disease progression, modest motor symptoms, no significant cognitive impairments, and absence of depression. Overall, the TD subtype typically exhibits a more benign disease progression and less functional disability that other subtypes.

Non-tremor dominant (ND) subtype: Aged 55 or over at disease onset, with bradykinetic motor features without or, only mild, resting tremor.	ND subtype commonly presents the characteristic motor phenotype scores, significant levels of cognitive impairment (being executive function most clearly affected), mild depression and, generally, more rapid disease progression and higher prevalence of dementia than the EDO and TD subgroups. This subtype also presents more severe cortical LP than other subtypes, which has been associated with cognitive impairment.
Rapid disease progression (RDP) Subtype: Irrespective of age, the life expectancy is within 10 years after symptoms appear.	Without severe motor disability or cognitive impairment (no dementia) at onset compared with the other subtypes, the RDP subtype consistently demonstrates an aggressive course. It can sometimes present mild depression and it exhibits the fastest rate of disease progression with rapid motor progression and deterioration of ADLs.

Abnormal protein aggregates in PD, lead to neuropathology and its progression. Currently, disease variants are difficult to fully characterise before death. However, studies identifying differences in neuronal loss levels, abnormal protein accumulation patterns and selective vulnerability to LP based on PD clinical subtypes, could aid to fully determine their clinical phenotype and provide valuable information to be used in both clinical settings and future research.

1.1.7 Disease Progression

Thanks to the exceptional work of many scientific groups, during the last three decades, the understanding of PD's evolution has drastically changed and evolved. It is still not clear whether PD starts in the digestive system or the brain, however, there are key neuropathological events that occur in the CNS of PwP and characterise the core features of this condition.

The development of PD has been linked to various areas of the nervous system and it involves different types of neurons. However, much attention has focused on a specific neuronal group in a brain region associated with the cardinal motor symptoms and behavioural processes commonly presented in PD. This region, located in the midbrain, is the Subtantia Nigra (SN), and is composed of two main types of neurons: dopaminergic neurons (which utilise dopamine as a neurotransmitter and are the main source of dopamine within the CNS), and inhibitor GABAergic neurons (which utilise γ-aminobutyric acid (GABA) as a neurotransmitter) (Nair-Roberts et al., 2008; Oertel & Mugnaini, 1984). Dopaminergic neurons are darker than neighbouring neuronal areas due to the presence of neuromelanin, a dark pigment biosynthesized from L-DOPA (i.e., dopamine precursor), which can be detected by magnetic resonance imaging (MRI) (Fabbri et al., 2017). In 1910, Sano introduced the SN having two subdivisions, which has been continually supported since (Sano,

1910). The two subdivisions presented are: the Subtantia Nigra Pars Reticulata (SN_{PR}), mainly populated by GABAergic neurons, and the Subtantia Nigra Pars Compacta (SN_{PC}) forming the dorsal part of the SN, mainly populated by dopaminergic neurons (Nair-Roberts et al., 2008; Oertel & Mugnaini, 1984); the latter being the neuronal group that is directly related with PD's neuropathology. The SN_{PC} harbours a densely packed population of dopaminergic neurons and, in gross anatomical dissections, appears in dark colour. This characteristic is what gives the name to the SN, which is the Latin for "dark substance".

The SN_{PC} is a region of the brain that forms part of a major neural pathway known as nigrostriatal pathway. On this pathway, dopaminergic neurons from the SN_{PC} send projections to the striatum, a basal ganglia (BG) structure mainly populated by MSNs. The striatum (formed by the caudate and putamen, presented in **Figure 1.2**) modulated by dopaminergic inputs from the SN_{PC}, is part of two intertwined pathways within the BG (i.e., direct/indirect pathways presented in **Figure 1.2**). Both pathways form circuits that communicate via inter-neuronal networks between MSN and other structures of the BG. Altogether, to modulate voluntary motor control, by inhibiting unwanted movements, or facilitating movement accordingly (Calabresi, Picconi, Tozzi, Ghiglieri, & Di Filippo, 2014). Additionally, the subcortical nuclei of the BG, participate in complex interactions with cortical areas of the brain, forming circuit loops to regulate not only the motor but, also, cognitive and motivational aspects of movement (Ferrazzoli et al., 2018).

It is fundamental to comprehend the role of the dopaminergic system, neurotransmitters and their physiological functions, in order to understand the implications of a progressively damaged neuronal brain circuit on the distinct PD's symptoms and their timeline of appearance.

In Parkinson's, there is a progressive neurodegeneration of the nigrostriatal dopaminergic system that starts with the loss of dopaminergic neurons in the SN_{PC}, which leads to progressive deterioration of PD's symptoms. This neuropathological hallmark, also involves a concurrent depletion of dopamine levels in the BG and damage to other neuronal systems (Geibl, Henrich and Oertel 2019; Braak et al. 2003). Thus, the loss of dopaminergic projections towards the striatum and dopamine depletion within the CNS, causes an imbalance between the direct/indirect pathways and an overall excitatory drive of the BG output nuclei (i.e., the globus pallidus pars interna [GPi] and the SN_{PR}; **Figure 1.2**) that results in over-inhibition of thalamic neurons projecting to the cortex and, subsequently, a pathological global inhibition of motor cortex areas (Calabresi et al., 2014).

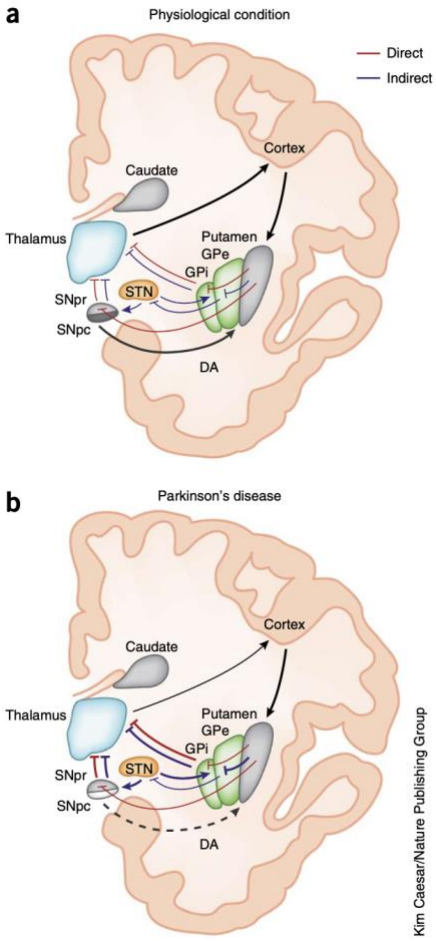

Figure 1.2 Schematic representation of the direct/indirect pathway classical model in the physiological condition and in PD (Calabresi et al., 2014). Reproduced with permission from Springer Nature.

As the disease advances, it has been observed that α-synuclein pathology progresses and can spread, both, neuron-to-neuron, and towards other interconnected brain regions outside the BG (Kordower et al. 2008; Li et al. 2008; see **Figure 1.1**). Thus, it has been suggested that LP initially appears in the olfactory bulb and lower brainstem regions, affecting those areas and the autonomic nervous system (ANS) first. This would correspond to Braak stages 1 and 2, and lead to the appearance of non-motor symptoms, such as constipation, anosmia, depression/anxiety, Rapid Eye Movement Sleep Behaviour Disorder (RBD), and also, in some cases, the presence of subtle motor features. Subsequently, the LP spreads into the midbrain and the basal forebrain concurrently with a severe loss of dopaminergic neurons in the SN_{PC}, which directly affects the striatum (Braak stages 3 and 4). The involvement of the BG, which over-inhibits the thalamus, leads to the development of a clinical phenotype of PD with a clearer motor picture, where tremor, rigidity, slowness and balance problems, among others, start to arise. Finally, LP reaches and affects cortical regions (Braak stages 5 and 6), linked to a worsening of the motor features (e.g., tremor, disturbance of voluntary movements,…) and non-motor symptoms, which include cognitive impairments (e.g., dementia), depression, sleep and sensory disruption, and emotional issues (Braak et al. 2003; Savica, Bradley and Mielke 2018; Noyce, Lees and Schrag 2016).

Overall, the Parkinson's journey has been thoroughly investigated, with a large number of studies following patient groups, in some instances, for over 25 years, providing notable understanding about PD development and progression (Diem-Zangerl et al., 2009; Duarte, García Olmos, Mendoza, & Clavería, 2013; Erro et al., 2013; López, Ruiz, Del Pozo, & Bernardos, 2010; Merola et al., 2011). Even now, there are unresolved, and apparently conflicting, views due to substantially variable rate of disease progression between individuals with PD (Jellinger, 2019). However, the increasing knowledge about Parkinson's pathology onset and progression, has led to identify the natural history of the disease and link it with its symptoms profile and timeline.

1.1.7.1 Diagnosis

The existing body of research on PD development and symptoms onset, suggest there is a prodromal phase of idiopathic PD before the cardinal signs become noticeable. The length of the prodromal phase is thought to vary between patients – and, probably, between different clinical phenotypes – and ranges from 2 to 50 years (Hawkes, 2008). For a patient that presents with the onset of characteristic symptoms in their sixties, it is suggested that the prodromal period may have been of 20 years length (Hawkes, Del Tredici, & Braak, 2010). The extensive length of this prodromal and pre-clinical phase delays the diagnosis and, therefore, the initiation of treatment strategies.

The loss of dopaminergic neurons in the SN_{PC} and, subsequently, a concurrent reduction in dopamine levels within the CNS, with a depletion of input in the striatum, are neurophysiological hallmarks that confirm PD diagnosis. However, the initial diagnosis is usually based on a clinical observation of the cardinal motor symptoms of Parkinson's (i.e., bradykinesia/hypokinesia, rigidity and tremor), medical history, physical examination and exclusion of other causes of parkinsonism (Tolosa, Wenning, & Poewe, 2006). Additionally, a levodopa challenge test is performed to complete this initial diagnosis. If the test is positive and the patient presents an excellent response to levodopa treatment, the result is indicative of PD but not conclusive. This test can help differentiate between PD and atypical Parkinsonism disorders that are clinically similar to PD: Progressive Supranuclear Palsy (PSP), Vascular Parkinsonism, Dementia with Lewy Bodies (DLB), Multiple System Atrophy (MSA) or Cortico-Basal Degeneration (CBD). However, patients with atypical Parkinsonism disorders can respond well, to some extent, to PD medication. In this case, given that PD diagnosis can only be confirmed through neuropathology (i.e., post-mortem analysis), functional neuroimaging can help differentiate inconclusive diagnosis.

Most MRI or Computed Tomography scans of PwP can appear uncomplicated and, if PD symptoms are present, getting a normal scan result does not exclude a possible PD diagnosis. However, Positron Emission Tomography (PET), presynaptic Dopamine-Transporter (DAT) Single-Positron Emission Computed Tomography (SPECT), and postsynaptic Dopamine D2 Receptors SPECT (D2-SPECT) scans, can be used with radioactive tracers to help identify potential nigrostriatal degeneration and loss of dopaminergic projections (Antonini & DeNotaris, 2004; Piccini & Whone, 2004). Their

outcomes can be indicative of PD and used to discriminate idiopathic PD from atypical Parkinsonism disorders. Additionally, these scans can help providing an accurate early diagnosis but, importantly, they are expensive and not always available.

Patients' signs and symptoms are still the first option to be considered when making a diagnosis. The main diagnostic criteria proposed for PD include: the United Kingdom PD Society Brain Bank (UKPDSBB; the first formal diagnostic criteria proposed for PD), Gelb's criteria (which requires the presence of at least two of the following cardinal features: resting tremor, bradykinesia, rigidity or unilateral onset), and the recently revised International Parkinson and Movement Disorder Society criteria (MDS-PD; which incorporates the previous two sets of diagnostic criteria) (Gelb, Oliver, & Gilman, 1999; Gibb & Lees, 1988; Hughes, Daniel, Kilford, & Lees, 1992; Postuma et al., 2015). Additionally, MDS-PD proposes the MDS-Unified Parkinson Disease Rating Scale (UPDRS) to examine all cardinal manifestations and introduces the important use of non-motor symptoms as additional diagnostic feature for PD. Although, at first, they were specifically designed for use in research, these set of criteria are innovative, complete and could be adopted by practitioners in routine settings as a general guide to the clinical diagnosis of PD. However, the use of these criteria among clinicians is still rather unusual (Marsili, Rizzo, & Colosimo, 2018).

1.1.7.2 Motor Symptoms

Despite recent advances in genetics testing and imaging techniques, PD still primarily remains as a clinically diagnosed condition. Therefore, a fast and correct diagnosis is crucial to start the therapeutic management as soon as possible. However, the neuropathological events precede the appearance of motor symptoms, and when those become apparent the nigrostriatal dopaminergic system has suffered severe degeneration with at least a 50–70% loss of striatal dopaminergic innervation (Brooks, 1998; Hou, Chen, Liu, Qiao, & Zhou, 2017; Noyce et al., 2016). As consequence, there is a lack of thalamic input and excitatory drive of the motor cortex neurons that is associated with a reduced ability of the motor system to execute voluntary motor plans. It is currently known that the cardinal signs of PD (bradykinesia/hypokinesia, rigidity and tremor) are directly related to the loss of striatal dopaminergic innervation and, therefore, this is the reason why PD has typically been considered as a motor disorder, secondary to BG dysfunction.

Characteristically, PD's motor symptoms present an initial unilateral asymmetry that usually remains, even though the symptoms progress and gradually spread towards the contralateral and lower limbs. This initial focal motor manifestation, is due to 60–70% reductions in dopaminergic inputs to the contralateral striatum (Rodriguez-Oroz et al., 2009) and is commonly characterised by akinesia (i.e., absence or loss power to initiate voluntary and spontaneous movement) and unilateral resting tremor in one of the upper extremities (Pallone, 2007).

Bradykinesia is characterised by reduced speed and amplitude of movements. PwP often describe it as 'weakening of the muscles', however, after examination, muscles strength is not affected (Geibl et al., 2019). Hypokinesia consists of reduced amplitude and frequency of spontaneous movement (Marsden, 1989). Altogether, the features akinesia–hypokinesia–bradykinesia imply slowed motor function. Rigidity is also a behavioural hallmark in PD. It is characterised by increased muscular tone at rest and increased resistance to stretching, resulting in stiffness of the limbs (Rodriguez-Oroz et al., 2009).

The combination of bradykinesia and rigidity, often affects the facial muscles resulting in hypomimia (i.e., a 'masked like' facial expression), hypophonia (leading to a soft monotonous speech), drooling and reduced arm-swing during walking. Additionally, the swallowing process and the limbs are affected leading to dysphagia and micrographia (i.e., small, cramped handwriting), respectively (Pallone, 2007).

Tremor is one of the most commonly recognised features of Parkinson's. It is typically present at rest and improves or stops with movement. Classically described as "pill-rolling" tremor, it is often the first symptom noticed. It is characterised by an alternating motion of the thumb and the forefinger at a 4-6 Hertz rate, and it usually starts unilaterally in the distal upper extremity before spreading to other limbs (Lemstra, Verhagen Metman, Lee, Dougherty, & Lenz, 1999; Rodriguez-Oroz et al., 2009).

Postural stability, ambulatory activity and gait speed are also affected in PD and deteriorate over time due to the progressive nature of the condition (Cavanaugh et al., 2015). Such impairments may increase the risk for individuals of falling, especially under dual-task conditions, which require the simultaneous performance of cognitive and motor tasks (e.g., walking while performing concurrent tasks such as talking or carrying an object) (Kelly, Eusterbrock, & Shumway-Cook, 2012; Raffegeau et al., 2019). Therefore, motor features, such as stooped posture, shuffling steps, postural instability and impaired gait, are also associated with an increased risk of falling. Additionally, in some cases, PwP can suddenly lose the ability to move, known as freezing of gait (FoG); a phenomenon that can be triggered by both mental aspects and visual inputs. Episodes of FoG in PwP are more common in crowded spaces, during time-restricted actions, stressful situations, whilst attempting to cross narrow spaces, during turning movements and, last but not least, initiating walking (i.e., "start hesitation") (Bloem, Hausdorff, Visser, & Giladi, 2004).

The expression of Parkinson's cardinal motor symptoms can significantly vary between patients, which has resulted in the description of different disease subtypes associated with distinct pathogenesis and rates of progression (Marras & Lang, 2013). Nevertheless, all the characteristic motor features are further exacerbated over time, and involve increased disability, fear of falling, low mood, and reduced quality of life (Schrag, Choudhury, Kaski, & Gallagher, 2015).

It is important to develop appropriate assessments to measure this diverse array of symptoms in order to be able to detect changes due to the course of the disease or in response to specific therapies. For instance, some of the measures that are further described in Chapter 2 (General Methods) are Timed Up and Go (TUG) and 6-Minute Walk Test (6MWT). Both measures can quantitatively analyse different traits of an individual's gait and walking features. TUG is a measure of the time that a participant needs to stand up from a chair, walk 3 metres and perform a 180 degree turn before returning to the chair and sitting down. 6-MWT is a measure of walking capacity through measuring the distance that a participant is able to cover in 6 minutes. In some cases, due to space availability, the 6-MWT is performed in 10-metre shuttles. Therefore, in both tests, participants are asked to perform a sequence of locomotor tasks, which involve turning, and can be used to analyse gait performance and changes in mobility (Falvo & Earhart, 2009; Morris, Morris, & Iansek, 2001). Moreover, both tests can identify changes in PD's specific traits, such as worsening bradykinesia and frequency of FoG, which can affect balance and increase the risk of falling, and postural problems, for instance kyphosis (i.e., increased convex curvature of the spine), which are motor features that are common in PwP.

1.1.7.3 Non-Motor Symptoms

The neurodegenerative events that characterise this condition also affect non-motor qualities and define PD as a multisystem disorder rather than a movement disorder. Together, both motor and non-motor symptoms, define the features of PD.

Non-motor symptoms are highly common in PD. They can be troublesome and more disabling than motor symptoms and, often, are under-recognised and under-treated (Bonnet, Jutras, Czernecki, Corvol, & Vidailhet, 2012). Frequently, some non-motor symptoms start appearing in the prodromal phase of PD, significantly earlier than the motor-symptoms, and the most common are: anosmia/hyposmia (loss/reduced sense of smell), constipation, depression/anxiety, apathy, sexual problems, fatigue, RBD and, eventually, cognitive dysfunction. Thus, motor features are just 'the tip of the iceberg' of this diverse disease.

Several non-motor symptoms are associated with a dysfunction of the ANS. Supported by autopsy studies and complemented by other research, Braak et al. (2003) demonstrated that the ANS, together with the lower brainstem and the olfactory bulb, were the earliest areas affected by PD neuropathology (Braak et al. 2003; Savica, Bradley and Mielke 2018). The ANS normally operates automatically and, although it is part of the PNS, there are a widespread of connections between the ANS and the CNS; for instance, through the BG. As the pathology progresses, it extends through the ANS affecting additional structures and their functions: the sympathetic ganglia, cardiac sympathetic efferents and the myenteric plexus of the gut (Cersosimo & Benarroch, 2012). The dysregulation of those areas has been linked to the appearance of non-motor symptoms such as bowel problems (e.g., constipation), dribbling, orthostatic dysregulation, sleeping problems (e.g., RBD, insomnia and

excessive daytime sleepiness) and digestive problems (e.g., reflux, upper gastrointestinal dysmotility).

Furthermore, with the progression of PD pathology, many non-motor symptoms become more prevalent and disabling. Additionally, cognitive impairments can arise (e.g., executive dysfunction) and develop into dementia, which can significantly challenge patients functioning.

1.1.8 Role of Aging

The process of aging is a multifactor phenomenon characterised by a gradual decline of several physiological functions and increased susceptibility to certain diseases, including PD (Rodriguez, Rodriguez-Sabate, Morales, Sanchez, & Sabate, 2015). Some of the initial noticeable signs that are common in Parkinson's, also naturally occur with aging. For instance, slower walking (i.e., bradykinesia) or increased number of falls, due to loss of balance and coordination, can be underestimated in PwP and easily blamed on aging. Hence, delaying the formal diagnosis of PD. This misperception can usually be clarified should symptoms change after taking Parkinson's medication (e.g., levodopa challenge test). Thus, keeping a record of symptom changes in relation to PD drug administration may be helpful. This is particularly important for research undergoing longitudinal studies, where recording medication changes throughout can aid in identifying whether outcome fluctuations occur due to a specific intervention or medicine changes.

Additionally, age is a well-established risk factor for idiopathic PD (Collier, Kanaan, & Kordower, 2011; Driver, Logroscino, Gaziano, & Kurth, 2009). Aged people present high incidence rates, and rapid increases in occurrence have been observed after the age of 60 years (Reeve, Simcox, & Turnbull, 2014; Van Den Eeden et al., 2003). This could be explained by the fact that with advancing age and senescence (i.e., biological aging), several processes that are essential for neuronal survival fail and contribute to neuronal loss in regions of the brain.

Numerous studies have detected and measured the neuronal loss that occurs within the brain due to aging. Interestingly, it has been observed that specific brain areas seem more vulnerable than others. The SN region is of particular interest, since dopaminergic neurons show increased vulnerability to aging and changes that can induce cell death (Dreher, Meyer-Lindenberg, Kohn, & Berman, 2008; Reeve et al., 2014). The processes that, alone or in conjunction, are sufficient to cause neuronal loss are: altered efficiency and changes in protein degradation pathways, mutations in mitochondrial DNA that affect ATP production, and build-up of oxidative products within the SN neurons caused by accumulation of reactive oxygen and nitrogen species with advancing age. Furthermore, processes such as dopamine metabolism, accumulation of iron and neuromelanin, and calcium handling (important for neuronal firing activity), experience age-related changes and can push dopaminergic neurons of the SN towards cellular death (Reeve et al., 2014). All these changes become stressors for the SN and weaken the dopaminergic neurons, which will not be able to overcome further

pathogenic insults that are observed in Parkinson's, such as: lysosomal dysfunction (which alters the autophagy-lysosomal pathway that regulates intracellular homeostasis), protein aggregation and misfolding (e.g., α-synuclein), neuroinflammation, activation of apoptosis and cell death pathways, among others (Mercado et al., 2017; Sarkar et al., 2016; Xu, Wei, Li, & Zhang, 2019). These Parkinson's-specific altered pathways individually, place dopaminergic neurons under significant oxidative stress with high concentrations of abnormal aggregates and damaged proteins. In order to cope with the increase demand of the degradation system, an efficient functioning of degradation pathways is crucial. However, as mentioned above, the overall competences of the degradation system are altered and progressively affected due to aging. Ultimately, all these factors together, could contribute to neuronal loss within the SN region. The main difference between healthy aged subjects and PwP, is the quantity of SN cell loss being significantly further accelerated in people suffering from Parkinson's. Moreover, it has been suggested that the neurodegeneration due to aging processes is not the only primary cause of idiopathic PD since the pattern of striatal dopamine loss observed in healthy aging differs substantially from the pattern observed in PD (Kish, Shannak, Rajput, Deck, & Hornykiewicz, 1992). Thus, supporting the multifactorial nature of PD pathogenesis.

In addition to age-related neuronal changes that can induce cell death, there is evidence that, with aging, non-neural cells (i.e., glia [e.g., microglia and astrocytes]) progressively start showing a dysfunctional and anomalous activity, which has also been observed in the PD brain. The altered activity of glial cells creates an environment of low-level inflammation ('neuro-inflammaging') important for the degeneration of dopaminergic neurons in the SN of aged brains and PD (Rodriguez et al., 2015). Furthermore, trophic support to neurons is vulnerable to aging and PD (Miranda, Morici, Zanoni, & Bekinschtein, 2019). Consistent with this idea, evidence from animal and human studies shows that both peripheral (i.e., circulating levels) and central (i.e., brain levels) concentrations of BDNF are reduced in aged primates, humans and rats (Hayashi, Mistunaga, Ohira, & Shimizu, 2001; Shimada et al., 2014; Silhol, Bonnichon, Rage, & Tapia-Arancibia, 2005). Consequently, a reduced BDNF expression has been associated with neuronal atrophy, reduced proliferative activity and neuronal death, which also occurs in PD and other neurological disorders (Murer, Yan, & Raisman-Vozari, 2001). This suggests that interventions aiming at increasing BDNF levels could prevent pathological changes in the CNS that are associated not only to aging but also to neurological disorders, including PD. Therefore, providing neuroprotection of vulnerable and affected areas such as the nigrostriatal system.

1.1.9 Quality of Life

PD is a condition that, apart from jeopardising mobility, can also have an impact on mental health, social interactions, behaviour and overall QoL. These factors, classed as non-motor, can directly impact PwP's willingness to engage with activities, such as exercise, that can have a beneficial impact for the prognosis and symptoms of this condition.

Fortunately, thanks to the amount of research completed in recent decades, the traditional view of PD as a purely motor disease has evolved and it is currently well acknowledged that PD is often associated with non-motor disturbances that can appear even before the motor symptoms (Poewe, 2008; Santamaria, Tolosa, & Valles, 1986). Although the awareness of the different forms of non-motor symptoms and their disabling ability has improved significantly, non-motor symptoms are often poorly managed by doctors and under-recognised, valued and treated in clinical practice, compared to motor symptoms, impacting negatively on QoL (Bonnet et al., 2012; Chaudhuri et al., 2006).

Medication aside, there are different factors that can positively impact PwP's QoL. For instance, engaging with group exercise has shown to positively influence some of these non-motor symptoms, and QoL overall. For instance, in Sheehy's et al. (2016) research, participants with PD that were part of a non-contact boxing-based group exercise program were recruited. Their research indicated that joining the exercise programme improved their physical competence, helped breaking taboos about PD, alleviated anxiety about the disease, reduced stress, sustained participation and cemented shared connections in the group. As participants gained experience, they welcomed modifications and increments of difficulty, however, some participants preferred to stay in a lower-level class (Sheehy, McDonough, & Zauber, 2016). Thus, it is important to tailor the interventions to accommodate all different participants to the programmes being developed. Additionally, it is important to bear in mind, that participants in group exercises can struggle with anxiety about seeing others at more advanced stages of PD, which can be overwhelming, disturbing and have an impact on dropout rates (i.e., known as downward affiliation comparison). However, Sheehy et al. (2016) observed that this view can be shifted to a downward dissociation comparison, where participants that gained more confidence with the exercise programme and performed better than others felt a boost of confidence and motivation to keep exercising (Sheehy et al., 2016). Hence, it is important that an intervention is long enough to be able to elicit all these changes. Exercise interventions such as aerobic exercise, dance, and Tai Chi, can significantly improve QoL in PD patients but they have to last at least 12 weeks to generate significant benefits (Chen et al., 2020). It has been observed that exercise programmes of short duration (i.e., 8 weeks or less) are not as effective as longer programmes (i.e., 12 weeks or more) at improving mobility, balance, cognition and QoL (Tidman & Skotzke, 2020). Taking part in a community-based program over a 12-month period that includes physical and mental activities has also been associated with the maintenance or improvement of both motor performance and QoL measured by an assortment of mobility and balance tests and the 39-item Parkinson's Disease Questionnaire (PDQ-39) (Stiles, Jaffe, Schwartz, Rossi, & Riley, 2020).

Finally, although exercise and social interaction induce many benefits, PwP can experience apathy, reduced motivation and/or emotional engagement, which can have an impact on PwP's involvement with social activities and attendance to exercise programmes (Simpson, McMillan, Leroi, & Murray, 2015). Moreover, Barone et al. (2009) found that apathy can be related with reduced QoL, which

was found to be associated with the worst score of the PDQ-39 when evaluating different non-motor symptoms (Barone et al., 2009).

PwP experience a wide range of emotional states in response to the life events that are strongly influenced by their condition (Tomagová et al., 2019). Therefore, it is important to account for the effects of both motor and non-motor symptoms in PwP and evaluate not only functional changes, but also emotional reactions, states and feelings experienced in order to design interventions that are able to improve or sustain QoL.

1.2 Current Interventions

During the last 20 years, there has been an increasing amount of research and trials attempting to develop pharmacological treatments to provide a neuroprotective effect as well as an improvement in surgical procedures. Although very promising results have been obtained, currently, there is no pharmaceutical or surgical therapy that tackles all three components together: providing a neuroprotective effect, stopping the disease progression and restoring the damage caused by the condition. To date, there is still no known cure for PD and the treatments that are available do not have an impact on the pathophysiology and progression of the disease. The symptomatic treatments include supportive therapies (e.g., rehabilitation) and medications. Medications have a palliative effect on the symptoms and help reduce them whilst supportive therapies try to improve QoL of PwP. However, there are potential interventions currently being studied and developed that are making great progress in respect of modifying the prognosis and progression of this disease.

In order to present real therapeutic potential for Parkinson's, an intervention has to target and act upon the three core components mentioned above: neuroprotection, slow or halt progression of PD, and restoration of damaged nerve cells. Currently, there is no single treatment that accomplishes these three components together, particularly, because the fundamental mechanisms of Parkinson's aetiology and progression are not fully understood. Moreover, patient heterogeneity presents a challenge for traditional-medicine approaches. A personalised-medicine approach designed for individual disease's profiles would be an optimal curative therapy. Nevertheless, promising disease-modifying therapies are emerging. For instance, cellular therapeutic options using a regenerative medicine approach, such as stem cell intracerebellar transplantation to the striatum and SN, could help in restore dopamine to its normal levels (Henchcliffe & Sarva, 2020). A combination of treatments could be the solution to successful treatment therapy for Parkinson's.

1.2.1 Emerging Therapies and Clinical Trials

In early 2019, Kern et al., in Australia, completed the enrolment and procedures for the first approved in-human neural stem cell-based clinical trial for PwP. The main purpose of this open-label, single centre, Phase 1 trial (ClinicalTrials.gov identifier: NCT02452723) was to evaluate the safety,

tolerability and preliminary effectiveness of transplanting human parthenogenetic neural stem cells (ISC-hpNSC) to patients with PD over a 12-month period (with a planned five-year, long-term follow-up). This study is currently underway, however, in the interim, an overview of the results has been made available in an abstract reporting no serious events related to the cell transplant, and a dose-dependent preliminary improvement of motor symptoms, QoL and participants' global functioning at 6 months (Kern et al., 2019). Although clinical trials with stem cells for PD are on the horizon and are far from discouraging, the understanding of the mechanisms of action of these cells is still incomplete and more research is needed with an appropriate number of participants involved. Furthermore, cell therapies do not mitigate the role of α-synuclein and the prion-like behaviour of its misfolded forms leading to LP (Brundin & Kordower, 2012). In that respect, immunotherapy has been used as a direct method against the abnormal accumulation of the protein α-synuclein, which is known to be associated with PD pathology. Initially, studies performed in animal models hypothesised that the use of specific antibody fragments against different epitopes (i.e., antigenic determinant, part of an antigen where antibodies bind) of α-synuclein could diminish the accumulation of pathogenic intracellular aggregates of α-synuclein in both cellular and preclinical models (Vaikath et al., 2019). This is meant to interrupt the prion-like spread of misfolded α-synuclein, entailing a potentially valuable disease halting mechanism. Firstly, Tran et al. (2014) proved *in vitro* and *in vivo* mice models of PD that α-synuclein monoclonal antibodies (Syn211 and Syn303) could reduce LP formation, prevent toxic α-synuclein uptake, prevent cell-to-cell spread, rescue tyrosine hydroxylase (TH) cell loss (TH identifies dopaminergic neurons in the CNS) and improve motor dysfunction (Tran et al., 2014). Additionally, Games et al.'s (2014) research provided further evidence of the therapeutic potential of α-synuclein immunotherapy for PD. Games et al. showed that by targeting α-synuclein's C-terminal region (CT) to avoid its CT-truncation, synaptic and axonal pathology could be attenuated. Furthermore, the accumulation of CT-truncated α-synuclein, which had been previously related with the formation of toxic fragments and pathogenic oligomerization and propagation of α-synuclein, was also reduced. Finally, the loss of TH fibers in the striatum, associated with the loss of dopaminergic input to the striatum present in PD, was able to be rescued, and motor and memory deficits improved in a mouse model of PD (Games et al., 2014).

To date, with the evidence of cellular and preclinical models, several clinical trials have evaluated the safety and tolerability of ascending doses of single and multiple intravenous infusions of anti-α-synuclein antibody infusions in humans (Jankovic et al., 2018; Schenk et al., 2017). These studies have demonstrated that PRX002 (a humanized monoclonal antibody targeting aggregated forms of α-synuclein) generally presented good safety and tolerability when intravenous infusions were applied. Additionally, Jankovic et al. (2018) found that, at the highest PRX002 dose (60 mg/kg), serum α-synuclein levels were reduced by up to 97%. However, cerebrospinal fluid (CSF) levels of α-synuclein did not significantly change although dose-dependent increases of PRX002 were observed (Jankovic et al., 2018). In contrast to the success achieved in the periphery, PRX002 concentrations in the CSF were not high enough to engage the monomeric α-synuclein. Recently,

this advanced study reached the clinical phase II study level with a randomized double-blind design (PASADENA Study, identifier: NCT03100149). Another key immunotherapy study is the SPARK study (Phase I identifier: NCT02459886, Phase II identifier: NCT03318523), a clinical trial currently in phase II, which studies a monoclonal antibody selective for the aggregated form of α-synuclein (named BIIB054) (Brys et al., 2019). The primary aim of this clinical trial is to evaluate the dose-related safety, immunogenicity, pharmacodynamic effects and pharmacokinetic profile of BIIB054, as well as its effects on the integrity of nigrostriatal dopaminergic nerve terminals. Apart from these leading immunotherapy studies with direct approaches in PwP, there are other clinical trials that are scheduled to be completed within the next year and will help further developing α-synuclein-targeting treatments (Zella et al., 2019).

Apart from immunotherapy, there are other promising approaches that have shown to have a vast potential as neuroprotective strategies. A large and growing body of literature has been investigating the use of NTFs as agents to promote neuroprotection and, therefore, nurture and support the affected neuronal populations. More recent attention has focused on the possibility of directly infusing NTFs into specific brain areas. In 2019, Whone et al. completed a pioneering clinical trial, called the GDNF trial, investigating the effects of GDNF in PD (Whone, Boca, et al., 2019; Whone, Luz, et al., 2019). They developed an intermittent intraputamenal (i.e., brain structure located at the base of the forebrain) delivery paradigm that was well tolerated and safe for the study participants. Additionally, they showed that the administration of GDNF did not evolve any immune reaction against the exogenous inoculated molecule (i.e., antibodies against GDNF were not detected). On the other hand, although their administration mode was more successful in delivering GDNF than in preceding trials, they failed to achieve clinical benefits since significant differences between the placebo and GDNF treatment groups were not observed. Clinical trials have mostly used GDNF administration, however, other NTFs, such as BDNF, seem promising in treating neurodegenerative conditions (Palasz et al., 2020). Evidence from animal models and the biological properties of BDNF, provide a valuable foundation to support BDNF's potential to: prevent neuronal cell loss, increase survival of dopaminergic neurons in the SN, protect dopaminergic projections to the striatum, and restore dopamine uptake in the striatum (Kim, Kareva, Yarygina, Kholodilov, & Burke, 2012; Razgado-Hernandez et al., 2015; Zhu, Li, He, Wang, & Hong, 2015). Similarly to other NTFs, the mode of delivery is key and could be performed either by intraparenchymal protein infusion or gene delivery using viral vectors, which have yet to be trialled for BDNF (Nagahara & Tuszynski, 2011).

It is important to bear in mind that, although holding a strong biological rationale and being a very promising and potentially useful strategy to provide neuroprotection and neuro-restoration, the use of NTFs is highly challenging. NTFs have a short in vivo half-life, poor bioavailability, and – although not null – marginal permeability through the BBB, which has been further discussed in section **1.6.2 Role of neurotrophins**. Furthermore, the duration of treatment of neurodegenerative conditions may last several years, hence, accessibility and delivery modes of the therapeutic options are important issues to be considered (Nagahara & Tuszynski, 2011).

The search and development of specific preclinical and clinical PD biomarkers is also crucial for tracking the effects of new potential therapies aimed at delaying, stopping or reversing the degeneration process that starts before the clinical symptoms become apparent. Additionally, biomarkers could be used to more easily detect "at-risk populations" and to design early suitable interventions to limit or even prevent the development of the disease. The development of novel biomarkers could also inform the effectiveness of specific types of medications and aid towards the development of personalised-medicine approaches for PwP.

1.2.2 Medical options

Once the diagnosis of PD is made, generally, treatment starts with palliative medical therapies that can improve the control of a diverse array of symptoms. Based on the patient's symptom profile (which, to some degree, can be predicted by disease duration) physicians can tailor specific therapeutic choices.

Around the late 1890s, Georges Marinesco and Paul Blocq published the first article that presented a possible association between Parkinson's and the SN by suggesting that a lesion of the midbrain could contribute to the characteristic motor symptoms observed in PwP (Hostiuc, Drima, & Buda, 2016). Later, in 1919, Constantin Trétiakof thesis work confirmed Marinesco and Blocq's hypothesis and stated that "*The results of our research lead us to say that there is an intimate relation between the substantia nigra and Parkinson's disease. It most likely is a cause-effect link*" (Hostiuc, Drima and Buda 2016, p. 4), and was the first to provide evidence of a substantial loss of pigmented nigral cells and the presence of LB in the remaining neurons in post-mortem analyses of brains of PwP (Geibl et al., 2019). It was almost 40 years later, when the Swedish neuropharmacologist Arvid Carlsson demonstrated that reserpine-treated rabbits presented depleted DA levels and a similar onset of motor deficits to parkinsonism. Moreover, his work showed that L-DOPA (i.e., the amino acid dihydroxyphenylalanine, immediate precursor of dopamine) fully antagonised reserpine, alleviated the symptoms that the animals presented and restored DA brain levels, for which he was awarded the Nobel Prize in Physiology or Medicine in 2000, together with Eric Kandel and Paul Greengard. These results suggested the role of a previously unknown brain amine (i.e., DA) as a CNS neurotransmitter. Soon after this, Ehringer and Hornykiewicz observed that there was a severe DA loss in the caudate and putamen (subcortical structures that belong to the striatum) of PD patients (Hornykiewicz, 1963). These findings were followed by several articles that evaluated the administration of L-DOPA to alleviate clinical symptoms of PD. Subsequently, based on the important work of George C. Cotzias, in the 1970s, the oral intake of L-DOPA was approved by the US Food and Drug Administration (FDA) as the first drug to treat PD (Geibl et al., 2019). Thus, the use of Levodopa (L-DOPA) became the earliest effective treatment for PwP.

L-DOPA is the chemical precursor to dopamine used to replenish the supply of dopamine, which cannot cross the BBB. Currently, there are more medications that are used to treat PD apart from L-

DOPA. These include: dopamine agonists (Pramipexole, Ropinirole, Amantadine), DOPA decarboxylase inhibitors (Benserazide, Carbidopa), catechol-O-methyl transferase (COMT) inhibitors (Entacapone, Tolcapone, Opicapone), monoamine oxidase B (MAO-B) inhibitors (Selegiline, Rasagiline, Safinamide), anti-cholinergic molecules (Trihexyphenidyl, Benztropine) and immunomodulatory therapies. However, several of these pharmaceutical agents are poorly soluble and not able to completely reach the CNS. Therefore, these drugs may be eventually metabolised, partially or completely, by the liver and this is when COMT inhibitors become useful (which prevent the breakdown of L-DOPA and prolong the duration of each dose) (Nutt et al., 1994).

Most patients, within 3 to 6 months of treatment, will achieve a substantial (60 to 80%) improvement of their motor symptoms through medical control. This drug-related improvement is usually consistent and reliable throughout the day and can last several years. However, over time and, especially, from the third year after diagnosis, PwP can develop motor fluctuations and the medication benefits wear off at a faster rate, meaning that the next medical dose will be needed earlier (i.e., up-titration) (López et al., 2010). When motor fluctuations are developed, practitioners can rely on additional lines of medical treatment, such as adding supplementary drugs (e.g., dopamine agonists can be added on the prescription of patients that initially started on L-DOPA). Usually, these medical adjustments can, to some extent, re-establish consistent control over the symptoms throughout the day. Nevertheless, over time, the development of motor fluctuations (i.e., motor performance changes), which is associated with disease duration, becomes inevitable and some patients will also develop dyskinesias (i.e., excessive involuntary bodily movements, including chorea and dystonia) (Cilia et al., 2014). PwP can experience some parts of the day with dyskinesias, which most typically occurs at peak-dose (on-phase), and episodes of freezing, bradykinesia and rigidity during the off-phase. In this situation, medication increases of dopamine levels will likely worsen peak-dose dyskinesias. However, a decrease in dopamine dose may worsen PD's symptoms and increase the length of the off-phase. Therefore, the therapeutic window is set above the threshold required to improve PD symptoms (start of on-state) and below the peak-dose dyskinesia (dyskinesia-threshold). Unfortunately, the therapeutic window narrows with time and the peak-dose dyskinesia threshold progressively decreases. Initially, the therapeutic window can last for about 4 to 6 hours and, after several years of treatment, it can diminish to 60-90 minutes (Obeso et al., 2000). However, contrary to what it was originally thought by clinicians, the onset of motor fluctuations and dyskinesias, and the narrowing of the therapeutic window, are not associated with the duration of levodopa therapy; instead, they are associated with disease progression and higher levodopa daily dose (Cilia et al., 2014).

Although it had been widely studied that the pathogenesis of PD targets the dopaminergic cells in the SN_{pc}, many uncertainties about PD's aetiology still remain. Moreover, it has been known for a long time that, in the course of PD, the dopaminergic pathway might not be the first affected region nor the only responsible for the bast array of symptoms that PwP present. Nonetheless, nowadays, dopamine replacement and restoration with the administration of L-DOPA or DA receptor agonists

still remains the gold standard therapy of symptomatic treatment for PD (Salamon, Zádori, Szpisjak, Klivényi, & Vécsei, 2020; Schade, Mollenhauer, & Trenkwalder, 2020).

1.2.3 Device-based Therapies

Unfortunately, motor fluctuations can eventually become uncoupled from the medication cycles, and become more inconsistent and unpredictable. At this stage, when palliative drugs fail to adequately control patients' symptoms, device-based therapies might be implemented: Deep Brain Stimulation (DBS; a surgically implanted neurostimulator delivers electrical stimulation to targeted areas in the brain [i.e., subthalamic nucleus] that control movement and block abnormal nerve signals that cause tremor, rigidity and walking problems), subcutaneous infusion of Apomorphine by a pump, and the newest option is Duodopa (Levodopa and Carbidopa [ratio of 4:1] in gel formulation which is infused by a pump directly to the small intestine). These three therapies have shown to reduce off-time and dyskinesias and improve activities of daily living (ADLs); nevertheless, more research is needed to effectively and directly compare these three therapies to help direct clinical management decisions (Antonini & Odin, 2009; Clarke, Worth, Grosset, & Stewart, 2009).

1.2.4 Non-medical options

In order to improve symptoms that cannot be relieved by taking medication, supportive therapies can help PwP maintain independence and improve QoL. The strategies of supportive therapies include: rehabilitation via physiotherapy (to avoid gradually developing muscles weakness due to lessened movement), occupational therapy (which may include adapting one's home and workplace to suit their needs), speech and language therapy (in order to improve the ability to move the facial muscles, show more facial expressions and breath) and psychological support (to treat depression or anxiety).

Furthermore, during the last decade there has been increased interest in developing exercise regimes not only to improve physical function in general but also to enhance neurobiological mechanisms that are believed to act on the three key components needed to develop curative therapies.

Consistent and sustained exercise activity can safely and effectively be maintained over multiple years for PwP. Thus, participation in such activities is feasible and beneficial for PwP (States, Sweeny, Rossi, Spierer, & Salem, 2017). The integration of physical activity to help manage the natural decline associated with PD started in the 1950s, however, with the publication of Gower's *Manual of Diseases of the Nervous System* in 1899, there was a period of time where recommendations for PD were based on the statement that "*life should be quiet and regular, freed, as far as may be, from care and work*" (Gowers, 1899), which ignored any previous observations suggesting that movement had the potential to improve PD. Nonetheless, with the publication of impactful experimental and animal studies, this view started to change and, up to the present time, a considerable amount of literature has been published showing that exercise interventions can

effectively maintain or even improve mobility, confidence, executive functions and QoL (Cakit, Saracoglu, Genc, Erdem, & Inan, 2007; Tanaka et al., 2009). It has been additionally suggested that exercise has the potential to slow Parkinson's progression, improve motor (e.g., functional mobility, ADLs, etc.) and non-motor symptoms (e.g., cognitive function, bowel and sleeping problems, etc.) by providing neuroprotection and boosting neuroplastic changes within specific brain areas (Alonso-Frech, Sanahuja, & Rodriguez, 2011; Campos et al., 2016; Cheng & Su, 2020; Ransmayr, 2011).

Specific beneficial effects of physical activity and different exercise modalities will be presented and discussed in the following sections.

1.3 Levodopa Equivalent Dose

Since its discovery in the 70s, L-DOPA has been considered the gold standard treatment for Parkinson's dopamine deficiency. However, L-DOPA has a short-half life and multiple daily doses are required to maintain its therapeutic levels in plasma. As the disease progresses, usually higher daily doses of L-DOPA are needed, which has been related with the onset of motor fluctuations, dyskinesias and the narrowing of its therapeutic window (i.e., swings in the therapeutic response to L-DOPA known as 'wear off' phenomena) (Cilia et al., 2014). In current practice, alternative pharmaceutical approaches are put in place with the aim to reduce L-DOPA related complications; for instance, other therapeutic choices include dopamine agonists, DOPA decarboxylase inhibitors, COMT inhibitors, MAO-B inhibitors, anti-cholinergic molecules (Trihexyphenidyl, Benztropine) and immunomodulatory therapies **(**see **Medical options** discussed in **section 1.2.2**).

The use of various pharmacological agents and different dose intensities hinders the interpretation and comparison of results from studies and clinical trials. To facilitate this, L-DOPA equivalent doses (LEDs) for each medical regimen can be calculated. The overall LED (also named levodopa equivalent daily dose [LEDD]) can be obtained by adding together the LEDs for each antiparkinsonian drug that are taken in one day. In 2010, Tomlinson et al. published a systematic review documenting the LEDs for Parkinson's drugs (Tomlinson et al., 2010). Tomlinson's team defined LEDs as "*that which produces the same level of symptomatic control as 100 mg of immediate release L-dopa (combined with a dopa-decarboxylase inhibitor)*" (Tomlinson et al., 2010). Following this protocol, they determined conversion factors that allowed the calculation of LEDD. LEDD provide an artificial, but useful estimate of the total daily antiparkinsonian medication that participants are receiving. Moreover, if used as a standard scheme, LEDD can allow comparisons between studies. Recently, Schade, Mollenhauer and Trenkwalder (2020), updated Tomlinson's et al. proposal by adding LED conversion factors for opicapone and safinamide (Schade et al., 2020).

In this thesis, participant's LEDD in all studies has been calculated using updated LED conversion formulae and protocols from published reports (Schade et al., 2020; Tomlinson et al., 2010). LEDD

has been thenceforth used to track changes in participant's treatment regimens and used as covariate in the analyses.

1.4 Effects of exercise on Parkinson's disease characteristics

Physical activity, defined as body movement produced by muscle action that requires energy expenditure, is an encompassing term that includes the subcategory of physical "exercise" (World Health Organization, 2009). Exercise is, therefore, understood as the term for physical activity that is planned, structured, and repetitive for the purpose of conditioning any part of the body to improve or maintain one or more components of physical fitness (Bouça-Machado et al., 2020; Caspersen, Powell, & Christenson, 1985; Dasso, 2019). There are different ways to prescribe exercise, but they all follow the FITT principles, which stands for Frequency (number of exercise sessions per day or the number of exercise sessions per week), Intensity (amount of effort the individual exerts), Time (length of each exercise session) and Type (mode of exercise performed), respectively (Cheng & Su, 2020). Generally, based on their type, exercises can be grouped in 4 different main modes: aerobic (cardiorespiratory), anaerobic (including strength, high-intensity interval training [HIIT], etc.), balance and coordination, and flexibility. Then, a multi-modal protocol refers to exercise interventions that are based on the combination of exercises of different components, such as aerobic and strength exercises, including other physical skills, such as balance and flexibility, or combining physical and mental exercises (e.g., adding memory games, counting backwards, etc.).

Research has shown that exercise is a valuable adjuvant for PwP. It helps managing PD symptoms and, there is evidence supporting that exercise can, additionally, improve the clinical efficacy of L-DOPA (Muhlack, Welnic, Woitalla, & Müller, 2007). Muhlacks' team observed a better motor response assessed with the UPDRS-III (motor examination part) after the exercise condition compared to a resting condition (Muhlack et al., 2007). They then hypothesised that continuous exercise contributed to the synthesis of central endogenous dopamine and its release into nigrostriatal regions enhancing dopaminergic sensitivity and neurotransmission (Muhlack et al., 2007). Despite these results, more studies, including RCT, are needed to confirm these hypotheses and to further investigate Parkinson's drugs metabolism in order to shed some light on the drug-exercise interactions. It is known that exercise alters liver blood flow, which may have an impact on drug metabolism (Niederberger & Parnham, 2021). However, these interactions are very complex and the pharmacokinetics of PD drugs on different exercise-related conditions (i.e., mode, intensity, duration) remain to be elucidated. It also remains unclear whether an improvement in PD's symptomatology after exercising happens due to the direct effects of exercise or the properties that exercise has related to its interactions with PD medication. Furthermore, another factor that determines exercise-induced adaptations is a change in blood flow distribution (Niederberger & Parnham, 2021). Blood flow changes can not only positively affect blood composition and the physiology of organs such as the brain but also have a negative impact on plasma protein binding of drugs. These key factors for the treatment of PD can ultimately have an impact on drugs' efficacy.

Awareness of the properties and complexity of the drug-exercise interactions has improved in the recent years, nonetheless, specific data on PwP about the effects of exercise on drug efficacy remains scarce.

Separately from the effect that exercise can have on the pharmacokinetics of drugs, it is important to develop treatments that are not only palliative but can protect individuals from developing PD, stop the condition or reverse its progression. During the past decade, a substantial amount of research has provided evidence of the ergogenic effects of exercise for PwP. Several reviews have been published, amalgamating the existing body of research on both human participants and animal trials providing evidence of the positive impact that exercise can have on both motor and non-motor symptoms (Cusso, Donald, & Khoo, 2016; LaHue, Comella, & Tanner, 2016).

Different mechanisms have been proposed to explain the exercise-induced benefits on both motor and non-motor symptoms, some of which will be further described below (see sections 1.4.1 and 1.4.2). However, these enhanced therapeutic underlying biological mechanisms can result in an increase of DA release that leads to improvements in motor function (through the activation of the dorsal striatum, which is the gateway to the BG – group of subcortical nuclei modulated by dopaminergic afferents from the SN_{PC}), enhanced mood, decision making, reward-related behaviour, motivation, and reduced apathy (through the activation of the ventral striatum, which receives projections of mesolimbic dopaminergic afferents) (Alexander, DeLong, & Strick, 1986; Cusso et al., 2016; Tanaka & Kirino, 2016). With this information in mind, Sacheli et al. (2018) designed a study to evaluate differences in DA release, reward signalling, and clinical features with PET scans between habitual exercisers and sedentary participants with PD (Sacheli et al., 2018). Participants were asked to complete 30 minutes of cycling on a bicycle ergometer at 60% of VO_2 reserve (i.e., percentage of the difference between maximum and resting VO_2) and PET scans, functional MRI, motor, and non-motor assessments were completed. Their results showed an increased DA release in the dorsal striatum of habitual exercisers, who also presented a greater ventral striatum activation, and lower apathy and bradykinesia scores, compared to sedentary participants with PD. This study provides evidence of the symptomatic benefits that habitual exercise can induce (i.e., exercise is associated with preservation of motor and non-motor function) and suggests that this beneficial response may result from synaptic changes (improved strength, density, and neuronal survival) due to an increased activity of dopaminergic pathways, through increased DA release, DA receptor density, or both, increased neurotrophic factors and immune response modulation, amongst others. A later study from the same group further evaluated the effects of aerobic exercise on evoked DA release and the activity of the ventral striatum comparing a group of PwP that completed an exercise intervention to a control group (Sacheli et al., 2019). Analyses revealed that aerobic exercise can modify the responsivity of the ventral striatum (likely through changes to the mesolimbic dopaminergic pathway) and increases evoked DA release in the caudate nucleus (part of the dorsal striatum) (Sacheli et al., 2019). This suggests that exercise-induced therapeutic effects could be

related to corticostriatal plasticity and enhanced DA release. These studies provided tangible evidence of the benefits of regularly engaging with exercise for PwP.

Although there is evidence showing that exercise is a valuable adjuvant for PwP that can improve medication uptake and efficiency, enhance DA release, and modulate the activity of different brain areas such as the striatum, the severity of motor symptoms does not always correlate with the degree of nigrostriatal dopaminergic degeneration that PwP present. The neuropathological events that precede the appearance of motor symptoms are linked to a severe degeneration of the nigrostriatal dopaminergic system and it is not until this deterioration has reached a 50–70% loss of striatal dopaminergic innervation that motor symptoms become apparent (Brooks, 1998; Hou et al., 2017; Noyce et al., 2016). This event, exposes the compensational ability of the brain, which is also known as Neuronal or Brain Reserve (BR), and allows the brain to operate without functional impairment until damage reaches a critical threshold reflecting the capacity of oneself to tolerate neuropathological events (Satz, 1993; Stern, 2002). This concept has also been defined in AD as Cognitive Reserve (CR), which refers to the differences observed between individuals in their susceptibility to brain changes due to aging or Alzheimer's pathology (where some can tolerate changes and maintain better function compared to others) (Stern, 2012). Stern suggests that 'reserve' acts as a moderator between pathology and clinical outcome (Stern, 2012). In PwP, it has been observed that despite presenting similar pathological changes (i.e., similar degrees of dopaminergic neuronal loss, dopamine depletion, etc.), there are individual differences in motor deficits. This is known as Motor Reserve (MR), an important conception for PwP. Importantly, MR can reflect an individual's capacity to tolerate Parkinson's dopaminergic loss by using compensation mechanisms (Palmer, Ng, Abugharbieh, Eigenraam, & McKeown, 2009). Thus, PwP with higher MR require lower doses of PD medications and presenting higher MR is associated with a lower risk of developing levodopa-induced dyskinesia and FoG (Chung et al., 2020). Moreover, Chung's et al. longitudinal study showed that initial MR can modulate the long-term prognosis in individuals with PD and that factors influencing initial MR in PwP could improve PD's progression and reduce complications related with its development (Chung et al., 2020). An important factor that can enhance MR in *de novo* individuals with PD is premorbid (i.e., preceding the occurrence of symptoms of a disease) exercise engagement.

Just as premorbid cognitive activity enhances an individual's CR, Sunwoo et al., evaluated whether premorbid engagement with exercise could enhance MR in PD (Sunwoo et al., 2017). Participants with mild striatal dopamine depletion that were physically more active showed significantly less motor deficits than groups of participants with similar striatal deterioration that were less active (Sunwoo et al., 2017). However, the more active group also presented a more abrupt decline in motor function related to the striatal dopamine activity in comparison to the other less active groups. This outcome has also been observed in individuals with AD with high CR (Stern, 2012). Researchers hypothesise that the rate of decline may be more rapid in individuals with higher CR and MR because the pathology has been tolerated longer than people with lower reserve.

The benefits of having a physically active lifestyle before PD's diagnosis have also been recently observed by other groups (Olsson, Svensson, Hållmarker, James, & Deierborg, 2020). Physical activity has been associated with an observed reduced risk of developing PD, however, the mechanisms undelaying this association are not fully understood. In order to explore this association further, Olsson et al. (2020) designed a prospective observational study and followed-up a big sample of long-distance skiers (n=197,685) and matched non-skiers from the general population (matching was done based on age, sex and region of residency; n=197,684). Olsson and colleagues were able to observe a reduced incidence (29%; Hazard ratio=0.71) of PD among long-distance skiers and suggested that this association was likely to be mediated by the levels of physical activity performed through life and MR, since this reduction was observed in both men and women. They also observed that this association became less strong with time (15-yrs. follow-up). With this evidence, researchers suggested that the presence of a reservoir of motor function protecting against PD could be the mechanism explaining the initial lower cumulative incidence for PD that, with time, converged with the non-skier group incidence (Olsson et al., 2020). Although Olsson et al. (2020) provided valuable results, their research presents limitations. For example, the levels of physical activity of non-skier participants were not measured. Therefore, this group may also include physically active participants. Nonetheless, researchers had previously characterised the skiers group and observed that the majority did exercise more than the general population (at least 4 hours a week) (Farahmand et al., 2003).

These studies emphasise the importance of being physically active as a potential general mechanism against neurodegenerative diseases such as PD highlighting its ability to delay the clinical appearance of PD's symptoms. More research is needed in order to elucidate whether the effects of having an active lifestyle and higher MR not only delay the diagnose of PD but also have an impact on the underlying pathology.

1.4.1 Effects of Exercise on Motor Function

Exercise has shown to elicit positive effects on PwP's symptomatology. However, it is important to narrow down the type of exercise and specify the effects that each modality elicits. For instance, hatha yoga has shown to provide improvements in balance and UPDRS motor scores, but not on gait kinematics in PwP (Elangovan et al., 2020). On the other side, group boxing showed significant improvements in gait (velocity and endurance) after participants attended 24–36 sessions of 90-min. each over a 12-week period (Combs et al., 2013). In the same study, a group of participants performed traditional exercise group (which included stretching and various seated exercises to improve range of motion, strengthening exercises, endurance training, and balance activities). Overall, both groups (boxing and traditional exercise) presented significant improvements in balance and mobility but only the boxing group significantly improved gait velocity and endurance (Combs et al., 2013). Also, a case study evaluating the effects of Tai Chi and the Lee Silverman Voice Treatment (LSVT) BIG home exercise program on an individual with stage 4 in the H&Y scale,

showed improvements in gait (i.e., walking velocity and step length) and balance (Pascal, Ehlers, & Hindman, 2018). Nonetheless, larger trials including more participants are needed.

The improvements in gait, balance, and mobility, described above, can help counteracting some of the cardinal signs of PD (bradykinesia, rigidity, and postural instability). However, it is important to bear in mind the complexity and high variability of Parkinson's symptomatology. The loss of automaticity of movements is another important feature in PwP that can affect some of the motor skills described above, such as balance and gait. Without pathology, automatic (unconscious) and voluntary (cognitive) control of movements interact to regulate motor performance. However, due to the diminished automaticity of movements present in PwP, individuals require a higher cognitive load to execute either motor or cognitive tasks (Redgrave et al., 2010). When referring to actions, there can be goal-directed (i.e., the relative utility and value of predicted outcomes is important for action selection) or habitual (i.e., stimulus–response, habitual control – which means that the action is habitual or automatic) actions (Redgrave et al., 2010). During the last decade, neurorehabilitation and exercise research in PD have evaluated and incorporated goal-based motor training (i.e., exercises based on the repetition and practice of goal-directed actions with progressive increases in intensity for the improvement or recovery of impaired motor function) (Petzinger et al., 2013). Some of the modalities that have already shown to provide benefits for PwP already include aspects of goal-based skill training. For instance, some aspects of Tai Chi training focus on dynamic postural control, which requires exercises that involve cognitive engagement and controlled weight shifting from the centre of gravity. Additionally, amplitude training also includes goal-based practice, especially when individuals are asked to put emphasis on producing large amplitude movements involving the whole body during the practice of a specific skill. Thus, PwP's observed benefits in gait, balance and mobility after engaging with a wide range of exercise modalities (e.g., Tai Chi, dancing, treadmill walking, boxing, amplitude training, etc.) could be elicited due to the beneficial effects of adding goal-based elements in these exercise modalities (Khuzema, Brammatha, & Arul Selvan, 2020; Petzinger et al., 2013).

The loss of automatic movements described above, can also be present as loss of automatic coupling between gait and posture, which, accompanied with impairments in response inhibition, visual perception and motor control, can lead to the appearance of FoG (Mak, Wong-Yu, Shen, & Chung, 2017). The automatic process of gait is regulated by descending pathways from the brainstem to the spinal cord that, modulated by the BG, involves steady-state stepping movements coupled with cognitive processing of posture-gait control, postural reflexes, head-eye coordination, and appropriate alignment of the body segments and level of postural muscle tone (Takakusaki, 2017). In PwP, damages in the BG circuitry (due to dysfunction of both the dopaminergic and cholinergic systems) and cognitive impairments presented can disturb posture-gait control, resulting in FoG or falling. Although it is usually deemed to be a "late" feature of PD, research has revealed that FoG can also occur in very early stages of idiopathic PD and, overall, 47% of PwP report having experienced episodes of freezing (Bloem et al., 2004; Jankovic, 2008). Exercise training can achieve

reductions in FoG by modifying long-term motor symptoms, physical functioning and PD's progression (Gao, Liu, Tan, & Chen, 2020; Mak et al., 2017). More precisely, walking training in forms of visually-cued treadmill training, auditory-cued walking training or anti-gravity treadmill training, as well as progressive lower limb strengthening and balance exercises, or adapted resistance training, have shown to improve FoG and mobility (Allen, Canning, et al., 2010; Baizabal-Carvallo, Alonso-Juarez, & Fekete, 2020; Frazzitta, Maestri, Uccellini, Bertotti, & Abelli, 2009; Silva-Batista et al., 2020). Additionally, goal-directed actions always require automatic processes of postural control (i.e., balance adjustments and muscle tone regulation) (Takakusaki, 2017). Therefore, exercise interventions including goal-based motor training approaches, as described above, have the potential to not only elicit general improvements in gait, balance and mobility, but also in FoG and risk of falling through the enhancement of neuronal control of motor programs (Takakusaki, 2017).

There remains a paucity of clear evidence on which modality of exercise is superior to improve PD symptoms, such as gait problems and cognition (Intzandt, Beck, & Silveira, 2018). Taken together, PD literature suggests that a mixed modality of goal-based exercises, yoga exercises, boxing, and aerobic exercise, amongst other types of exercise, could elicit more synergistic benefits than performing individual exercise modalities alone (Petzinger et al., 2013). Hence, giving a rational for using multi-modal exercise approaches for PwP, such as circuit training, where different functional exercises can be included without limiting them into one category of type of exercise.

1.4.2 Effects of exercise on non-motor functions

L-DOPA, currently the most effective treatment for PD symptoms, is more effective to palliate motor symptoms than non-motor symptoms (Hsueh et al., 2018). Thus, it is very important to develop therapeutic strategies that can improve non-motor symptoms since depression and cognitive deficits are the leading cause of reduced QoL and can affect up to 50% of patients with PD (Aarsland et al., 2021; Hsueh et al., 2018).

As discussed in this literature review, several studies have shown the beneficial effects that exercise has in numerous aspects of PD, emphasising that exercise training should be recommended to all patients with PD. However, due to this disease pathology, ANS functioning might become affected and, therefore, there are some aspects related to ANS dysfunction that should be considered. PD patients usually present attenuated cardiovascular responses during exercise, which must be carefully interpreted. The degeneration of brain areas that are important for an optimal cardiovascular control can lead to an inability to increase sympathetic drive and, therefore, affect cardiovascular responses that will present a reduced heart rate, reduced beat-to-beat heart rate variability (HRV) and inability to elevate blood pressure (BP) (Akbilgic et al., 2020; Sabino-Carvalho & Vianna, 2020). Blunted cardiovascular responses in healthy adults are considered as possible adaptations to an involvement with chronic exercise, however, for PwP these could become an important component of exercise intolerance and decrease exercise efficiency. Hence, special adjustments to these altered autonomic

responses must be implemented in patients with PD enrolling with physical activity programmes and interventions. For example, using subjective measures of exercise perceived effort (i.e., using the Rating of Perceived Exertion [RPE] scale) instead of heart rate measurements. More studies in the field are needed, nevertheless, Akbilgic et al. (2020) recently reported that by measuring the electric activity of the heart (which is directly linked to ANS functioning) with sophisticated electrocardiogram (ECG) analysis, can provide informative data and, additionally, identify subjects at high risk of developing PD (Akbilgic et al., 2020).

Animal models that mimic the hallmark changes of PD have paved the way to study the effects of exercise on non-motor symptoms, such as cognitive impairments, mood and sleep. In 2018, Hsueh et al. examined the effects of performing voluntary exercise on non-motor functions (i.e., cognitive function and depressive behaviour) in 6-hydroxydopamine (6-OHDA) lesioned rats. (Hsueh et al., 2018). They compared rats that had unlimited access to a running wheel to a group that were not allowed to exercise and observed that voluntary running wheel exercise could reduce the impairment of depressive-like and cognitive behaviour. Their results also suggest that the underlying mechanism of the beneficial effects of performing voluntary exercise could be explained by the upregulation of neuroprotective proteins (such as BDNF), whom presented significantly higher BDNF levels in the lesioned side of the striatum of 6-OHDA rats than the non-exercisers (Hsueh et al., 2018). However, in animal models, it is important to consider the amount, site and timing of the neurotoxin that will be used to create the PD model as well as the duration, timing and intensity of the exercise intervention in relation to the administration of the neurotoxin (Crowley, Nolan, & Sullivan, 2019). Unfortunately, these factors vary noticeably between currently published studies.

In addition to animal studies, the evaluation of the effects of exercise on cognition was also assessed in PwP. To date, several research studies have been reviewed. Although studies differ widely regarding FITT principles, the overall conclusion is that exercise interventions are a unique approach for PwP that not only alleviate the classic motor symptoms, but also improve a range of non-motor symptoms (Cheng & Su, 2020; da Silva et al., 2018; Intzandt et al., 2018; Murray, Sacheli, Eng, & Stoessl, 2014; Reynolds, Otto, Ellis, & Cronin-Golomb, 2016). Moreover, especially if implemented at early stages, exercise has proved to be a feasible adjunct treatment approach with wide-ranging benefits related to mood, cognition, and sleep, amongst others, for PwP (Reynolds et al., 2016). A recent RCT, investigated the effects of 3 different types of exercise (functional mobility, multi-modal and cognitive) and 2 different lengths (4 and 8 months) on psychological and cognitive aspects in people with PD (Gobbi et al., 2021). Gobbi's et al. (2021) reported that functional mobility and cognitive training showed the potential of maintaining executive function, attention, and working memory in PwP. Furthermore, contrary to what Tanaka et al. (2009) observed in their training study, the multi-modal training intervention did not substantially maintain or improve executive function features (Tanaka et al., 2009). One plausible explanation given by the authors was that their multi-modal intervention included lower-complexity activities with combinations of aerobic characteristics that may not have reached their target zone. On the other side, the low-complexity of these activities

allowed participants to socialise more, which was reflected in significantly lower levels of physical stress only observed after 8 months of engaging with multi-modal exercise (Gobbi et al., 2021). Thus, Gobbi's et al. results conflict with a previously published study that found that engaging with a multi-modal exercise program for 6-months can improve executive function measured with the Wisconsin Card Sorting Test (WCST) in PwP (Gobbi et al., 2021; Tanaka et al., 2009). In 2018, da Silva's group (2018) published a comprehensive review evaluating the last 10 yr RCTs on the effects of exercise on cognition in PwP (da Silva et al., 2018). This review integrates evidence from different exercise interventions (e.g., adapted tango for PwP, cognitive training combined with motor training [stretching, strengthening and axial mobility exercises], and treadmill training) showing the positive and significant effects of physical exercises on cognitive function in PwP (da Silva et al., 2018). Interestingly, cognitive training alone also has the potential of improving short-term memory, working memory and depression (Petrelli et al., 2014). Nonetheless, the nature of cognitive training (i.e., structured *vs* non-structured) defines which cognitive and affective functions can be improved differently.

It seems plausible to hypothesise that training both cognitive and motor function simultaneously could, therefore, provide more benefits for PwP. There is research suggesting that cognitive training combined with motor training can improve participants' independent performance of ADLs (II domain of the UPDRS), balance (measured using the Berg Balance Scale and the Unipedal Stance Test) and cognition (measured with the Montreal Cognitive Assessment [MoCA]) (Pompeu et al., 2012). However, a subsequent study investigating the effects of adding cognitive training to motor physiotherapy in comparison to motor physiotherapy alone showed that both interventions similarly improved balance (measured with the the Balance Evaluation Systems Test [BESTest]) and UPDRS scores, and that combined physiotherapy with cognitive training was not superior to physiotherapy alone (Terra, Barboza, Almeida, Bueno, & Smaili, 2020). Nonetheless, Terra's et al. outcomes suggest that, whilst balance improvements were maintained after 3 months of follow-up in both intervention groups, only the combined modality of physiotherapy and cognitive training provided lasting significant improvements in ADLs (domain of the UPDRS), which are important to maintain PwP's functional independence.

Further work evaluating the effects of combining motor training plus cognitive training in PwP is needed to further evaluate whether addressing both modalities together can potentiate the improvement of motor and non-motor outcomes. Moreover, most studies evaluating the effects of exercise on PD symptomatology only show improvements in either motor or non-motor symptoms (see **Table 1.2**), however, more research is required to evaluate those outcomes concurrently. Nonetheless, it is important to bear in mind that exercise dose matters, as demonstrated by the association of exercise frequency with less PD severity and better cognitive status, which emphasises the importance of exercise for PwP (Oguh, Eisenstein, Kwasny, & Simuni, 2014).

1.4.3 Role of multi-modal exercises vs single type exercises

In addition to the abovementioned beneficial effects that exercise can elicit in PwP, published research has also provided evidence about the importance of the specificity and the purposeful nature (i.e., goal-directed movements) of the training programme. For instance, to improve dynamic balance, confidence and centre of mass excursion, a specific programme based on core stability (following the principles of motor relearning and skill acquisition) was significantly better than doing non-specific exercise (involving general physical exercises of active joint mobilisation, muscle stretching, and motor coordination) (Cabrera-Martos et al., 2020). These outcomes are important to promote the maintenance and/or improvement of ADLs, which require appropriate mobility (e.g., walking, reaching, transfers, etc.), and are tightly linked with a reduced probability of risk of falling.

Collectively, the majority of research findings reported in this chapter consistently point towards an exercise-induced overall improvement of motor and non-motor function in PwP. In view of all the diverse characteristics and benefits of the different mentioned exercise modalities, the idea that a multi-modal approach could be more beneficial for PwP started to emerge. During the last decade, the development and implementation of multi-modal exercise interventions has been an area of interest amongst research in older adults and PwP (see **Table 1.2**). However, studies evaluating multi-modal exercise programmes as a holistic approach to address both the motor and non-motor deficits of PwP have not yet been undertaken in the United Kingdom (see **Table 1.2**).

A multi-modal exercise protocol is not only based on the combination of exercises for different components (e.g., aerobic, strength, flexibility, coordination, etc.), it is also focused on improving PwP's specific impairments. Multi-modal exercise approaches have the potential to include components that are specially selected because they are the most affected in PwP due to PD's symptomatology (i.e., increased rigidity, bradykinesia, FoG, gait problems, etc.). They also allow the combination of physical and mental exercises to additionally include cognitive training (such as adding memory games, counting backwards, etc.). Thus, the benefits of multi-modal programmes go beyond the advantages elicited by traditional specific methods (improvement of strength, flexibility, coordination, etc.) and can also improve PD's specific deficits. They can be delivered in the form of group class, which is a safe and feasible format for PwP that has shown to provide more benefits than individual training for PwP (Rawson et al., 2019). Moreover, multi-modal classes have a high adherence, can be long-lasting exercise programmes and help promoting a long-term active-life style (Pereira et al., 2012).

Furthermore, exercise presents several general health benefits that are also imperative for PwP. For instance, exercise has a protective effect for noncommunicable diseases (e.g., cardiovascular disease, stroke, diabetes, and some types of cancer), is associated with improved mental health and delayed onset of dementia (Langhammer, Bergland, & Rydwik, 2018).

Table 1.2 Characteristics of key studies included in the literature review on multi-modal exercise. NP = not provided, con = control, d = days, int = intervention, m = months, y = years.

Study & Location	Participants	Intervention	Assessments	Main Results
(Gianoudis et al., 2014) Melbourne, Australia	N = 162 A (int) = 68 A (con) = 67 Older adults	**Frequency**: 3d/week **Intensity**: 3 to 8 RPE (Borg Scale 1 – 10) **Type**: community-based multi-modal exercise **Time**: 60 min **Duration**: 12 m **Adherence**: 93% (59% compliance) **Location**: community setting	**Physical function**: Three-repetition maximum testing, Timed Stair Climb Test, 30 Second Sit-to-Stand, Four Square Step Test, and the Timed up-and-go (TUG) test with a cognitive task	Multi-modal exercise can be successfully delivered in a community setting and is an effective approach to improve musculoskeletal and functional performance in older adults with risk of falling
(Gobbi et al., 2009) São Paulo, Brazil	N = 34 A = 67 H&Y I – III	**Frequency**: 3d/week **Intensity**: NP **Type**: (1) multi-modal exercise, (2) adaptive programme **Time**: 60 min **Duration**: 6 m **Adherence**: NP (participants had to attend at least 70% of the sessions to be included) **Location**: university facilities	**Physical function**: Berg Balance Scale, the TUG test	Both exercise programmes improved mobility in PwP
(Gobbi et al., 2021) São Paulo, Brazil	N = 152 (107 analysed) A = 69 H&Y I – III	**Frequency**: 2d/week **Intensity**: NP **Type**: (1) multi-modal exercise, (2) functional mobility, (3) mental/leisure **Time**: 60 min **Duration**: 8 m **Adherence**: NP (participants had to attend at least 70% of the sessions to be included) **Location**: university facilities	**Cognitive function**: MMSE, Clock Drawing Test, Wechsler Memory Scale-Revised, Wechsler Adult Intelligence Scale III, Digital span, Wisconsin Card Sorting Test, Verbal Fluency test, Corsi block-tapping test	The multi-modal training intervention did not substantially maintain or improve executive function due to the lower complexity of activities included
(Pereira et al., 2012) Campinas, Brazil	N = 33 A (int) = 67 A (con) = 72 H&Y I – III	**Frequency**: 3d/week **Intensity**: 60-80% HRmax **Type**: multi-modal exercise **Time**: 60 min **Duration**: 6 m **Adherence**: NP (participants had to attend at least 70% of the sessions to be included) **Location**: university facilities	**Physical function**: Berg Balance, TUG, Posture Locomotion Test **Cognitive function**: MMSE (although it is not discussed in the results)	Participants' mobility (measured with the TUG) improved after 6 months

(Tanaka et al., 2009) São Paulo, Brazil	N = 20 A (int) = 65 A (con) = 65 H&Y I – III	**Frequency**: 3d/week **Intensity**: 60-80% HRmax **Type**: multi-modal exercise (in groups of 10 participants) **Time**: 60 min **Duration**: 6 m **Adherence**: NP (participants had to attend at least 70% of the sessions to be included) **Location**: university facilities	**Cognitive function**: Wisconsin Card Sorting Test, Wechsler Adult Intelligence Scale III	The multi-modal exercise programme was able to improve executive function in PwP after 6 months
(States et al., 2017) New York, USA	N = 46 A = 64 H&Y I – III	**Frequency**: 2d/week **Intensity**: NP **Type**: community-based, group exercise programme (no control group) **Time**: 60 min **Duration**: 1, 3 and 5y (in blocks of 3 x 10 weeks) **Adherence**: 59% (1y), 39% (3y), 29% (5y) **Location**: university wellness, recreation, and athletic centre	**Physical function**: gait speed, 6-minute walk test (6MWT), TUG, Berg Balance, grip strength	Participants' physical function was maintained over the course of the study
(Vaughan, Morris, Shum, O'Dwyer, & Polit, 2012) Gold Coast, Australia	N = 49 A = 69 Older women	**Frequency**: 2d/week **Intensity**: 3 to 6 RPE (Borg Scale 1 – 10), 126-128 bpm music **Type**: multi-modal exercise **Time**: 60 min **Duration**: 4 m **Adherence**: NP (participants had to attend at least 85% of the sessions to be included) **Location**: community (in halls)	**Physical function**: 6MWT, TUG test, the One-legged Stance test **Cognitive function**: California Older Adult Stroop Test, Controlled Oral Word Association Test, Letter-Number Sequencing test, Deary-Liewald Reaction Time Task, Trial Making Test (TMT A and B) **Biomarkers:** plasma BDNF	The multi-modal exercise programme was able to improve cognition, physical function and BDNF levels in older woman compared with controls.

It was towards the early years of this century that exercise guidelines started to put emphasis on the importance of performing multi-modal exercise for older adults, including strengthening exercises, cardiovascular, flexibility and balance training, to reduce the decline in physical functioning, morbidity and mortality associated with aging (American College of Sports Medicine Position Stand, 1998). From then, the first studies evaluating the potential of multi-modal exercise programmes started to emerge, overall, providing evidence of a positive effect on falls prevention (Baker, Atlantis, & Fiatarone Singh, 2007). However, a systematic review evaluating the literature available at that time noted the limited amount of studies evaluating multi-modal exercise for older adults and/or comparing it to single-modalities with robust interventions (Baker et al., 2007). Nonetheless, research in older adults provided valuable information and evidence of the beneficial effects of multi-modal exercise training. A RCT in older women engaging with a multi-modal class twice a week for 16-weeks compared to a resting control group showed that the multi-modal exercise could improve not only cognition and physical performance, but also plasma concentrations of BDNF (Vaughan et al., 2014). Improvements in neurocognitive performance were reflected in an enhanced verbal fluency, information processing speed, and a better ability to attend, concentrate, think flexibly and resist distraction. Improvements in physical performance showed significant improvement in the 6MWT, TUG and the One-legged Stance test (OLST), compared to the control group after the multi-modal intervention. Additionally, this was the first study to demonstrate that BDNF, as a marker for neurogenesis and possible mediator of the induced improvements in cognitive functioning, could significantly increase after engaging with multi-modal exercise (Vaughan et al., 2014). The authors from these studies emphasised that further research was needed to generalise their results to clinical populations, such as PwP. However, these findings paved the way for the design of studies evaluating multi-modal exercise for PwP.

Subsequently, in 2009, Gobbi et al. investigated whether multi-modal exercise could improve PwP's functional independence by measuring functional balance and mobility; components that are crucial to perform ADLs (Gobbi et al., 2009). To do this, researchers compared a multi-modal programme (with activities to work on aerobic capacity, flexibility, upper and lower limb strength, motor coordination, and balance) to an adaptive programme (which included lower complexity exercises for flexibility, strength, motor coordination, and balance). Results showed that, after 6 months participation, both exercise programmes were able to significantly improve functional balance and mobility, but did not provide a clear answer as to which was the optimum mode for PwP (Gobbi et al., 2009). Although this study has several strengths, additional relevant information would be required to interpret these findings further. For instance, quantitative measures of intensity at which participants completed both programmes and the amount of physical activity that participants engaged with outside of the study sessions, since it is believed that those factors could influence the study outcomes (O'Callaghan et al., 2019; Schenkman et al., 2018). A study investigating the effects of doing 12-weeks of moderate-intensity continuous training (MICT) compared to HIIT found that HIIT was more effective at increasing serum levels of BDNF that MICT (O'Callaghan et al., 2019). Additionally, a phase 3 clinical trial called SPARX3 is currently investigating further whether

performing high-intensity endurance exercise for 12 months can attenuate PD's progression more than engaging with moderate intensity exercise (ClinicalTrials.gov identifier: NCT04284436).

Then, in 2009, Tanaka and their team were the first to evaluate whether multi-modal exercise could improve executive functions in PwP (Tanaka et al., 2009). PD participants engaged with a multi-modal exercise programme for 6 months and researchers measured executive functions whilst controlling for concentrated attention, anxiety, and depression symptoms. Compared to a control group that maintained their daily routine and did not take part in any regular or structured exercise, the multi-modal exercise intervention was able to improve participants' capacity for abstraction (i.e., "*the ability to concentrate on the essential aspects to a specific context and ignore other aspects present that had accidental or less relevance*") and mental flexibility (i.e., "*the capacity to respond to contingencies, by inhibiting an inappropriate response and seeking an appropriate one*"), both components of executive functioning (Tanaka et al., 2009). The outcomes of a recently published systematic review of available information supported these results and reported that PwP's executive function, attention, and memory tended to improve after different modes of exercise, including multi-modal exercise (i.e., combining resistance, aerobic and coordination exercises) (Stuckenschneider et al., 2019). Based on their findings, researchers suggest that combined exercise might be best to improve global cognitive functions (Stuckenschneider et al., 2019).

The effects of multi-modal exercise have been assessed in different populations and the evidence reviewed has demonstrated the beneficial effects of these programmes on several occasions (Fraga et al., 2021; Gobbi, Barbieri, Vitorio, Pereira, & Teixeira-Arroyo, 2011; Nascimento et al., 2014; Pereira et al., 2012; States et al., 2017; Stuckenschneider et al., 2021; Tanaka et al., 2009; Vitório et al., 2011). In particular, multi-modal exercise interventions are of particular relevance for PwP due to its capacity to improve physical function and targeted physiological systems, and to overall reduce PD's disability (Ellis & Rochester, 2018). Moreover, a recent review concludes that multi-modal exercise may be the most effective training protocol for PD and recommends PwP to perform it routinely during the week and in the long term, to maintain benefits on endurance, resistance, and flexibility (Martignon et al., 2020). However, it is common that studies evaluate outcomes of physical performance, cognition, and neuroprotective biomarkers separately (as reported in **Table 1.2**). Or else, include short observation periods that might not be long enough to result in meaningful or long-lasting changes. Moreover, although there is evidence showing that multi-modal exercise tends to increase the bioavailability of neurotrophic factors (e.g., BDNF and insulin-like growth factor-1 [IGF-1]), the need to elucidate the biological mechanisms underlying these responses still remains (Stuckenschneider et al., 2021; Vaughan et al., 2014). Therefore, the development of studies enrolling PwP in exercise interventions with durations longer than six months are required to evaluate whether the functional balance and mobility gains observed in shorter duration programmes can be maintained or enhanced.

1.4.4 Acute vs chronic effects of exercise in PD

Acute exercise may be defined as a single bout of physical activity (Basso & Suzuki, 2017). On the other end of exercise training spectrum, chronic exercise is understood as a repeated amount of single bouts of exercise over a short or long-term period of time (Sellami et al., 2018). Additionally, it is important to distinguish between the effects that exercise can elicit, which can be divided into acute (i.e., transient responses) and chronic responses (i.e., consequence of the frequent repetition of isolated bouts of exercise that can produce more permanent adaptations) (Thompson et al., 2001). Acute exercise bouts have shown to improve executive functioning and enhance motor skill consolidation in PwP (Ridgel, Kim, Fickes, Muller, & Alberts, 2011; Steib et al., 2018). Chronic exercise programmes of 2 to 10-weeks duration have also shown to elicit improvements; however, those effects can be short-lived and lost when measured at a 3-months follow-up (Morris, Iansek, & Kirkwood, 2009; Nocera, Horvat, & Ray, 2009; Tidman & Skotzke, 2020). However, research suggests that longer exercise interventions for 6-months or more, can enable better and longer-lasting improvements compared to shorter programmes. The latter may be insufficient exposure to exercise stress to elicit long-term changes (i.e., chronic effects) in functional and cognitive performance (Gobbi et al., 2009).

Due to the progressive nature of PD, it is not expected that the symptoms would stop after taking part in a short exercise intervention. Thus, the development of training regimes that elicit longer-lasting benefits are imperative. Similar to Tanaka's et al. study (2009), Pereira and colleagues designed a 6-month multi-modal intervention which investigated the effects of engaging with multi-modal exercise on balance, mobility and clinical status of PwP (Pereira et al., 2012). Overall, their research provided evidence of the feasibility and high adherence with enduring benefits for PwP. In addition, they showed that multi-modal exercise was able to significantly improve mobility (Pereira et al., 2012). Although they recommend the enrolment of PwP on long-lasting multi-modal exercise programmes and reported valuable research outcomes, the evaluation of longer-term interventions to help promote long-term engagement with exercise and the maintenance of an active life for PwP is still needed. With regard to this, States et al. (2011) studied the feasibility of an on-going, community-based, multi-modal and group-based exercise programme for PwP and provided evidence that this setting was safe, feasible, and effective in maintaining physical functioning after 1, 3 and 5 years (States, Spierer, & Salem, 2011; States et al., 2017). This research provides PD-specific information and adds to the literature that explored whether chronic exercise was feasible for an elderly population, suggesting that chronic exercise is safe and without harmful side effects in the elderly and PwP (Sellami et al., 2018).

On the other side, acute studies, which can be performed in the laboratory under controlled conditions, have enabled the investigation of the potential underlying mechanisms that might elicit exercise-induced benefits in PwP. Moreover, the evaluation of acute responses to exercise bouts across a longer-term exercise intervention can enable the study of how much these acute responses

relate to the long-term outcomes (i.e., chronic effects). This is important because many previous research studies have only assessed acute short-term effects of exercise (for instance, on BDNF) based on the assumption that this response predicts chronic effects. This is a large assumption, and the precise relationship between what is seen in the immediate acute responses to exercise and chronic adaptations needs to be further explored, especially in PwP.

There remain several aspects of the FITT principles of exercise prescription for PwP about which a more detailed understanding is required. For instance, the dosing and timing of exercise need clarification. The adaptations that exercise can elicit require that the volume and difficulty of the exercise must challenge the body and create an overload, which can be different amongst individuals (Ellis & Rochester, 2018). Thus, further work is needed to explore and confirm the optimal dose of the diverse exercise modes available for PwP.

1.5 Current recommendations for exercise for people with Parkinson's

Although the integration of physical activity as a complement to manage the overall functional decline associated with PD was initially introduced during the 1950s, it was not until later in the 1980s when the first experimental studies and more research in the area were published (Gibberd, Page, Spencer, Kinnear, & Hawksworth, 1981; Hurwitz, 1989; Mitchell, Mertz, & Catanzaro, 1987; Palmer, Mortimer, Webster, Bistevins, & Dickinson, 1986; Szekely, Kosanovich, & Sheppard, 1982). Subsequently, several studies have investigated the potential that exercise may have for PwP and there have been attempts to create specific exercise guidelines for PwP. However, they lack specificity in terms of exercise testing and prescription. Due to the use of different and non-standardised methodologies in exercise testing and sample analyses, the comparison between study outcomes is challenging, which hinders the elaboration of appropriate guidelines for exercise prescription in PwP. To facilitate this, the standardised recommendations proposed by Basso and Suzuki in 2017 were followed in the studies presented in this thesis (Basso & Suzuki, 2017).

Exercise guidelines are the different public recommendations for exercise prescription that provide objective information about the type, time, intensity, frequency, volume, and progression that individuals of different populations should receive. General guidelines state that all adults are recommended to complete at least 150 minutes of moderate, or 75 minutes of intensive aerobic exercise per week, and two days a week of balance, flexibility or strength training for all major muscle groups (NHS, 2019). More health benefits are achieved if the levels of physical activity are higher, however, a recently published systematic review defends that the current threshold-centred messaging of those guidelines might create a barrier for less physically active individuals who can get clinically relevant health benefits by simply becoming more physically active (NHS, 2019; Warburton & Bredin, 2017). Moreover, specific guidelines are required for PwP, which should be designed in accordance with the latest exercise guidelines for PwP, combined with a personalised

approach taking into account individual's history, comorbidities, barriers, and preferences (ACSM, 2016, 2017).

In early 2020, Martignon et al. published specific guidelines for clinical testing and tailored exercise prescription for each stage of PD (Martignon et al., 2020). Their review evaluates exercise-based approaches to the management of symptoms at each stage of the disease, the most suitable clinical exercise testing, training programs based on testing outcomes and PD's stages and the effects of exercise on antiparkinsonian drugs to suggest the most effective exercise–medication combination. However, they also highlight the lack of standardized parameters for exercise testing and prescription in published studies. Despite their valuable work, future studies and a new guideline for exercise in PD are still required, and should focus on FITT principles to understand better which combination of these elements are optimal for PwP (Barbieri, Kalva-Filho, & Torriani-Pasin, 2020). Subsequently, in October 2020, Radder et al. published a systematic review updating the work of the widely used meta-analysis "*European Physiotherapy Guideline for Parkinson's Disease*" with newly published strategies and knowledge to summarise the evidence on exercise modalities in PwP (Keus et al., 2014; Radder et al., 2020). It has been suggested that Radder et al. (2020) meta-analysis could serve as a guideline for exercise prescription for PD, although specific studies are still needed to directly compare different exercise interventions with standardised training regimes, along with frequency, intensity, duration, and progression of training load. Evaluation of which regime is the most appropriate for PD subgroups, which would include the underrepresented group of people with young onset PD, is also needed. Finally, the feasibility of implementing these exercise modalities in the community is critically needed (Barbieri et al., 2020; Radder et al., 2020).

More recently, the Parkinson's Foundation and the American College of Sports Medicine (ACSM) released new exercise recommendations that expanded on previous version of the ACSM's Guidelines on Exercise Testing and Prescription and Exercise Management for Persons with Chronic Diseases and Disabilities (ACSM, 2016, 2017; Parkinson's Foundation, 2020). One important addition in these newly published recommendations, was the inclusion of balance and agility exercises, including multi-tasking, as one of the four exercise domains that are safe and effective for PwP (together with aerobic exercise, strengthening and flexibility) (Parkinson's Foundation, 2020).

Guidelines published to date tend to focus on exercise strategies that improve the cardinal motor signs of PD (motor) and QoL, without including information about non-motor outcomes. Thus, more work is required, and comprehensive guidelines need to be provided to enable a standardised approach to exercise testing in PwP.

1.6 Potential underlying biological mechanisms of the beneficial effects of physical activity on PwP

Exercise is a powerful tool for prevention, maintenance and treatment of whole-body metabolic health and brain functions in healthy adults (Smith et al., 2011; Thyfault & Bergouignan, 2020). Moreover, regular exercise has the ability to prevent and/or mitigate chronic diseases and lower overall mortality rates, since there is a linear relationship between levels of physical activity and health status (Pedersen, Klarlund & Saltin, 2015; Sallis, 2015).

The remarkable effects that exercise can elicit on a physiological level, not only relate to the widely recognised impact on skeletal muscle metabolism, but also result in metabolic adaptations in multiple other tissues and organs (Thyfault & Bergouignan, 2020). Beyond the beneficial potential that exercise has proved to evoke in healthy individuals, there is mounting evidence showing the unequivocal beneficial and minimal deleterious effects that it has for PwP (Pedersen, Klarlund & Saltin, 2015). For instance, apart from the potential to lower UPDRS scores, exercise can also improve underlying metabolic derangements, including inflammation, mitochondrial dysfunction, and glucose metabolism in PwP (Krumpolec et al., 2017; Tomlinson et al., 2013).

A common neuropathological feature of PwP is a progressive loss of dopaminergic neurons from the SN_{PC} (Salamon et al., 2020). However, despite extensive studies using various animal models and clinical studies, PD's aetiology is still unknown. Nonetheless, in recent years, numerous researchers have sought to understand the underlying mechanisms of PD's pathophysiology to determine and find molecules that are able to protect dopaminergic neurons and/or can halt or reverse the degeneration that PwP experience. Most of the *in vivo* studies using animal testing originate from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-OHDA animal models. Questions have been raised about the use of animal subjects modelled with toxin experiments due to the extensive complexity of PD. However, the use of these animal models has been fundamental for the development of therapeutic interventions, some of which have later been tested in clinical trials and proved effective in PwP (such as exercise) (Crowley, Nolan and Sullivan 2019). Overall, animal research has revealed exercise can lead to greater angiogenesis, a broad increase in cerebral blood flow, reduce oxidative stress and neuroinflammation, and increase the expression of neurotrophic factors (e.g., BDNF, GDNF) (Petzinger et al., 2015). Additionally, a review of the current available literature reviewing animal and human studies suggests that the multiple mechanisms by which exercise contributes to improvements in both mental and physical health in humans, also involve the upregulation of neurotrophins (including BDNF), reduction of neuroinflammation and improvements of cerebral circulation (such as increased BBB permeability) (Stimpson, Davison, & Javadi, 2018). Overall, these changes lead to an enhancement of neurogenesis, improving the capacity for adaptive brain plasticity (e.g., due to reductions in age-related white/grey matter loss) (Stimpson et al., 2018). These exercise-enhanced underlying mechanisms that result in both physical and cognitive improvements, are especially relevant for PwP. In particular, these include exercise-induced

increases in neurotrophins and cerebral blood flow, which occur within those brain circuits in the basal ganglia and cerebellum that are involved in motor control, and also contributes to the upregulation of neurotrophins to different brain areas. The enhanced production of BDNF due to exercise has several benefits important for PwP that are discussed in the section below.

1.6.1 Decreased Neurodegeneration

As mentioned in **section 1.2** (**Current Interventions**), there is still no known cure for PD and the pharmacological or surgical treatments that are currently available for PwP do not have an impact on the pathophysiology and progression of the disease. These treatments are used for symptomatic control and mainly offer short-term benefits before a worsening of PD symptoms and drug adverse reactions happen. Therefore, further investigations aimed at collectively providing a neuroprotective effect, stopping the disease progression, and restoring the damage caused by the condition, are imperative.

Due to the limited effect that pharmacological and surgical procedures have to treat symptoms and restrict Parkinson's progression, non-pharmacological interventions have received an increased attention during the last few decades (e.g., acupuncture, cognitive training, different forms of physical activity [e.g., exercise], patient education, reflexology, self-management program, spa therapy, cueing training, music therapy, neuromuscular therapy, non-invasive brain stimulation such as repetitive transcranial magnetic stimulation [rTMS], transcranial direct current stimulation [tDCS], etc.). In particular, there is mounting evidence that exercise can induce neuroprotection, slow down the disease progression and attenuate, or even reverse, nigrostriatal neurotoxicity and degeneration (Ahlskog, 2018; Conceição, Moura, & Pauli, 2019; Lau, Patki, Das-Panja, Le, & Ahmad, 2011). Initially, these concepts were investigated in studies performed with experimental animal models of PD. Although animal studies results cannot be directly extrapolated to humans, animal models such as rodents or pigs, present some genetic similarities with humans and allow direct analysis of areas that are restricted in humans, such as the brain (Klein et al., 2011). However, in animal studies it is important to take into consideration the time of administration and dose of the drugs used to induce PD, since variations on those produce different effects and therefore the outcomes of those studies cannot be directly compared. The non-standardisation of these factors could explain why a large number of animal studies have reported beneficial neuroprotective and restorative effects of exercise in models of PD, but some studies did not (Hou et al., 2017). Nevertheless, most data show that, when appropriately dosed, physical activity and exercise can reduce the risk of developing PD, protect the residual dopaminergic neurons and/or directly restore the dysfunctional cortico-basal ganglia motor control circuit through an exercise-triggered production of endogenous neurotrophic factors (Hou et al., 2017).

It is currently known that these results could potentially be inferred to humans. There is a large amount of epidemiological data suggesting that exercise can prevent the development of PD in

humans (Hou et al., 2017; Paillard, Rolland, & de Barreto, 2015; Petzinger et al., 2015). Moreover, as mentioned previously herein, exercise can increase BDNF in PwP, which provides neuroprotection and can help counteract the onset and progression of PD.

Overall, many studies have investigated the protective effects of exercise for PwP and provided evidence that the targeted effects of exercise on the nigrostriatal pathway may include an increase in DA release, downregulation of dopamine transporter expression (which increases the levels of extracellular DA), reduced striatal DA loss, partial preservation of midbrain dopaminergic neurons and preserved, or restored, dopaminergic terminals (Ellis & Rochester, 2018; Sacheli et al., 2019). However, it is important to note that although cerebral exercise-dependent structural and functional changes have been observed, these are general and diffuse in nature and require careful interpretation and further research on this subject matter is required (Ellis & Rochester, 2018).

Nonetheless, taking all the information together, there is abundant evidence defending that exercise should be prescribed to vulnerable populations as a preventive measure and to PD patients as a non-pharmacological component of their treatment. Moreover, exercise is a universally available and side effect-free, medicine.

1.6.2 Role of neurotrophins

Having discussed the growing evidence indicating that physical activity and exercise can generally prevent chronic diseases such as PD, slow Parkinson's related degeneration, improve symptomatology (both motor and non-motor), improve QoL and promote overall health, this section will now discuss one of the most documented and proposed underlying mechanisms of the neuroprotection effects of exercise: the role of neurotrophic factors.

The principal neurotrophic factors known to mediate the effects of exercise on the brain are BDNF, IGF-1 and vascular endothelial-derived growth factor (VEGF). They work collectively to modulate exercise-related benefits in brain plasticity, functioning and health. More specifically IGF-1 and BDNF regulate the effects of exercise on learning and also modulate depression, while IGF-1 and VEGF regulate exercise-dependent stimulation of angiogenesis and hippocampal neurogenesis (Cotman, Berchtold, & Christie, 2007). These molecules work in concert with intertwined regulatory pathways, but they all converge on BDNF signalling as a final common downstream mechanism mediating exercise effects on neuronal plasticity and learning (Cotman et al., 2007). BDNF then binds to tropomyosin-related kinase receptors B (TrkB), which regulate growth and survival of cells by controlling the Ras-PI3K-Akt signalling cascade, and neuronal differentiation and neurite development through GRB2-Ras-MAPK-Erk signalling (Gupta, You, Gupta, Klistorner, & Graham, 2013; Hernandez-Baltazar et al., 2019). In the SN_{PC}, BDNF is required for the generation, differentiation, and/or survival of dopaminergic neurons (Baquet, Bickford, & Jones, 2005).

GDNF is also an extensively studied neurotrophic factor with important neuroprotective and neurotrophic effects on dopaminergic neurons (Hou et al., 2017). However, compared to BDNF, GDNF is a slightly bigger molecule and it does not cross the BBB, which means that its peripheral (i.e., blood) and central (i.e., brain) levels are independent (Ahlskog, 2018; Pan et al., 1998). BDNF also presents a similar problem. It's molecular size is large and it does not readily pass through the BBB, however, there is evidence suggesting that peripheral BDNF crosses the BBB by a saturable transport system that maintains its intact form (Fumagalli, Racagni, & Riva, 2006; Pan et al., 1998). Moreover, Klein et al. (2011) investigated how accurately peripheral BDNF reflects central BDNF expression in brain tissue by analysing BDNF levels in the blood and brain in three different mammalian species (rat, pig and mouse) (Klein et al., 2011). Researchers found that peripheral BDNF concentrations of rats and pigs were positively correlated with their central BDNF levels. Moreover, pig plasma BDNF levels were comparable to previously reported plasma BDNF values in humans. Thus, demonstrating a close link between blood and brain and supporting the potential of using blood BDNF as predictor of brain BDNF levels (Klein et al., 2011).

Moreover, although preclinical data obtained from different toxin animal models suggests that GDNF have potent effects on survival and neurite outgrowth from dopaminergic neurons (i.e., it can rescue dopaminergic neurons and their projections in the nigrostriatal pathway), in the brains of PwP with α-synuclein pathology GDNF might not have the same ability to rescue dopaminergic neurons (Barker et al., 2020). Additionally, GDNF is normally expressed at low levels in the adult human brain and its expression levels are not usually hampered in PwP (Howells et al., 2000). Therefore, BDNF is proposed as a promising biomarker candidate in PD studies as it stands as a potent dopaminergic neurotrophin, normally expressed in the healthy adult nigrostriatal dopaminergic system that is present in lower levels in PwP (Parain et al., 1999; Rahmani et al., 2019; Scalzo et al., 2010). Moreover, in some instances BDNF levels seem to correlate with motor impairment, which may be a compensatory mechanism in response to brain damage that occurred in the initial years of the disease, and have the potential to increase after exercise interventions, which is clinically relevant for PwP and can be applied at any stage of PD (Frazzitta et al., 2014; Howells et al., 2000; Parain et al., 1999; Scalzo et al., 2010; Schmidt-Kassow et al., 2012).

1.6.2.1 BDNF and pro-BDNF

More than 35 years since its discovery, BDNF has been intensively studied, as evidenced by the thousands of publications currently available on this particular neurotrophic factor (Barde, Edgar, & Thoenen, 1982). There has also been a substantial amount of research exploring exercise-induced changes in BDNF levels in healthy and mostly young populations (Jiménez-Maldonado, Rentería, García-Suárez, Moncada-Jiménez, & Freire-Royes, 2018). The precise effect of exercise-induced BDNF responses is a much-debated topic amongst published literature, however, results overall show that BDNF levels increase after performing exercise, especially, immediately after (Ross, Saladin, George, & Gregory, 2019). Research improving the sampling temporal resolution, have shown that

the exercise-induced increases in BDNF levels start rising whilst performing exercise that lasts more than 20-min. and return to baseline levels after 10-min. of recovery (Schmidt-Kassow et al., 2012). These transient elevations of BDNF need to be further investigated in order to understand the long-term effects of exercise on BDNF responses, however, they seem to follow an hormetic response (Gradari, Pallé, McGreevy, Fontán-Lozano, & Trejo, 2016). Although BDNF levels appear to be significantly higher in blood immediately after exercise, but return to/or below baseline levels afterwards, muscle biopsies revealed a 50% increase in BDNF protein levels 24-h. later, which suggests that BDNF expression in target tissues may remain elevated for 24–72 h after high-intensity exercise (Matthews et al., 2009). This evidence insinuates that the overall effects of a single session of high-intensity aerobic exercise on BDNF expression may last several hours to several days post-exercise.

It is also important to consider the exercise dosing. A significant dose-response relationship between exercise intensity and BDNF concentration have been reported in most animal studies evaluating BDNF dynamics, although some groups have also reported controversial results (de Almeida et al., 2013; Jiménez-Maldonado et al., 2018; Knaepen, Goekint, Heyman, & Meeusen, 2010; Walsh & Tschakovsky, 2018). Nonetheless, this hypothesis was tested in humans and a similar pattern was observed; that is, peripheral levels of neurotrophic factors were significantly increased after exercise in an intensity-dependent manner, researchers suggest (Jiménez-Maldonado et al., 2018; Ross et al., 2019; Schmidt-Kassow et al., 2012). However, most of these studies were carried out with young healthy participants, which makes it difficult to translate to other populations such as those affected by PD. Both the nature of the exercise intervention and the individuals' characteristics, determine BDNF responses. Thus, healthy individuals might require higher exercise intensities to induce BDNF responses, whereas low-to-moderate exercise might be strenuous enough to trigger BDNF responses in individuals living with chronic conditions (e.g., PD) (Knaepen et al., 2010). Further studies are required to determine exactly how exercise affects BDNF kinetics in PD, PD's pathology, and progression.

Considering all the evidence mentioned above and the fact that studies report the important roles that BDNF have within the SN_{PC} in the generation, differentiation, and/or survival of dopaminergic neurons, it is tempting to propose the exogenous administration of BDNF as a therapeutic opportunity to preserve/restore dopaminergic neurons and improve PD's symptomatology (Baquet et al., 2005). Unfortunately, this idea is rather difficult to put in practice. Firstly, as already mentioned, the size of this molecule is reasonably large, which limits BDNF's diffusion to the brain parenchyma from the periphery. Secondly, its short half-life on plasma and its rapid uptake by the liver, compromise BDNF's therapeutic potency and effectiveness (Fumagalli et al., 2006; Hernandez-Baltazar et al., 2019; Pardridge, Kang, & Buciak, 1994). Also, the duration of the treatment, BDNF infusion or the appropriate rate of BDNF delivery in order to avoid neurotrophin degradation by proteases and other inactivating enzymes, are currently unknown (and could be different depending on the brain region). Additionally, the molecules that cross the BBB should be

designed to be site-specific for the nigrostriatal system to ensure the successful distribution of BDNF to the targeted area. Other forms, or delivery, or cell transplantation to induce the secretion of neurotrophic factors, are promising and currently being studied in animal models, however, their application in human participants would be rather invasive (Fumagalli et al., 2006; Hernandez-Baltazar et al., 2019). Nonetheless, some pre-clinical and clinical resources related to BDNF and its therapeutic role have been patented (Hernandez-Baltazar et al., 2019). An alternative suggested strategy to overcome all the above-mentioned problems could be the endogenous modulation of BDNF. For instance, with exercise, which has the ability to increase not only BDNF peripheral levels, but also BDNF's expression (Gómez-Pinilla, Ying, Roy, Molteni, & Reggie Edgerton, 2002; Hötting, Schickert, Kaiser, Röder, & Schmidt-Kassow, 2016; Rahmani et al., 2019; Vaughan et al., 2014; Vaynman, Ying, & Gomez-Pinilla, 2004).

Taking together important concepts about BDNF's physiology and its effects, it is important to mention that, although the main site in the body of BDNF expression is the brain, there are also non-neural tissues involved in its secretion to circulation, such as endothelial cells, platelets, skeletal muscle and liver (Máderová et al., 2019). These different expression sites relate to the diverse functions that BDNF's has in regulating metabolism, angiogenesis, myogenesis and muscle regeneration to improve peripheral glucose uptake/utilization, diabetic hyperglycaemia, skeletal muscle fatty acid oxidation, and protecting from motor neuronal degeneration (Colombo et al., 2013; Matthews et al., 2009; Pedersen et al., 2009). Moreover, it is important to bear in mind that, although the brain represents a major contributor to circulating BDNF levels during exercise, platelets bind, store and release BDNF upon activation, which can be influenced by exercise (Fujimura et al., 2002; Walsh & Tschakovsky, 2018). Therefore, measuring BDNF in platelet-poor plasma avoids measuring platelet-derived BDNF to a higher extent than measuring BDNF in platelet rich plasma (Gejl et al., 2019). Peripheral blood measurements of BDNF have been shown to be highly dependent upon the analytical methods and the biological medium in which BDNF is analysed (Gejl et al., 2019). Hence, it will be crucial for strong research studies to ensure a standardised and accurate measurement of BDNF throughout an intervention. This sample type issue is further discussed in a later chapter of this thesis.

Finally, it is also important to consider the precursor protein of BDNF, pro-BDNF. Pro-BDNF is cleaved by proteases (intracellular or extracellular) to release the pro-domain and the mature form of BDNF (Borodinova & Salozhin, 2017). Like BDNF, neurons can release endogenous pro-BDNF following an action potential, however, its active biological functions antagonise the pro-survival functions of BDNF. Thus, pro-BDNF is known to facilitate cell death (neuronal apoptosis) and to negatively regulate synaptic plasticity and transmission (Hempstead, 2014). It has been suggested that the pro-BDNF/BDNF ratio could be an indicator of neuronal health. Therefore, bearing in mind that BDNF can be released upon platelet activation, any use of plasma pro-BDNF/BDNF ratio as a potential biomarker for neurocognitive health should take into account platelet activation status, or consider the use of platelet-poor plasma as a biological medium. In relation to exercise, there is very

little published research on pro-BDNF regulation, biosynthesis, and post-translational processing after exercise. Although Brunelli et al. (2012) observed that pro-BDNF content was modulated by a cycling incremental test to exhaustion or exercising at individual anaerobic threshold, showing an increase of pro-BDNF intracellular concentrations, further research is needed to investigate the dynamics of pro-BDNF, especially after exercise, and its biological effect in PwP (Brunelli et al., 2012). So far, animal studies suggest that exercise could have important implications for brain plasticity through increases in the activity of tissue-type plasminogen activator (tPA), which is a serine proteinase that facilitates pro-BDNF cleavage into mature BDNF (Ding, Ying, & Gómez-Pinilla, 2011).

1.6.2.2 BDNF val66met polymorphism

PD is a multifactorial condition influenced by both genetic and environmental factors. Differences in the diverse array of symptoms developed, age at the time of diagnosis, duration of the disease and speed of progression, are some of the factors that contribute to increasing individual variability in Parkinson's. During the last two decades, genotype has gained immense attention and research has justified that it is another important factor that could play a crucial role and explain the diverse display of physiological and cognitive responses to physical activity and exercise in PwP.

BDNF is highly expressed in the central nervous system and studies show that it plays a particularly important role in PD. To promote synaptic plasticity, neuronal growth, differentiation and survival, BDNF binds to TrkB receptor to activate downstream signalling mediators regulated by the Ras-PI3K-Akt cascade (Gupta et al., 2013). BDNF and TrkB are expressed in 70% of dopaminergic neurons in the SNpc and are crucial for the establishment and protection of those neurons (Baquet et al., 2005; Cagni et al., 2017). Accordingly, in animal models of PD, it has been observed that BDNF can not only sustain the survival of dopaminergic neurons, but also protect them against neuronal death induced by the neurotoxic effects of 6-OHDA and N-methyl-4-phenylpyridinium ions (MPP^+) (Spina, Squinto, Miller, Lindsay, & Hyman, 1992). Although studies that examined the association between gene mutations or polymorphisms present conflicting results, there is evidence indicating that alterations in the BDNF gene may contribute to PD pathogenesis. Moreover, a decrease (or absence) of the neurotrophic effects of BDNF could be an etiologic factor of PD and accelerate neuronal damage and PD symptomatology.

The expression of BDNF is genetically inherited and can present single nucleotide polymorphisms (SNPs), which are the most common type of genetic variation. The rs6265 SNP in the BDNF gene is a G to A change that results in the substitution of a valine (Val) to methionine (Met) at codon 66 (Val66Met) in the pro-domain region in the terminal exon of that gene (region p13-p14 of chromosome 11) (www.ncbi.nlm.nih.gov/gene/627#reference-sequences). Thus, the combination of Val and Met alleles results in three different Val66Met genotypes: GG (Val/Val), AG (Val/Met) and AA (Met/Met), and the genetic model of inherited SNPs may be dominant (Met carriers vs. Val/Val),

codominant (Met/Met vs. Val/Met vs. Val/Val), or recessive (Met/Met vs. Val carriers); however, it still remains unclear whether the Met allele is dominant, codominant, or recessive (Tsai, 2018).

Several studies have explored how the Met variant of Val66Met polymorphism ($BDNF_{MET}$) links to anatomical and behavioural phenotypes in humans. It has been suggested that a relationship exists between this variant and the prevalence of depression, anxiety and poor cognitive performance in healthy individuals (Egan et al., 2003; Hariri et al., 2003; Notaras, Hill, & Van Den Buuse, 2015). However, its relevance to neuroclinical disorders remains unclear. The inconsistency observed in published data about the association between the BDNF gene and neuronal disorders could be explained by ethnic differences. Shen and colleagues reviewed the distribution of alleles and genotype for the Val66Met polymorphism in healthy subjects and observed significant differences depending on both region and ethnicity; the percentage of people carrying $BDNF_{MET}$ was higher among an Asian population compared to a Caucasian population (Shen et al., 2018).

In vitro studies have shown that $BDNF_{MET}$ seems to impair BDNF's regulated secretion, BDNF's distribution into neuronal dendrites and BDNF's binding to the high affinity TrkB (Chen, Bath, McEwen, Hempstead, & Lee, 2008; Chen et al., 2004; de las Heras et al., 2020), which suggests that the region encompassing the Met substitution could present a specific trafficking signal required for efficient BDNF transport and sorting. This idea was supported by the finding of sortilin, a transport protein that interacts with the BDNF region that includes the Met substitution (Chen et al., 2005). Thus, the $BDNF_{MET}$ alteration decreases the interaction of BDNF with this trafficking protein affecting the delivery of BDNF to the regulated secretory pathway and the physiological roles of this neurotrophin.

It is believed that activity-dependent secretion of BDNF in $BDNF_{MET}$ carriers could be decreased and reduce the BDNF response to exercise. Lemos et al. (2016) demonstrated, in healthy subjects, the presence of the BDNF Val66Met polymorphism could impair peripheral vascular reactivity and serum BDNF responses to exercise training (Lemos et al., 2016). It has also been proposed that the absence of $BDNF_{MET}$ (i.e., carrying the Val allele of Val66Met) could enhance the effect of aerobic exercise on neuroplasticity, improve response to rehabilitation and motor recovery compared to $BDNF_{MET}$ carriers (Mang, Campbell, Ross, & Boyd, 2013). However, de las Heras et al. (2020) questions whether $BDNF_{MET}$ carriers could actually benefit more from engaging with physical activity compared to Val carriers, who could be more vulnerable to the lack of sufficient physical activity (de las Heras et al., 2020). Therefore, the contribution of BDNF's polymorphisms on BDNF responses to exercise needs to be clarified.

1.7 Effects of Covid-19 on PwP

Since December 2019, the COVID-19 pandemic has placed a strain on society and health care systems, severely affecting lives and changing the whole world. Recent evidence has shown that its

causative agent, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can exacerbate the clinical spectrum of neurological diseases, such as PD (Ferini-Strambi & Salsone, 2020). Although significant progress has recently been made trying to explore this relationship, there are still numerous questions that remain unresolved, and the COVID-19 susceptibility for PwP, and people suffering from other neurological disorders, requires further investigation. It has been reported that COVID-19 aggravates the clinical course of PD by exacerbating motor and non-motor symptoms, increasing anxiety and, subsequently, affecting QoL (Cilia et al., 2020; Ferini-Strambi & Salsone, 2020). However, this information needs to be clarified by cross-sectional and longitudinal studies with larger sample sizes investigating the relationship between clinical changes and COVID-19 severity, systemic inflammation (i.e., cytokine levels), and SARS-CoV-2 detection in CSF. On the other hand, additional research has suggested that the high COVID-19 vulnerability presented in PD is likely to be associated with older age rather than the neuropathology itself. Elderly individuals presenting severe COVID-19 symptoms on hospital admission are at higher risk for mortality related to COVID-19 (Ferini-Strambi & Salsone, 2020; Li et al., 2020). Thus, research findings suggest that, although PwP may present respiratory muscle rigidity and several other comorbidities, PD by itself does not appear to increase the risk of being infected by SARS-CoV-2 and developing COVID-19 (Ferini-Strambi & Salsone, 2020).

Overall, many health care systems have been affected and were not prepared to make telemedicine available to patients. Consequently, most elective procedures and interventions (such as DBS, infusion therapies, specific exercise therapies, etc.) had to be postponed during the pandemic, presenting an unprecedented challenge for many people, and especially PwP (Fasano et al., 2020). The health community should use the COVID-19 pandemic as an opportunity to evolve, promote and standardise digital initiatives for patients, including those with PD.

This crisis happened over a very short period of time and has significantly changed people's daily routines, forcing the population to adapt and be flexible to this new situation. These adaptations require appropriate cognitive functioning, which depends on the optimal functioning of dopaminergic pathways. In PD, these pathways are affected due to the loss of dopaminergic neurons. Thus, PwP can present cognitive impairments (of considerable heterogeneity), which can lead to cognitive and motor inflexibility, putting PD patients at risk of chronic stress under challenging circumstances like the current pandemic (Helmich & Bloem, 2020; Robbins & Cools, 2014). Another significant negative consequence of the pandemic is the reduction of physical activity levels, due to being encouraged to 'stay at home', closed gyms and regular group activities being cancelled (e.g., the weekly multi-modal exercise class discussed in study 2 presented in Chapter 4). This is imperative for PwP because exercise is a valuable supplementary intervention for this population, and it helps in managing the symptoms of PD. Thus, any cessation, or decrease of physical activities will most likely have a negative impact on PwP. Hence, a loss of fitness during the lockdown may worsen motor (strength, aerobic capacity, coordination, balance, etc.) and, moreover, non-motor (i.e., insomnia, constipation, depression, etc.) symptoms in PD.

As the SARS-CoV-2 virus spread across the globe, drastic measures had to be implemented by the governments in order to slow down infection rates and the spread of COVID-19. Although being necessary, this has affected PwP daily-life activities and well-being. During the Italian lockdown (period between 20th of April and 2nd of May 2020), Schirinzi's team surveyed 74 PwP about their motor activity habits before COVID-19 pandemic and during lockdown, as well as the perception of their own health (Schirinzi et al., 2020). Up to 60% of participants perceived that during the pandemic their condition worsened, and this perception was not dependent on age, sex, age at onset or disease duration (Schirinzi et al., 2020). However, PwP started to successfully use technology-based assistance and were keen to enrol on management strategies to continue physical activity at home, which emphasises the need to develop and promote digital alternatives (e.g., the weekly multi-modal exercise class discussed in **Chapter 4** [study 2] was transformed into an online Parkinson's specific exercise class, which is discussed in **Chapter 7** [study 5]).

1.8 Thesis Aims and Hypotheses

Taken together, the overall purpose of this thesis is to evaluate how exercise provides a positive therapeutic intervention to maintain or improving PD symptoms with the potential to delay the progression of PD to more severe stages. These will be evaluated through different studies, and it is expected to show a reduced decline, or maintenance in physical and cognitive functions (meaning functional maintenance) through the engagement with an exercise intervention.

The first aim of this thesis is to evaluate the feasibility of running a weekly community-based exercise class for PwP tightly linked with research. This study requires regular assessments of physical function, cognition, and biomarkers (i.e., BDNF), which have not been assessed concurrently in previous research investigating the effects of a community-based multi-modal exercise for PwP (see **Table 1.2**). The study outcomes will be evaluated using both quantitative and qualitative methods and it is expected that the exercise class and the study measurements will be able to be performed in a community setting and maintained in the long-term. Secondly, this study will be able to the assess the chronic effects of engaging with the multi-modal exercise programme by using an array of functional, cognitive, neurobiological, mood and QoL measures and following up participants that engaged for 1, 2 and 3 years with the community-based exercise class. It is hypothesised that participants results will be maintained or improved over time compared to their baseline.

Thirdly, biomarker levels, cognitive and physical function changes will also be monitored in both healthy older adults (HOA) and non-active PwP (na-PD), whom will be used as comparison groups to better understand the rate of PD's progression and the impact that the multi-modal exercise intervention might have on regular exercisers with PD. Moreover, in order to explore whether genotype had an effect on the results from, the rs6265 SNP in the BDNF gene (/i.e., presenting the Val/Met or Met/Met genotypes opposed to the Val/Val genotype) was also investigated. HOA are expected to outperform both groups of participants with PD, however, the MM exercising group of

PwP is anticipated to present lower functional decline and better scores and biomarker levels over time than the na-PD group due to the expected beneficial effects of MM exercise.

With regard to the measurement of samples with complex matrices, such as serum and plasma, or other unvalidated sample types such as saliva, it required the researcher to validate the appropriate diluent prior to running the assay. Thus, the reagent diluents used for each of the sample types analysed in this thesis (serum, plasma and saliva), were optimised beforehand by using PBS supplemented with specific percentages of animal serum in order to mimic the complexity of each sample matrix. The omission of this step could significantly alter the performance of the immunoassay, which could be one of the reasons why incongruences in BDNF levels have been reported in the literature.

The thesis also aims to investigate the kinetics of the neurotrophins BDNF and pro-BDNF in PwP after an acute exercise bout and to understand how BDNF and pro-BDNF kinetics behave in different conditions. It is hypothesised that after each bout of acute exercise, BDNF levels will increase, even more so when cycling is combined with cognitively challenging tasks. However, research about pro-BDNF regulation in PwP is scarce. Therefore, this work will facilitate a better understanding of these neurotrophins production in relation to exercise and start informing the PD community on the best exercise intervention to enhance optimal BDNF and pro-BDNF production and regulation.

Finally, following a standard focus group methodology, the researcher aimed to gain in-depth understanding of the regular exercisers' views about the multi-modal exercise class, as well as the change toward an online delivery due to the coronavirus disease 2019 (COVID-19) pandemic. Related to the second point, the focus group discussing the online delivery of the multi-modal exercise class, through discussions about the feasibility, practicality, and participant's thoughts of this modality, contributed to the development of guidelines for the online delivery of exercise for PwP.

Chapter 2. General Methods

2.1 Ethics Approval

All studies within the thesis had ethical approval from the School of Sport and Exercise Sciences' Research Ethics Advisory Group (SSES REAG) at University of Kent (see **Table 2.1** and **Appendix G**) and were conducted in conformity with the Declaration of Helsinki.

Written informed consent was obtained from each participant before they participated in any project. Participants were only to be included if they were living independently and able to provide consent (i.e., no compromise in capacity to consent).

Table 2.1. Ethical reference numbers for all research studies presented in each chapter.

	Ethical Reference Number
Chapter 4 – Studies 1 and 2	Prop 04_2016_2017 (**Appendix A**) Prop 61_2017_18 (**Appendix B**) Prop 63_2018_19 (**Appendix C**)
Chapter 5 – Study 3	Prop 45_2018_19 (**Appendix D**)
Chapter 6 – Study 4	Prop 28_2019_20 (**Appendix E**)
Chapter 7 – Study 5	1_2020_21 (**Appendix F**)

2.2 Participants' criteria and recruitment

Participants involved in the studies included people with mild-to-moderate PD (Hoehn & Yahr [H&Y] stage I, II, III or IV), or healthy older adults (HOA), with all their faculties intact to enable them to complete the assessments for each project. Participants were required to present with low-risk status and be free from any limiting medical conditions (except PD) that would put them at risk during exercise according to the Physical Activity Readiness Questionnaire (PAR-Q). Likewise, HOA also completed the PAR-Q. Participants with PD completed a pre-exercise screening form, requesting background medical information that was used to identify risks and limitations for exercise linked to PD, and symptoms indicating any underlying cardiovascular diseases (CVDs). Participants also completed the Mini-Mental Parkinson (MMP), a screening test for cognitive impairment, and the short version of the International Physical Activity Questionnaire (IPAQ) to report their pre-assessment physical activity levels during the 7 days prior to the assessments.

Participants were excluded if they had any other neurological disease (apart from PD), cognitive decline (i.e., delirium or dementia), any significant physical and/or sensory impairment.

To control for the effect of medication dosage on the study outcomes, participants were instructed to perform each assessment in an "on-medication" state (which closely corresponds to their daily living more accurately than doing it on the "off-medication" state), except for drug naïve participants, and

take their medication approximately 45-mins. to 1-hr. before starting the intervention or assessments, similar to the protocol Plotnik et al. (2011) described in their study (Plotnik et al., 2011). Any dosage or medication changes during the duration of the studies were recorded and were not grounds to exclude participants in any analysis.

Participants were encouraged not to engage in strenuous exercise activities the day before each study visit, to refrain from consuming food and fizzy drinks 1-hr. before testing and to avoid caffeine and alcohol 24-hrs. prior to an assessment.

Participants were recruited through Parkinson's UK Research Support Network, printed and electronic advertisements placed on notice boards on various digital channels, support groups (i.e., Medway Working Age Group [MWAG]), and specific centres (i.e., European School of Osteopathy [ESO]).

2.2.1 Testing location

In collaboration with the MWAG and Parkinson's Equip, researchers at University of Kent helped in establishing a Parkinson's-specific exercise group in 2016. The group was based in the St Mary's Island Community Centre (Island Way W, St Mary's Island, Chatham ME4 3EP, UK). In order to maintain a familiar environment, it was decided to undergo the testing assessments in the Community Centre for the class participants that opted to take part in the research project described in study 2 (**Chapter 4).** However, in April 2019, there was a change in location and the testing assessments were moved to the laboratories at the Medway Building (the Medway Campus, School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, ME4 4AG, UK). The reasons for this venue change were to improve the feasibility of the assessments (additional blood and saliva) and avoid time restrictions to the testing schedule.

Apart from study 2 (included in Chapter 4), the studies included in this thesis were completed in two different locations based on participants preference or availability to travel to the testing site. Participants who were initially tested in a particular location, were repeatedly assessed at the same venue.

Thanks to working in collaboration with the ESO, participants recruited from the area of Maidstone, or able to travel there, were tested in Boxley House at the ESO (Maidstone, ME14 3DZ, UK). Therefore, researchers travelled to the testing site on several occasions with the necessary equipment (i.e., portable freezer, centrifuge, pipettes, consumables, scales, stadiometers, sphygmomanometers, stethoscopes, pulse oximeters, dynamometers).

The other University of Kent testing location were: Medway Park (Mill Road, Gillingham, ME7 1HF, UK) and Medway Building on the Medway Campus (School of Sport and Exercise Sciences, University of Kent, Chatham Maritime, ME4 4AG, UK).

Finally, on some occasions (e.g., study 2 included in Chapter 4), single-session testing days were organised. Participants were scheduled in intervals of 15-30 minutes and the whole group was assessed through one or two subsequent days. All the different testing assessments were separated in stations (samples, cognitive measures, health and functional measures) and completed in the same order. Different researchers help performing each of the measurements and, wherever possible, same researcher completed the same assessments throughout the length of the study for test-re-test reliability. For the studies presented in the other Chapters the same PhD Student completed all the assessments and participants were tested individually.

2.3 Health Measures and Pre-Testing Assessments

2.3.1 Pre-Testing Forms and Questionnaires

A battery of forms and questionnaires were completed before each assessment in order to assess participants. A general and a PD-specific pre-testing Health Screening Questionnaire were completed before each testing session to optimise participant safety during assessments.

Participants with PD were also asked to provide the following information: PD History (i.e., diagnosis date, age at diagnosis and side effected [left, right, both]), type of symptoms experienced, PD severity (i.e., H&Y Scale) and PD Staging (i.e., early disease, moderate disease, advanced disease).

2.3.2 Anthropometric Measurements

Body weight was assessed using an analogue scale (SECA 761, SECA, Hamburg, Germany). Height was measured with a stadiometer with 0.01 cm precision (SECA 213, SECA, Hamburg, Germany). Participants were instructed to maintain an upright vertical position, keeping their feet parallel and their head positioned in the Frankfurt plane.

The body mass index (BMI) was calculated using the formula BMI = weight (kg)/height $(m)^2$, and the parameters were based on data from the World Health Organization (1995).

BP and heart rate (HR) were taken at rest with the participant in a seated position. BP was measured using the technique of Riva-Rocci/Korotkoff with a sphygmomanometer (Carescape V100, GE Medical Systems, Wisconsin, USA). Resting HR (RHR) was taken with a pulse oximeter (Model B-50DL, BIOSYNC, Qinhuangdo, China).

Following the ACSM guidelines, participant's waist circumference (WC) was measured with the participant standing, feet together, arms at the sides, and abdomen relaxed (Kaminsky, 2014). Without compressing the skin and using an inelastic retractable 1.5m measuring tape (The Hanger Store, Chelmsford, UK), a horizontal measure was taken at the narrowest part of the torso from the anterior aspect (above the umbilicus and below the xiphoid process). Alternatively, the mid-point between the lowest lateral rib and iliocristale landmark was used (Ma et al., 2013).

2.3.3 Short version of the IPAQ (IPAQ Short form)

The IPAQ short form was used to measure the types of intensity of physical activity and inactivity (i.e., sitting time) that people do as part of their daily lives (Craig et al., 2003). The total weekly physical activity was estimated by weighting the reported minutes per week within each activity category by their assigned metabolic equivalent (MET) energy expenditure estimate (see Ainsworth et al. 2000 for MET intensity levels for each activity). IPAQ uses these measures to estimate the levels of total physical activity in MET-min/week.

The IPAQ has been developed and tested for use in populations with an age range of 15-69 years. However, Hurtig-Wennlf, Hagstrmer and Olsson, in 2010, modified the short version IPAQ for adults to be used in the elderly (≥65-yr.; named IPAQ-E) and showed that it provided acceptable estimates of physical activity that were in line with the other questionnaires. The IPAQ-E includes the same questions as the IPAQ short form but in different order. With this in mind, it was decided to use the short version IPAQ for the studies included in this research. The decision of using the short version of the IPAQ was based on the age range of our participants (49 – 83-yr.), with the majority of our participants being within age range for the IPAQ short form. Moreover, Tomioka et al. (2011) evaluated the reliability and validity of the IPAQ short form among adults aged 65 years and older and found that it is also a useful tool for assessing physical activity in elderly adults (Tomioka et al., 2011).

The IPAQ short form asks about three specific types of activity undertaken in four different domains/situations (*i.e.,* there are 7 questions in total about the individuals' last 7-day recall of physical activity). The types of activity include: vigorous-intensity activities, moderate-intensity activities and walking. The domains/situations can include: leisure time physical activity, domestic and gardening (yard) activities, work-related physical activity and transport-related physical activity.

To obtain the total score for the IPAQ short form, the duration (in minutes) and frequency (days) of walking, moderate-intensity and vigorous-intensity activities were multiplied by their MET score and summed up. Therefore, multiplying the MET score of an activity by the duration and frequency within the last 7 days provides a score in MET-min/week.

2.4 Functional Assessments

A battery of tests widely used in Parkinson's research and clinical settings were adopted to provide an objective real-world and familiar assessment of mobility and physical function. All the tasks were explained using a standardised script and demonstrated beforehand at every assessment visit to ensure reliability of measurements. Furthermore, participants were rested prior to each assessment and successive evaluations were performed at the same time of the day to maintain consistency. RPE and/or HR were taken immediately following each test as a measure of subjective effort.

2.4.1 6-Minute Walking Test (6MWT)

The 6MWT is a widely used sub-maximal test that measures walking capacity, speed, balance, agility and aerobic endurance, as the distance that can be walked in 6 minutes (i.e., 6-Minute Walk Distance [6MWD]). It was first developed by the American Thoracic Society (ATS), who published comprehensive guidelines in 2002, and initially designed to assess patients with cardiopulmonary issues (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002). However, it has been gradually introduced to other conditions and, in 2017, Kobayashi, Himuro and Takahashi confirmed the clinical validity of the 6MWT for people with moderate PD (Kobayashi et al., 2017).

The 6MWT is self-paced; participants choose their own exercise intensity and are allowed to stop if necessary. Although they are instructed to "*walk as far as possible in the 6 minutes*", participants usually do not achieve maximal exercise capacity during this test (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002). Since most ADLs are performed at sub-maximal levels of exertion and do not require maximal efforts, the 6MWD may better reflect function related to physical ADLs. Therefore, timed walking tests are valid measurements to predict community walking in PD. Moreover, it is recognised that healthy adults outperform PwP in walking tests (including the 6MWT); both speed and step length are reduced in PD compared to controls (26.5% and 23%, respectively) (Garber & Friedman, 2003; Rochester et al., 2004). However, rather than being related to fatigue or lower maximal aerobic capacity (VO_2 peak), PwP seem to obtain lower 6MWT results due to gait and balance impairments (Garber & Friedman, 2003). FoG can particularly impact walking performance in the 6MWT because the 10-m walking course necessitated frequent turning. Thus, improvements in those parameters could be reflected in an increased 6MWD (Garber & Friedman, 2003).

The set-up consisted in placing cones 0.5-m from each end of a 10-m shuttle walking course to be used as turning points. Therefore, this test involved straight-line walking and turning. Chairs were placed at either side along the room/hallway (to allow participants to rest should they need to) and tape markings on the floor represented each measured metre of the course. Before the start of the test, participants were briefed with a standardised script. During the test, the same standardised

encouragement was given to each participant, and no other means of verbal encouragement were provided to avoid any influence on the participant's effort or walking speed. Participants walked back and forth along the 10-metre track and were asked to stop where they were exactly after 6-mins. Immediately after stopping, participant's HR was recorded with a pulse oximeter (Model B-50DL, BIOSYNC, Qinhuangdo, China). The distance covered in the last shuttle was recorded to the nearest completed metre and the total number of shuttles walked within 6 minutes was recorded on the data collection sheet. The participant's RPE (6-20 scale) at the end of the test was also documented (Borg, 1998). The 6MWD was obtained by multiplying the total number of shuttles by 10 (plus the distance covered in the last shuttle).

2.4.2 Timed up-and-go (TUG)

The TUG test is a mobility test commonly used to screen for falls risk both for inpatient and community settings. It was developed in 1991, initially designed for the geriatric population and recommended as a routine screening test for falls, gait and balance in older people (Podsiadlo & Richardson, 1991). Its use is advocated by the American Geriatric Society, the British Geriatric Society and the National Institute of Clinical Evidence (NICE) published guidelines (National Institute for Health and Care Excellence 2013; Panel on Prevention of Falls in Older Persons, American Geriatrics Society and British Geriatrics Society 2011). However, it has also been suggested that it should not be used in isolation to identify community-dwelling older people at increased risk of falling (Barry, Galvin, Keogh, Horgan, & Fahey, 2014). Instead, it should be used alongside other assessments.

In this thesis, TUG was part of the battery of assessments used to evaluate participant's physical function. More specifically, it was used to assess mobility during goal-directed locomotor tasks (i.e., TUG), which are often impaired in PwP (Morris, 2006). The reliability of measurements for the TUG test has successfully been evaluated in PwP and demonstrated a good association with falls occurrence; a cut-off score of 11.5-sec. has been proposed (Morris et al., 2001; Nocera et al., 2013). Moreover, TUG test has shown to be a useful measure of mobility in PwP that can detect differences in performance between people with PD and the elderly without PD (Morris et al., 2001).

The TUG test measures the time it takes a participant to rise from a chair (approximate seat height of 46 cm), walk at their normal walking pace towards a line on the floor that is 3-m away, turn 180-degrees, return to the chair and sit down. The timing for this test started at the signal "*three, two, one, go*!" and stopped when the participant was seated. The test was repeated 3 times and the average time used in analysis. At the end of each attempt, participants were asked to answer how they felt by rating the level of effort that this activity required on the RPE scale (6-20) (Borg, 1998). A faster time indicates better functional performance in the TUG test. Participants were asked to wear their regular footwear and were allowed to use walking aids (the same equipment and conditions were used in subsequent assessments).

2.4.3 1-Minute Sit-to-stands (1-STS)

The 1-STS test is a measure of lower body muscular strength and endurance; factors that play significant roles in ADLs. In PwP these attributes are reduced and can negatively affect walking velocity and fall risk (Allen, Sherrington, Canning, & Fung, 2010; Frykberg & Häger, 2015). Moreover, muscle strength (i.e., force) and power (i.e., force*velocity) determine the ability to perform physical activities and are important indicators of health as well as strong predictors of mortality (FitzGerald et al., 2004; Myers et al., 2002; Ruiz et al., 2008). Thus, sit-to-stand measures can provide clinically relevant outcomes of function and mobility in PwP. Furthermore, the 1-STS test shows high correlations with other tests of exercise capacity (e.g., 6MWT), is reliable in PwP, a responsive alternative for measuring exercise capacity, reproducible, valid and provides prognostic information (Bohannon & Crouch, 2019; Ozalevli, Ozden, Itil, & Akkoclu, 2007; Petersen, Steffen, Paly, Dvorak, & Nelson, 2017; Puhan, Siebeling, Zoller, Muggensturm, & Riet, 2013).

The 1-STS test was administered using a chair (without arms and an approximate seat height of 46 cm) placed against the wall to prevent it from moving. Participants were instructed to sit in the middle of the chair, keeping their back straight, with their feet approximately at shoulder-width apart and placed on the floor at an angle slightly back from the knees, with one foot slightly in front of the other to help maintain balance. Participants were told to keep their arms crossed at the wrists and held against the chest. However, some participants needed to use their arms to help propel them out of the chair. Any changes in technique, or the use of additional support were noted and maintained in subsequent study visits.

The timing for this test starts at the signal "*three, two, one, go*!" and captures the number of times that the participant can stand (to a fully erect and straight position) and sit down, on a regular chair, in 1 minute. Participants were encouraged to complete as many full stands as possible and at the end of that minute they were asked to answer how they felt by rating the level of effort that this activity required on the RPE scale (6-20) (Borg, 1998). The number of completed sit-to-stands was recorded and incorrectly executed movements were not counted.

The 1-STS test records the number of sit-to-stands a person can complete in 1 minute rather than the amount of time it takes to complete a pre-determined number of repetitions, hence allowing the assessment of a wide variety of ability levels with scores ranging from 0 (for those who cannot complete 1 stand) to greater then 20 for more capable individuals.

2.4.4 Grip Strength (GS)

The Southampton protocol was followed in order to perform bilateral GS measurements to assess the maximum isometric strength of the hand and forearm muscles (Roberts et al., 2011; Sousa-Santos & Amaral, 2017). GS test-retest reliability has been assessed in PwP and shows that is a reliable and

simple measurement that, in conjunction with other tests (e.g., 1-STS), can be used as a predictor of health, systemic weakness and increased mortality (Rantanen et al., 2000; Villafañe et al., 2016).

Figure 2.1 Protocol to perform a grip strength measurement.

Participants performed the GS test seated in a chair with arm rests (approximate seat height of 46 cm), their feet flat on the floor and their hips as far back in the chair as possible. An analogue Hand Grip Dynamometer (T.K.K. 5001 Grip – A, Takei Scientific Instruments, Niigata, Japan) was used to perform the measurement on each hand. Participants were instructed to rest their forearms on the arm of the chair in a neutral position (with their wrist just over the arm of the chair; see **Figure 2.1**). The handle was adjusted to the grip of each participant so that their thumb (facing upwards) was round one side of the handle the dynamometer and the four fingers were around the other side. The individual adjustments that were made for each participant were recorded and kept the same for each visit. Whilst participants held the dynamometer, the researcher rested the base of the dynamometer on the palm of their hand to support its weight to negate the effect of gravity on peak strength (avoiding the restriction of any movement). Next, participants were instructed to follow the subsequent instructions: "*I want you to squeeze as hard as you can for as long as you can until I say stop. Squeeze, squeeze, squeeze, stop*" (participants were asked to stop when the needle stopped rising). Finally, grip strength was read in kilograms from the outside dial (rounded to the nearest 1 kg). This measurement was then repeated in the other hand and two further measures were taken with each hand, alternating sides. Hence, a total of three readings for each arm were taken and the average between the three measurements was used in statistical analyses. Hand dominance was also recorded (i.e., left, right or ambidextrous).

2.5 Cognitive and Mood Assessments

2.5.1 Mini-Mental Parkinson's (MMP)

The MMP is a cognitive screening tool developed by Mahieux et al. and derived from the Mini-Mental State Examination (MMSE) tool that measures attention, conceptualisation, construction, initiation/perseveration and memory (Folstein, Folstein, & McHugh, 1975; Mahieux, Michelet, Manifacier, Boller, & Guillard, 1995). The MMP includes seven subsections that are completed in the following order (each subsection's maximal score presented in brackets, with a total score of 32): 1) orientation (10), 2) visual representation (3), 3) attention/mental control (5), 4) two-set verbal fluency (3), 5) visual recall (4), 6) shifting (4) and 7) concept processing (3). The researcher administers the test verbally and participants provide the answer to each task that are evaluated with a score. The sum of the scores for each subsection provides the final score. The MMP has shown good test-retest reliability and a score of 27 or less out of 32 showed the ability to predict cognitive impairment on neuropsychological testing with 86% specificity and 73% sensitivity (Caslake et al., 2013).

2.5.2 Trail Making Test A and B (TMT-A and TMT-B)

The Trail Making Test (TMT) is a neuropsychological test that has two parts, part A and part B. Both parts, consist of 25 circles distributed over a sheet of paper. The aim of both parts of the test is to complete the task as quickly as possible, therefore, the score of each part represents the amount of time (in seconds) required to complete the test. In Part A (TMT-A), the circles are numbered from 1 to 25 and participants had to connect the numbers in ascending order by drawing a continuous line. In Part B, the circles included both numbers (from 1 to 13) and letters (from A to L), and participants had to link the cercles in ascending order alternating numbers and letters (e.g., 1-A-2-B-3-C-4-D, etc.). A sample sheet was completed by the researcher to demonstrate the test to participants beforehand. In both parts, the numbers and letters are placed in a semi-random fixed order over the paper to avoid the overlap of any of the connecting lines.

The TMT (A and B) is a test of visual attention (it provides information about visual search speed, scanning, speed of processing) and task switching (it tests mental flexibility, as well as executive functioning), that can be used in PwP (Olchik et al., 2017; Reitan, 1958; Tombaugh, 2004).

2.5.3 Clock Drawing Test (CDT)

The CDT is a screening tool that can be used to assess the cognitive state of participants by measuring spatial dysfunction and neglect (Agrell, Berit, 1998). The CDT can help with the diagnosis of cognitive dysfunction (e.g., dementia) and it can also discriminate between PwP and healthy individuals (Scarpina, Paschino, Priano, & Mauro, 2020). There are multiple scoring systems and no

general consensus to which system is consistently superior in terms of validity. Therefore, it has been suggested that "simpler is better", and the qualitative assessment of a 'normal' test result versus an 'abnormal' could also be sufficient to establish a baseline for follow-up (Mainland, Amodeo, & Shulman, 2014).

For this thesis, it was decided to simplify the test and, in order to facilitate the objective analysis of clock face completion, each participant was provided with a piece of paper with a 10cm black circle (Watson, Arfken, & Birge, 1993). Participants were asked to *'Imagine this is a clock face. Please put in all the numbers on the clock face and set the hands at ten past eleven*.' No other instructions were given (Death, Douglas, & Kenny, 1993; Shulman, 2000). The scoring system followed was: 1 point for all the numbers being in the correct order, 1 point for the numbers being in the correct spatial position, 1 point for inserting two hands of the clock and 1 point for the correct time.

2.5.4 Brief Old People Quality of Life Questionnaire (OPQOL-Brief)

The OPQOL-brief is a shorter version of the full 35-item Older People's Quality of Life Questionnaire (OPQOL), which has shown to have excellent applicability to cognitively normal older people and people suffering from mild or moderate dementia (Bowling, Hankins, Windle, Bilotta, & Grant, 2013). The OPQOL-brief starts with a preliminary single item on global QoL coded from "very bad" (=1) to "very good" (=5). This initial item is not included in the total score. The questionnaire follows with 13 items (e.g., enjoying one's life, looking forward to things, staying involved with things, and feeling safe where one lives, etc.) that are summed for a total score that ranges from 13 to 65. Participants have to select the response that best describes their view for each item: "strongly disagree", "disagree", "neither agree nor disagree", "agree" and "strongly agree", each with a score of 1–5, respectively. Higher scores represent higher QoL.

2.5.5 Brunel Mood Scale Questionnaire (BRUMS)

The BRUMS is a 24-item mood scale that measures 6 identifiable affective states (i.e., anger, confusion, depression, fatigue, tension and vigour) through a self-report inventory, with participants rating a list of adjectives on a 5-point Likert scale ranging from 0 ("not at all") to 4 ("extremely"), based on subjective feelings. Each identifiable affective state includes four related items and, overall, can reach a score between 0 and 16. The instruction set used was how participants 'have been feeling in the past week, including today' or 'how do you feel right now'. BRUMS is a sport-specific variation of the Profile of Mood States (POMS) developed and validated by Terry and his colleagues (Terry, Lane, & Fogarty, 2003).

2.6 Blood collection

2.6.1 Finger Prick

Finger-tip capillary blood was collected in K_2EDTA microvettes® (Microvette® CB 300 K_2EDTA, Germany) and then centrifuged with a portable centrifuge (Mini-centrifuge, CAT No. 12-006-901, Fisher Scientific UK Ltd., Loughborough, UK) for 10 minutes at 2700 *g* at room temperature. The supernatant was transferred into microcentrifuge tubes and stored at -80ºC for later analysis.

2.6.2 Venepuncture

Blood samples were collected from the antecubital vein into one 6ml K_2EDTA coated purple vacutainer™ and one 6ml serum red vacutainer™ (Fisher Scientific, Loughborough, UK).

Blood collected into K_2EDTA vacutainers was used to obtain different types of plasma. Using a compact bench top centrifuge (Heraeus Megafuge 8R Small Benchtop Centrifuge, Thermo Scientific, UK), platelet rich plasma (PR-P) was obtained by centrifuging the purple vacutainer at 1500 *g* for 10 minutes at 4°C within approximately 30 minutes of collection. The supernatant (i.e., plasma) was subsequently aspirated into microcentrifuge tubes and stored immediately at -80°C for later analysis. In order to obtain platelet poor plasma (PP-P), an additional step was completed and, after the first centrifugation, PR-P samples were further centrifuged at 10,000 *g* for 10 minutes at 2-8 °C (AccuSpin Micro 17R, Fisher Scientific UK Ltd., Loughborough, UK). The supernatant (i.e., PP-P) was then aspirated into microcentrifuge tubes and stored immediately at -80°C for later analysis.

In order to obtain serum from the serum vacutainer™ tubes, samples were allowed to clot for 1-hr. at room temperature. Next, the red vacutainers were centrifuged at 1500 *g* for 10 minutes at 4°C. The supernatant (i.e., serum) was subsequently aspirated into microcentrifuge tubes and stored immediately at -80°C for later analysis.

2.7 Saliva collection

2.7.1 Passive drool

Unstimulated saliva samples were collected in pre-weighted universal tubes for 4 minutes using the passive drool technique (an extra 2 minutes were allowed if participants did not reach the minimum amount of sample that was required within the initial 4 minutes, i.e., up to the bottom line separating the conical part of the tube). Consumption of alcohol, food, fizzy drinks and water were documented prior to the saliva sample collection.

Saliva samples were collected following the passive drool technique because research had found that 96% of the samples collected with this technique were above the assay limit of detection for BDNF, compared to samples collected using a Salivette collection device (only 65% of the samples had BDNF levels above the limit of detection) (Mandel, Ozdener, & Utermohlen, 2011).

Initially, participants sat quietly in the laboratory and were asked to swallow any saliva present in their mouth. In order to start the saliva collection, participants were instructed to lean forward and passively drool into the universal tube with minimal orofacial movements. Immediately after collecting the sample, the universal tubes were weighed to the nearest centigram and centrifuged at 17000 *g* for 5 minutes (AccuSpin Micro 17R, Fisher Scientific UK Ltd., Loughborough, UK).

Assuming a saliva density of 1g/ml, saliva flow rate was estimated by dividing the sample volume by collection time. The supernatant was then collected and aliquoted in microcentrifuge tubes that were immediately stored at -80 °C for later analysis.

2.8 Samples analyses

2.8.1 ELISA Analysis

After thawing the samples of interest, BDNF and pro-BDNF concentrations were determined using the DuoSet Enzyme-linked immunosorbent assay (ELISA) Development System (cat #DY248 and cat #DY3175, respectively, R&D Systems Europe Ltd., Abingdon Science Park, UK). The same ELISA kit was used to determine BDNF levels in all sample types mentioned in this manuscript (cat #DY248). The same DY3175 kit was used to measure pro-BDNF in different sample types. All assays were performed using the manufacturer's recommended buffers and substrates, and in-house optimised diluents (process explained in Study 1 presented in **Chapter 3**). Each sample was assessed in duplicate (unless otherwise stated), and BDNF and pro-BDNF were interpolated from a serial concentration standard curve prepared with the standard provided in the DY248 and DY3175 kits, respectively, which was included on each assay plate/assay run.

Therefore, an in-house developed method of sandwich ELISA optimised for serum, plasma and salivary BDNF was used for the assays, which specifically measured BDNF and did not show cross-reactivity or interference with similar neurotrophins, such as b-NGF, NT-3 and NT-4, and the trophic factor GDNF. Additionally, another in-house developed method of sandwich ELISA optimised for serum and different types of plasma was used for the analysis of pro-BDNF.

ELISA is an immunological method that has been commonly used to detect and quantify BDNF in human samples. The particularities of this method will be described in detail in **Chapter 3**. In brief, the ELISA used in the analyses required the antigen of interest (i.e., BDNF or pro-BDNF) to be

captured by a specific antibody bound on a solid surface (i.e., individual wells of the 96-well microtitre plate). Then, a detection antibody conjugated to an enzyme is added and binds to the 'captured' antigen of interest. When substrate is subsequently added to the well, the enzyme's activity produces a fluorescent and measurable product that will be used to calculate the concentration of BDNF or pro-BDNF, our antigens of interest.

ELISA development requires gathering all the required components and involves the construction of the assay. Furthermore, an additional step to ensure that the assay results would be robust and accurate was included. Thus, an ELISA optimisation was completed, which involved testing and adjusting specific components.

Recommended and already optimised concentrations for the capture and detection antibody pairings were used (as described in **Chapter 3**). However, prior to analysing any of the final samples, we performed a small pilot study in order to develop the reagent diluent based on each sample type used (see study 1 presented in **Chapter 3**).

2.8.1.1 ELISA methodological steps to measure BDNF

BDNF concentration was assayed using the DuoSet ELISA Development System (cat #DY248, R&D Systems Europe Ltd., Abingdon Science Park, UK).

First of all, the capture antibody was diluted to the final concentration (2 µg/ml) in phosphate-buffered saline (PBS), and 100 µl of diluted capture antibody was immediately put into each well of a 96-well flat bottom plate. Subsequently, the plate was sealed with an adhesive strip and incubated overnight at room temperature. In the next morning, the wells were aspirated and washed with 400 µl/well of PBS containing 0.05% Tween-20 (PBST; R&D systems cat #WA126) for a total of three times. Performing thorough washes between each step of the ELISA is essential to remove non-bound reagents and decrease background signal, which would increase the signal to noise ratio. Afterwards, the plate was blocked with reagent diluent provided in the DY248 kit (1% BSA; R&D systems cat #DY995) by adding 300 µl/well to prevent unwanted and/or non-specific bindings of antibodies or other proteins to the plate during subsequent steps. The plate was then sealed and incubated for at least 2 hours at room temperature. During the incubation time, the standards and samples were prepared. Serial dilutions of the standards were prepared from a stock solution that was obtained after reconstituting the standard vial with 0.5 mL of reagent diluent. The highest standard (1,500 pg/mL) was obtained from diluting the stock solution (140,000 pg/mL) and, thereafter, 2-fold serial dilutions were performed with appropriate optimised reagent diluent to obtain a seven-point standard curve with the following concentrations: 1500 pg/mL, 750 pg/mL, 375 pg/mL, 188 pg/mL, 93.8 pg/mL 46.9 pg/mL and 23.4 pg/mL. Additionally, the samples were diluted in their appropriate optimised sample diluent following their specific dilution factors, being 100×, 5×, 5× and 2× for serum, PR-P, PP-P and saliva, respectively. Finger-tip capillary blood was considered to be PR-P

and, therefore, a dilution factor of 5× was applied in the samples from study 2 (**Chapter 4)**. However, some values fell outside of the higher standard range, and it was decided to apply a 6× in subsequent studies 1 and 6, presented in **Chapter 3** (for the HOA and na-PD groups) and **Chapter 6**, respectively.

After the incubation time required to block the plate with reagent diluent, the 96-wells were again aspirated and washed three times with PBST. Next, 100 µl of the standards and diluted samples were pipetted into each well in duplicate (unless otherwise stated) and allowed to incubate for 2 hours at room temperature after sealing the plate. Then, the aspiration/wash step was repeated three times with PBST to remove unbound material and minimise the potential for high background signal. After removing the entire buffer from the wash, 100 µl of the biotinylated detection antibody (diluted to its working concentration of 25 ng/ml in reagent diluent) were added to each well. After incubating for 2 hours at room temperature, the plate was aspirated and washed three times with PBST to wash away unbound antibodies. Immediately after, 100 µl of the working dilution (200-fold dilution) of streptavidin conjugated to horseradish-peroxidase (HRP) were added to each well and incubated for 20 min at room temperature out of direct light. Using biotinylated secondary antibodies followed by streptavidin-HRP improves the sensitivity of the assay. With the interaction biotin-streptavidin, four molecules of streptavidin bind to biotin, hence, achieving amplification and offering a 4:1 increase in signal, which allows the detection of smaller quantities of analyte.

After this step, the plate was aspirated/washed three times with PBST. After this final washing procedure, 100 µl of substrate solution (tetramethylbenzidine [TMB]; R&D systems cat #DY999) were added to each well and incubated for 20 min at room temperature out of direct light. In this step, the TMB substrate is converted by the Streptavidin-HRP and produces a blue product with a colour intensity proportional to the amount of analyte (i.e., BDNF) present in the sample. The reaction was terminated by adding 50 µl of stop solution to the wells (2N Sulfuric acid; R&D systems cat# DY994), which turns the blue colour into yellow. Straightaway, the 96-well plate was placed into a microplate reader (Fluostar OPTIMA, BMG LABTECH Ltd., Aylesbury, UK) which briefly mixed the wells before measuring the absorbance of the coloured reaction product of each well at 450 nm (wavelengths of 530 nm and 610nm were also read in order to subtract them from the readings to improve accuracy). Then, the microplate reader outputted the wells' respective optical density (OD) values. The more analyte is present in the sample, the higher the absorbance and, therefore, the obtained OD values.

The duplicate readings for each standard and sample were averaged, and the averaged zero standard OD was then subtracted from all readings. In order to obtain the concentrations of the samples in each plate, a standard curve for each individual plate was created using software capable of generating a four-parameter logistic (4-PL) curve-fit (http://elisaanalysis.com and https://myassays.com/). The concentrations of the samples in each plate were subsequently calculated according to the standard curve from their plate. Finally, since samples had been diluted, the

concentration read from the standard curve was multiplied by their dilution factor. BDNF was expressed as pg/mL.

For each study using this technique, samples from any single subject were analysed on the same plate.

The intra-assay CVs for BDNF measurements in PP-P, PR-P, serum and saliva were 14%, 7%, 4% and 13%, respectively, from duplicate samples across all plates assessed in this thesis. The intra-assay CVs for pro-BDNF measurements in PP-P and PR-P were both 6%, from duplicate samples across all plates assessed in this thesis.

2.8.1.2 ELISA methodological steps to measure Pro-BDNF

Pro-BDNF concentrations were assayed using the DuoSet ELISA Development System (cat #DY3175, R&D Systems Europe Ltd., Abingdon Science Park, UK). The procedure followed the steps described in **section 2.8.1.1**, noting that the working concentration for specific reagents were different than those used for BDNF. Specifically, the capture and detection antibodies were diluted to their final concentration (4 μg/ml in PBS and 500 ng/ml in reagent diluent, respectively). The highest standard (5,000 pg/mL) was obtained from diluting the stock solution (180,000 pg/mL) and a seven-point standard curve was obtained, by serial dilution, with the following concentrations: 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 313 pg/mL 156 pg/mL and 78.1 pg/mL. The samples were diluted in their appropriate optimised sample diluent with their specific dilution factor, being 5× for PR-P and PP-P. Serum's dilution factor is not reported due to presenting some difficulties which are further discussed in Chapter 3.

In order to obtain the concentrations of the samples in each plate, a standard curve for each individual plate was created using a software capable of generating a 4-PL curve-fit (https://www.myassays.com).

The intra-assay CVs for pro-BDNF measurements in PP-P and PR-P were 6%, in both instances, from duplicate standards and samples across all plates analysed in this thesis.

2.8.2 BDNF val66met polymorphism

The expression of BDNF can present SNPs. The rs6265 SNP in the BDNF gene is a G to A change that results in the substitution of a Val to Met at codon 66 (Val66Met) in the prodomain region in the terminal exon of that gene (region p13-p14 of chromosome 11) (www.ncbi.nlm.nih.gov/gene/627#reference-sequences). The combination of Val and Met alleles results in three different Val66Met genotypes: GG (Val/Val), AG (Val/Met) and AA (Met/Met).

It is imperative to bear in mind important concepts. Deoxyribonucleic acid (DNA) is the hereditary material in humans. DNA is composed of a double helix to form double-stranded DNA, which carry genetic information. The two linear strands that form the DNA are complementary and run opposite to each other, or anti-parallel, and twist together. These can be referred to as plus and minus strand, amongst other names.

Some databases, such as the NCBI dsSNP database, only include the information regarding one DNA strand (in this case, the 5'→3' plus strand). However, the rs6265 SNP, located in the human BDNF gene, is oriented in the minus strand (3'→5'). Therefore, the single nucleotide variation (SNV) for rs6265 in the plus strand is C>T or C/T, whilst in the minus strand is G>A or G/A. This means that the wild type allele (encoding the Val) will have a C at the SNP position of the plus strand, whereas the alternate allele will have T in the SNP position of the plus strand (https://www.ncbi.nlm.nih.gov/snp/rs6265?vertical_tab=true#clinical_significance). Therefore, there are two allele specific primers in the BDNF gene: the allele specific primer 1 (ASP1) amplifies the allele that contains a C base at the SNP position on the plus strand, whereas the allele specific primer 2 (ASP2) amplifies the alternate allele with a T base at the SNP position on the plus strand. Hence, presenting the GG genotype in the minus strand is equivalent to presenting CC in the plus strand. This information is important to order the appropriate primer for the Polymerase Chain Reaction (PCR)-based genotyping technique that is described below.

2.8.2.1 DNA Purification

Study 2 (Chapter 4) participants were included in the analyses.

Genomic DNA was isolated from saliva or serum samples using a commercially available genomic DNA extraction kit named (DNA Mini Kit, QIAgen GmbH, Hilden, Germany) according to the manufacturer's instructions. In brief, 200 µl of sample was added to a microcentrifuge tube with 20 µl of QIAGEN Protease. After adding 200 µl of buffer AL, the mix was vortexed and incubated for 10 min at 56°C. Subsequently, 200 µl of ethanol was added to the sample. The mixture was then pipetted into a QIAmp Mini spin column and centrifuged at 6000 *g*. Two more elution steps removed any residual contaminants from the mix.

Each blood and saliva sample were subjected to the same protocol as described above and once all the extractions where completed, the purified DNA samples were immediately frozen at -80°C until analysis.

2.8.2.2 SNP Genotyping

Genotyping of the rs6265 SNP in the BDNF gene from saliva and serum samples was completed using a RNase H2 enzyme-based amplification (rhAmp) assay, a PCR-based genotyping technique (Assay ID: CD.GT.WSKT3824.1; Integrated DNA Technologies, Coralville, Iowa, USA).

A LightCycler 96 (Roche, Basel, Switzerland) instrument was used for amplification and detection under thermal cycling conditions of: one pre-incubation cycle of 10-min. at 95 ºC (for enzyme activation); 40 amplification cycles of 10-sec. at 95ºC (for denaturation), 30-sec. at 60ºC (for annealing), and 20-sec. at 68ºC (for extension). Fluorescence from the probe was measured at the end of each amplification cycle (during extension). Controls were added to the assay.

To compare the effects of exercise on physical function and cognition, participants were categorised according to their Val66Met genotype: GG homozygotes (Val/Val), AG heterozygotes (Val/Met) and AA homozygotes (Met/Met). In subsequent analyses, individuals with Val/Met or Met/Met genotypes were combined (Met carriers) and compared with individuals with the Val/Val genotype.

2.9 Medication: Levodopa Equivalent Dose

In this thesis, participant's LEDD has been calculated using updated LED conversion formulae and protocols from published reports and used as covariate in the analyses to account for changes in participant's treatment regimens (Schade et al., 2020; Tomlinson et al., 2010).

2.10 General statistical analysis

Data are presented as mean ± SD unless otherwise stated, with the level of significance set at $P<0.05$. Sample size estimation was performed for each study using previously published data and an alpha level and power set at 0.05 and 0.80/0.90, respectively, as stated in each chapter (G*Power software, version 3.1.9.6). All statistical analyses were performed using common statistical software packages (SPSS 27 [IBM, Armonk, NY], GraphPad Prism Software version 8 for MacOS [GraphPad Software, San Diego, CA, USA] and R, version 4 [www.r-project.org]). Data were checked for normal distribution and, in cases where the assumption of normality was violated, data were natural log (Ln) transformed prior to analysis. If this step was not sufficient, non-parametric tests were performed. Measures of effect size were calculated to determine the magnitude of the effects in a standardized metric and reported as partial eta-squared (η^2_p) (small = 0.01, medium = 0.06, large = 0.14) or Cohen's *d* (*d*) for paired samples T-Test (small = 0.20, medium = 0.50, large = 0.80) (Cohen, 1988).

Chapter 3. Study 1 – Optimisation of ELISA Assays for BDNF and Pro-BDNF and Comparison of Different Sample Types in Healthy Adults and People with Parkinson's

3.1 Abstract

Introduction: The link between neurotrohpins, such as BDNF and its precursor pro-BDNF, and neurological conditions, such as PD, has received significant attention over the past two decades. Research suggests that BDNF levels are reduced in PwP and it appears plausible to develop interventions aimed at increasing systemic BDNF levels, such as physical exercise. BDNF can be measured in several sample types (e.g., plasma, serum and saliva). However, peripheral BDNF is currently analysed with inconsistent methodologies, presents poor reproducibility and is highly variable, which compromises the reliability and validity of these measurements. **Aims:** to investigate the utility and develop specific reagent diluents for each different sample type with the purpose of optimising and improving the accuracy of BDNF and pro-BDNF measurements, and to compare BDNF and pro-BDNF levels from participants with Parkinson's (na-PD) and healthy older adults (HOA). **Methods:** using monoclonal antibodies and sandwich ELISAs, 6 different combinations of reagent diluent were evaluated to closely match the matrix of each sample type, which were obtained from 3 healthy volunteers. Spike/Recovery assays were performed to determine the optimal reagent diluent combination for each sample type. Subsequently, sandwich ELISAs were performed to measure BDNF and pro-BDNF levels in platelet-poor plasma, platelet-rich plasma and serum samples from na-PD (n=11) and HOA (n=16). Participants' BDNF genotype was also investigated with PCR assays. **Results:** Four and two diluent optimisation trials were completed to obtain acceptable recovery results for BDNF and pro-BDNF, respectively. Different concentrations of reagent diluent were required for each sample type and analyte. Although no clear effect of BDNF genotype on neurotrophin levels of HOA or na-PD participants was observed, levels of pro-BDNF were significantly higher in na-PD. BDNF levels tend to decrease over time in both groups. **Conclusions:** It is key to use the appropriate combination of reagent diluent for each sample type and analyte to ensure accurate measurements. The addition of pro-BDNF in conjunction with BDNF measurements might help tracking and understanding neurotrophin fluctuations in PD.

3.2 Introduction

BDNF is one of the most studied neurotrophins and a candidate biomarker for the diagnosis and monitoring of therapeutic prospects for brain disorders, such as PD or Alzheimer's Disease. The mature form of BDNF is produced by the cleavage of its precursor, pro-BDNF (Seidah & Chretien, 1999). *In vivo,* BDNF and pro-BDNF bind to two major receptors, which dictate their antagonistic roles within the CNS. On one hand, BDNF regulates the growth, development, and survival of specific neuronal types through the activation of its high-affinity TrkB receptor (see Chapter 1 for more information). It has also been revealed that BDNF can promote the survival of all major neuronal types affected in PD (Murer et al., 2001). On the other hand, pro-BDNF preferentially binds to the pan-neurotrophin receptor ($p75^{NTR}$) and potentiates neuronal death and synaptic withdrawal, amongst other proapoptotic functions (Gupta et al., 2013). BDNF and its precursor were first discovered in the brain, however, it is currently known that both of them are present in blood (Le Blanc et al., 2020). Although being one of the most promising biomarkers and able to be measured in several sample types, BDNF's use is limited. BDNF's results published in the literature currently show poor reproducibility, likely due to sample collection and storage (pre-analytical stage), sample analysis (analytical stage) and assay-related (e.g., intrinsic assay quality, appropriate preparation of reagents, etc.) differences between studies. Thus, in order to track changes due to pathology or interventions and obtain reliable measures of BDNF (and similar biomarkers, such as pro-BDNF), it is important to standardise the methodology and be consistent. As mentioned, assay-related factors are also key for the appropriate measurement of BDNF.

BDNF concentrations can be quantitatively determined by ELISA. For this assay, standard curves are determined using the kit-provided human BDNF diluted in reagent diluent at eight known concentrations. Samples also need to be diluted at their specific dilution factor with reagent diluent. The use of a correctly optimised reagent diluent is crucial to ensure an optimal assay performance. This step is not commonly reported in the literature and could be one of the reasons why BDNF results vary considerably amongst published studies. The diluent suggested by the DuoSet packages (#DY248 and #DY3175) is suitable for most cell supernatant samples. However, the measurement of samples with complex matrices, such as serum and plasma, or other unvalidated sample types such as saliva, is not guaranteed and may require the researcher to validate the appropriate diluent prior to running the assay. Sample matrix is a general term that refers to all the components present in a sample except for the analyte of interest. Each sample type has a specific matrix that can affect the response and detection of the analyte that is being measured, which is known as matrix effects and can lead to inaccurate quantitation. Thus, matrix effects should be addressed in bioanalytical method development and validation, such as ELISAs using samples that are unvalidated or have complex matrices.

It is important to assess the efficiency of the assay in detecting all the analyte present, as well as accounting for the matrix effects so that diluents used for both samples and standards have similar

matrix and, therefore, similar matrix effects on the final analyte measurement. Research supports that the optimisation of analyses conditions and the use of appropriate reagents can reduce or correct for matrix effects (Chiu et al., 2010). Thus, the sample types used in this thesis (i.e., serum, plasma and saliva) and the standards used in each plate, required optimised reagent diluents, which were obtained by using PBS supplemented with specific percentages of animal serum in order to mimic the complexity of each sample matrix (described below). The omission of this step could significantly alter the performance of the immunoassay, which could be one of the reasons why incongruences in BDNF levels have been reported in the literature.

Taken together, the first aim of this chapter was to evaluate whether the optimisation of specific reagent diluents was needed for each sample type used in the analyses. As suggested by the data, each sample type required a specific reagent diluent. Thus, different optimised reagent diluents were developed for each sample type and the accuracy of the assay and the evaluation of potential interference in the sample matrices were determined by Spike/Recovery assays. With this information in mind, the second aim of this study was to apply the acquired knowledge in the subsequent assays for the sample analyses required throughout this thesis. Given the relevance of neurotrophic factors in PD, specially BDNF, an observational study was completed to evaluate plasma (platelet-poor and platelet-rich) and serum levels of BDNF in na-PD and HOA and how those change over time. Moreover, pro-BDNF was also measured to provide an insight into this precursor levels and obtain pro-BDNF/BDNF ratio, which has been proposed as a potential biomarker for neurocognitive health (Le Blanc et al., 2020).

3.3 Methods

3.3.1 Participants

Three healthy volunteers (2 females and 1 male) from the University of Kent participated in the developmental phase of the study and provided samples (blood and saliva) for the optimisation trials that were completed prior to the final ELISA assays that were performed to analyse the samples from 11 na-PD and 16 HOA participants (the demographics of na-PD and HOA participants are provided in study 2 [Chapter 4], which provides a longitudinal evaluation of the physical and cognitive functions of these participants and a comparison with a group that completed an intervention). HOA and na-PD participants did not engage with any structured exercise or interventions provided by the researchers and the na-PD group described themselves as non-active people with PD.

In each of the optimisation trials, samples from the same subjects were used across all the plates for the Spike/Recovery assays. Specifically, the 1st and 2nd optimisation trials were run with the samples from the same volunteer, across the different reagent diluent combinations, in duplicates. The 3rd

optimisation trial was completed with the samples of another volunteer. The 4th optimisation trial included the samples of 3 volunteers across all the reagent diluent combinations used.

3.3.2 Reagent diluent optimisation process

The first step of the reagent diluent optimisation process requires the preparation of different assay reagent diluents, which will be assessed for each specific sample type in order to find the most appropriate mixture for each sample matrix. To quantify an analyte in a sample (i.e., BDNF and pro-BDNF), it is necessary to create a standard curve of the analyte in a matrix that closely matches the sample. For example, when working with serum samples to measure BDNF, the assay requires a standard curve of the analyte in an analyte-depleted solution. This specific solution, known as reagent diluent, may be obtained from combinations of various reagents. Thus, various diluents should be tested, such as PBS supplemented with Bovine Serum Albumin (BSA), fetal bovine serum (FBS) or Goat Serum (GS), amongst other options. When the assay requires samples to be diluted, both the sample and the standards are diluted and run with the same combination of reagent diluent. Subsequently, a Spike/Recovery experiment is performed to assess whether each of the proposed matrices are suitable for each sample type.

Initially, the first reagent diluent is performed by supplementing PBS with BSA at 1% (which is the recommended standard assay diluent by the manufacturer for less complex samples like cell supernatant). Subsequently, increasing concentrations of GS are used to create several reagent diluents that will be analysed for each sample type until optimal signal is obtained (for the DuoSet assays, GS is the manufacturer recommended animal serum to use). All the combinations assessed are: reagent diluent that came with the assay (1%BSA in PBS) and various combinations of PBS supplemented with different percentages of GS (cat #DY005, R&D Systems Europe Ltd., Abingdon Science Park, UK). Therefore, 6 different combinations of reagent diluent were originally prepared: a) 1%BSA, b) 10% GS, c) 20% GS, d) 30% GS, e) 40% GS and f) 50% GS.

Next, ELISAs were run for serum, platelet rich plasma (PR-P), platelet poor plasma (PP-P) and saliva (see **Table 3.1**), as described in Chapter 2 (see **sections 2.8.1.1** and **2.8.1.2**). Each of these 6 combinations of reagent diluent were used to prepare both the samples and the standards. Hence, each reagent diluent combination to be assessed required its specific standard curve of the analyte in a matrix that attempted to closely match the samples.

Subsequently, Spike/Recovery assays were performed, as described below in **sections 3.3.2.1** and **3.3.2.2**. The % recovery for each sample type was calculated and used to determine the optimal reagent diluent combination of PBS and GS for each type. A total of four diluent optimisation trials were performed to obtain acceptable recovery results for BDNF. Two diluent optimisation trials were performed to obtain acceptable recovery results for pro-BDNF.

Finally, using the results obtained from the previously described steps, plasma and serum plasma samples were analysed following the steps described in Chapter 2 (see **sections 2.8.1.1** and **2.8.1.2**).

3.3.2.1 BDNF Spike/Recovery Assays

It is not normally known how much of the analyte of interest is present in the sample being analysed. Therefore, it is particularly difficult to ascertain whether the assay used has successfully extracted and measured the analyte from the sample matrix. During ELISA assay development, Spike/Recovery assays are commonly performed to determine whether the value obtained from a sample is accurate, or whether there are factors in the sample matrix interfering with the measurement. Therefore, a known amount of recombinant protein is "spiked" into a sample and run in the ELISA. The resulting concentration, or "recovery" of the spiked material, demonstrates if the expected value can be measured accurately. If the recovered value differs significantly from the amount expected (i.e., the recovery value is not within 80-120%), this can be a sign that some factor in the sample matrix may be causing a falsely elevated, or falsely depressed value. Each sample type has specific matrix and the diluent used in ELISAs must mimic the complexity of each sample matrix. If the diluent has not been optimised, it can affect the recovery of spiked samples. Thus, 6 different combinations of reagent diluent were evaluated with Spike/Recovery assays to find the most appropriate diluent for each sample type used in this thesis.

In order to assess this, a known amount of recombinant protein (named spiking stock) is "spiked" into a sample and, subsequently, analysed in the ELISA. The spiking stock was initially obtained from the standard that was included in the DuoSet kit. The standard is stored at a higher concentration than its working concentration and it is generally recommended that the spiking stock solution should have a concentration approximately 10-times the highest assay standard (i.e., 1,500 pg/mL). This is then used to spike the samples, with the aim that the concentration of the spiked sample falls in the middle-to-high end of the standard curve range. Therefore, in order to create spiked samples that would fall within middle-to-high end of the detectable range of the standard curve, the following steps were followed. An initial 140,000 pg/ml stock solution was created after reconstituting the standard with the appropriate volume specified by the DuoSet package. Then, 10 ul of stock solution were added into 990 ul of PBS to create 1 ml of spiking stock solution at a concentration of 1,400 pg/ml. General recommendations suggest spiking the samples with a 10× spiking stock. Hence, 30 ul of the spiking stock solution were added to 270 ul of each sample (these volumes are based on needing 300 ul in order to be able to run each sample in duplicates; i.e., 100 ul per well). That is, an increase of approximately 140 pg/ml in each sample concentration was expected (i.e., 140 pg/ml is the amount spiked into the sample). With this information, the percentage recovery (% recovery) for each sample can be calculated (presented with a formula below). When the matrix for the standards matches the sample matrix, the % recovery is better.

R&D Systems recommendations state that a recovery range of 80-120% is deemed acceptable.

$$\% \text{ Recovery} = \frac{Spiked\ sample\ value - Unspiked\ sample\ value}{Amount\ spiked\ into\ the\ sample} * 100$$

3.3.2.2 pro-BDNF Spike/Recovery Assays

In order to assess what % of GS was the best to avoid samples matrix interferences for pro-BDNF, Spike/Recovery assays were also performed for this analyte of interest. Thus, a known amount of recombinant pro-BDNF protein was prepared (creating a spiking stock of pro-BDNF) and "spiked" into samples that were subsequently analysed in the ELISA.

The spiking stock was initially obtained from the standard that was included in the DuoSet kit (#DY3175). The standard vial, stored at a higher concentration than its working concentration (i.e., 5,000pg/mL), was reconstituted with the appropriate volume specified by the DuoSet package (#DY3175) and an initial 180,000 pg/mL stock solution was created.

Then, 20 ul of stock solution was added into 1,485 ul of PBS to create 1,500 ul of spiking stock solution at a concentration of 2,400 pg/ml. General recommendations suggest spiking the samples with a 10× spiking stock. Hence, 30 ul of the spiking stock solution was added to 270 ul of each sample (these volumes are based on needing 300 ul in order to be able to run each sample in duplicates; i.e., 100 ul per well). That is, an increase of approximately 240 pg/ml in each sample concentration was expected (i.e., 240 pg/ml is the amount spiked into the sample). The % recovery for each sample was calculated as explained in **section 3.3.2.1 BDNF Spike/Recovery Assays.**

3.3.3 BDNF measurements

The samples collected for this study were analysed following the ELISA method described in Chapter 2 (see **section 2.8.1**).

3.3.4 Statistical Analysis

The Shapiro–Wilk test was used to assess for the normality of BDNF data. When the assumption of normality was violated, data was log-transformed. Otherwise, if transformations were not successful or comparisons between small sample sized groups were required, appropriate non-parametric tests were used. Continuous variables are presented as mean and SD, or standard error of the mean (SEM), as stated, or median and range when distribution deviated from normal. Correlations between data sets were analysed using nonparametric Spearman's rank method. Comparisons between BDNF concentrations in the different sample types were analysed using two-way ANOVA tests. The significance level was set at 0.05, using software packages (SPSS 27 [IBM, Armonk, NY], GraphPad Prism Software version 8 for MacOS [GraphPad Software, San Diego, CA, USA] and R, version 4 [www.r-project.org]).

3.4 Results

3.4.1 Results of the BDNF Spike/Recovery Assays

Intra-assay coefficient of variation (CV), a measure of intrinsic assay quality, was assessed by comparing BDNF values measured twice on the same plate, for each subject. The first reagent diluent optimisation assay presented some challenges that were reflected in a considerably high CV (11% and 23%, across two plates) and was used as initial pilot study that would be repeated until assay conditions would be deemed optimal. Depending on the source, it is stated that the suggested CV for duplicates should be ≤ 20%.

On this initial pilot study, all the 6 different combinations of reagent diluent were examined: 1%BSA, 10% GS, 20% GS, 30% GS, 40% GS and 50% GS. The assay results showed concentrations between 20 and 50% of GS proved to be appropriate for serum samples, showing acceptable recovery values between 80-120% (the following recovery values were obtained for each of the GS combinations ranging from 20 to 50%: 96, 116, 80 and 85% recovery, respectively). PR-P showed favourable (yet not optimal) recovery results for the 30% and 40% GS combinations (64% and 121% recovery, respectively). GS combinations between 10% and 40% provided the best recovery results for the PP-P sample type (97, 111, 73 and 71% recovery, respectively). Finally, the best recovery result for saliva samples was obtained using 10% GS (which was the only GS combination that provided an acceptable recovery of 80%). The best reagent diluent combinations for each sample type obtained in the optimisation trials are presented in **Table 3.1**. It is worth noting that, to choose the best combination of reagent diluent, all the non-spiked sample values were taken into consideration. For instance, in the 1st optimisation trial for PP-P, the 20% GS combination (which had a recovery of 111%) was chosen over the 10% GS combination (which presented an overall recovery of 97%) due to some of the non-spiked samples presenting non-detectable levels of BDNF with this combination of GS.

Based on the results from the 1st optimisation trial, it was decided to perform a 2nd and 3rd optimisation trials that provided valuable outcomes that would help suggesting which combinations were optimal as reagent diluent for serum, PR-P and PP-P (which were 40% GS, 40% GS and 30% GS, respectively; presented in **Table 3.1**). In the 2nd optimisation trial, all the 6 different combinations of reagent diluent were examined: 1%BSA, 10% GS, 20% GS, 30% GS, 40% GS and 50% GS. In the 3rd optimisation trial, which was focused on the optimisation of plasma and serum samples, only 20, 30, 40 and 50% GS combinations of reagent diluent were assayed, which seemed to be the combinations that mimicked best plasma and serum matrices.

After these 3 trials, saliva samples did not provide acceptable recovery results and a 4th optimisation trial was subsequently performed for this sample type. Taking into account the performance of saliva in the previous Spike/Recovery assays, a wider range of reagent diluent combinations were used: 1%

BSA, 5% GS, 10% GS, 15% GS, 20% GS, 30% GS and 40% GS. The combination of 1% BSA provided a recovery of 55%, which was the best result we could achieve for saliva. The remainder reagent diluent combinations were deemed not acceptable. To overcome this issue, it was decided that for subsequent analyses of saliva, a spike recovery test should be included for every sample that would be analysed. Consequently, samples with recovery values significantly below 80% or above 120% would be deemed as not accurate and, therefore, excluded. The results from the saliva analyses using this approach are presented in study 2 (**Chapter 4).**

The handling of finger prick plasma (FPP) samples, as explained in **Chapter 2**, did not require a second centrifugation step to remove platelets. Therefore, it was considered to be PR-P and a combination of 40% GS that was appropriate for the dilutions of this sample type. Nonetheless, on the 4th optimisation trial, the % recovery of FPP for 40% GS was evaluated and showed to be 84% (which is a recovery value considered optimal).

Table 3.1 Best combinations of optimised reagent diluent obtained after four optimisation assays. Data presented as percentage of GS supplementing PBS to create the Reagent Diluent (percentage recovery).

	Reagent diluent supplementation with GS (% Recovery)			
Sample type	**1st Optimisation**	**2nd Optimisation**	**3rd Optimisation**	**4th Optimisation**
Serum	20% GS (96%)	40% GS (97%)	40% GS (104%)	-
PR-Plasma	40% GS (121%)	40% GS (106%)	40% GS (73%)	-
PP-Plasma	20% GS (111%)	30% GS (113%)	30% GS (86%)	-
Saliva	10% GS (80%)	*	*	1% BSA (55%)
Finger Prick	-	-	-	40% GS (84%)
Assay CVs	17%	6%	<7%	<5%

**None of the suggested combinations of the reagent diluent provided % recoveries higher than 33% for saliva. Furthermore, the 3rd optimisation was only performed with PBS supplemented with 20, 30, 40 and 50% of GS and none of them were deemed suitable for saliva (i.e., recoveries were ≤ 25%). Hence, a 4th optimisation trial was performed with a range of GS concentrations focused on: 1%BSA, 5% GS, 10% GS, 15% GS and 20% GS. The results showed high variability between samples and all the % recoveries were below optimal values (i.e., <80-120%).*

3.4.2 Results of the pro-BDNF Spike/Recovery Assays

A reagent diluent optimisation trial for pro-BDNF was performed by supplementing PBS with BSA and increasing concentrations of GS until optimal signal was obtained. The combinations assessed were: a) 1%BSA, b) 10% GS, c) 20% GS, d) 30% GS, e) 40% GS and f) 50% GS. Subsequently, the ELISA optimisation trial was run for the following sample types: PP-P, PR-P and serum, as described in in **Chapter 2**. Saliva and FPP were not included in the pro-BDNF optimisation trials due to not being sample types to be used in the final sample analyses. Finally, the % recovery for each sample type was calculated and used to determine the optimal reagent diluent combination of PBS and GS for PP-P, PR-R and serum.

On this pro-BDNF optimisation trial, a concentration of 10% GS proved to be appropriate for PR-P and PP-P samples, showing satisfactory recovery values of 101% each. Serum showed favourable recovery results with the 30% GS combination (91% recovery). However, the values obtained for serum samples had to be estimated based on a 4-PL curve-fit obtained from the standards because pro-BDNF concentrations in serum presented values below the lowest standard. To further evaluate this, an additional optimisation trial for serum pro-BDNF was performed using different dilution factors for serum (i.e., first a 100× was used, then a 3× and 2×). None of the dilution factors used provided measurable levels of pro-BDNF in serum and, therefore, it was decided not to include this sample type for further analyses of pro-BDNF.

3.4.3 BDNF and pro-BDNF measurements results

Using the information obtained from the optimisation trials, samples collected in the studies 2 and 4, presented and discussed in Chapter 4 and 6, were analysed following the optimised ELISA protocol described in this chapter (i.e., using specific reagent diluents for each sample type). Moreover, samples collected in study 2 presented in Chapter 4 (regarding na-PD and HOA participants) were evaluated in the observational study presented hereunder. Participants' characteristics are presented in **Chapter 4** (see **Table 4.1**).

This section evaluates whether biomarker levels (BDNF and pro-BDNF) change over time (i.e., 6 months) in 16 HOA and 11 na-PD participants who did not take part in the MM exercise intervention. These participants classed themselves as "not active" and were not given any instructions regarding their physical activity levels. Biomarker level differences between both groups were also investigated. BDNF was measured in several sample types: PP-P, PR-P and serum. Pro-BDNF was measured in PP-P and PR-P. A small number of samples fell outside the standards range and their BDNF concentration was extrapolated from the standards curve (3 PP-P samples from the HOA group, 3 PR-R samples from the HOA group and 2 PR-P sample from the na-PD group).

The possible effect of BDNF genotype was considered and BDNF polymorphisms (Met carriers and individuals with the Val/Val genotype) were included as covariate in the analyses. It was observed that controlling for this variable did not affect the behaviour of any of the dependent variables. Moreover, BDNF and pro-BDNF levels of HOA and na-PD were not significantly different across sample types. Therefore, in this study, there is not a clear effect of BDNF genotype on the participants' data. See Chapter 4 for a further description of the genotyping techniques used and outcomes.

In order to evaluate longitudinal changes in BDNF and pro-BDNF and use the widest temporal window available for this study (i.e., 6 months), the following analyses evaluate BDNF and pro-BDNF changes between baseline measurements (i.e., 1st assessment) and 6 months later (i.e., 3rd assessment) and across different sample types.

3.4.3.1 Plasma BDNF (PP and PR)

Individual levels of BDNF measured in PP-P at baseline and 6 months later are presented in **Figure 3.1**.

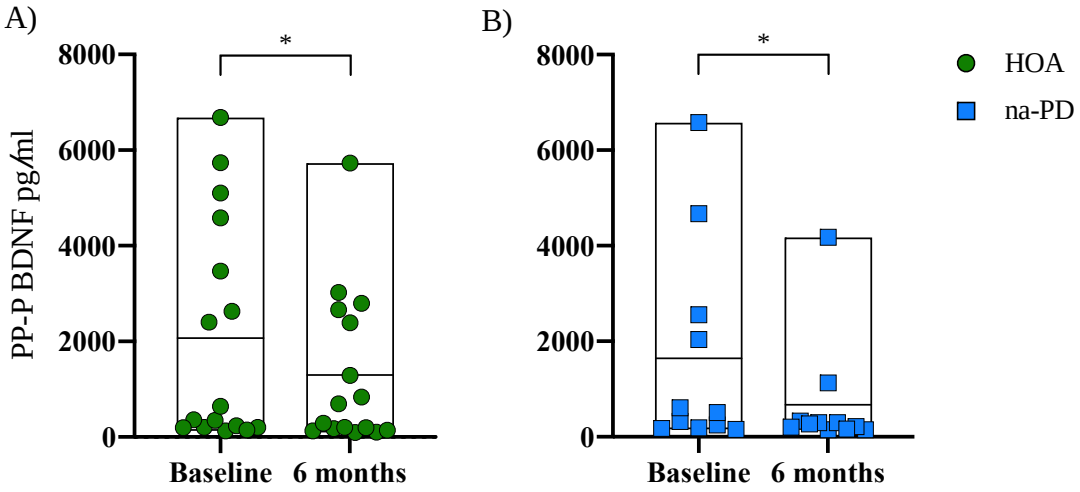

Figure 3.1 Individual responses and changes of platelet poor plasma BDNF over time separated by group. Floating bars represent the maximum (top), the mean (line) and the minimum (bottom) values. A) HOA, healthy older adults (circles). B) Na-PD, non-active people with Parkinson's (squares). *Significant difference between time points ($p<0.05$).

A two-way ANOVA on log-transformed data of PP-P BDNF revealed a non-significant interaction between group and time on PP-P BDNF levels ($F(1,25)=0.733$, $P=.400$, $\eta^2_p=0.028$). Although PP-P BDNF levels did not significantly differ between groups ($P=.472$), PP-P BDNF levels did significantly decrease in both HOA and na-PD groups after 6 months ($P=.007$; 2 and 48% median percentage decrease, respectively).

Individual levels of BDNF measured in PR-P at baseline and 6 months later are presented in **Figure 3.2**.

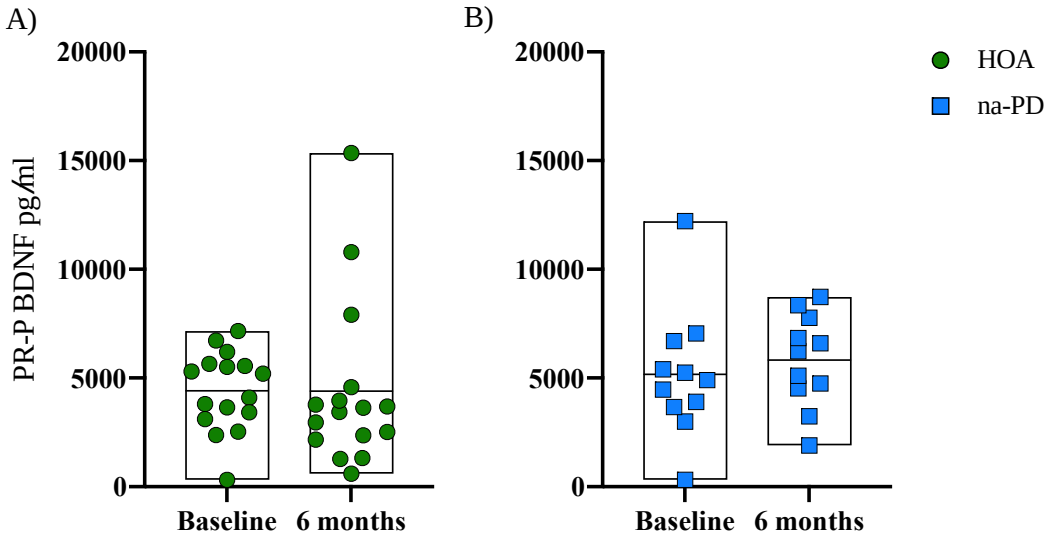

Figure 3.2 Individual responses and changes of platelet rich plasma BDNF over time separated by group. Floating bars represent the maximum (top), the mean (line) and the minimum (bottom) values. A) HOA, healthy older adults (circles). B) Na-PD, non-active people with Parkinson's (squares).

A two-way ANOVA revealed a non-significant interaction between group and time on PR-P BDNF levels ($F(1,25)=0.222$, $P=.642$, $\eta^2_p=0.009$). PR-P BDNF levels did not significantly differ between groups ($P=.217$), or after 6 months ($P=.662$).

3.4.3.2 Plasma pro-BDNF (PP and PR)

Individual levels of PP-P pro-BDNF at baseline and 6 months later are presented in **Figure 3.3**.

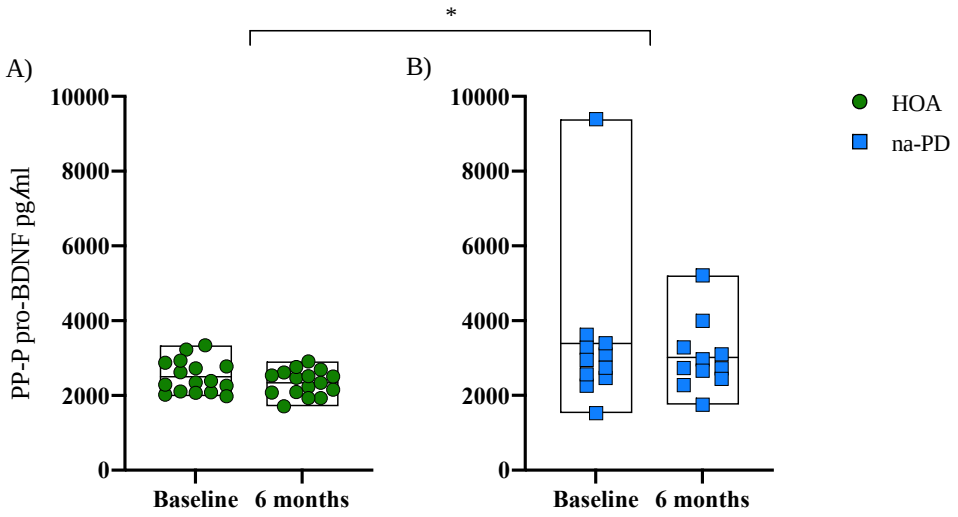

Figure 3.3 Individual responses and changes of platelet poor plasma pro-BDNF over time separated by group. Floating bars represent the maximum (top), the mean (line) and the minimum (bottom) values. A) HOA, healthy older adults (circles). B) Na-PD, non-active people with Parkinson's (squares). *Significant difference between groups ($p<0.05$).

A two-way ANOVA on logged-transformed data of PP-P pro-BDNF revealed a non-significant interaction between group and time on PP-P BDNF levels ($F(1,25)=0.097$, $P=.758$, $\eta^2_p=0.004$). Although the main effect of time on PP-P pro-BDNF levels is not significant ($P=.117$), PP-P pro-BDNF levels did present significant differences across the HOA and na-PD groups. The na-PD group presented significantly higher levels of PP-P pro-BDNF compared to the HOA group ($F(1,25)=4.797$, $P=.038$, $\eta^2_p=0.161$).

Individual levels of PR-P pro-BDNF at baseline and 6 months later are presented in **Figure 3.4**.

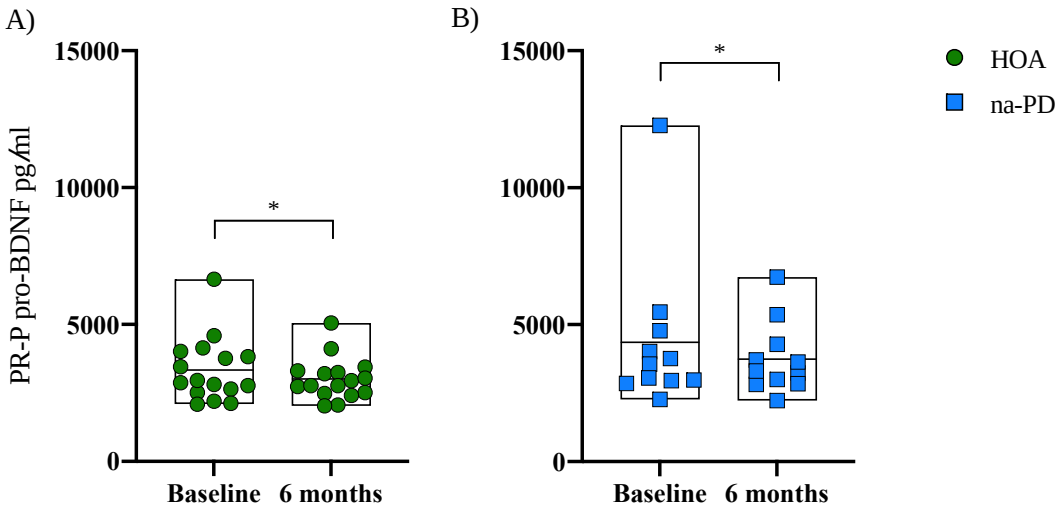

Figure 3.4 Individual responses and changes of platelet rich plasma pro-BDNF over time separated by group. Floating bars represent the maximum (top), the mean (line) and the minimum (bottom) values. A) HOA, healthy older adults (circles). B) Na-PD, non-active people with Parkinson's (squares). *Significant difference between time points ($p<0.05$).

A two-way ANOVA revealed a non-significant interaction between group and time on PR-P BDNF levels ($F(1,25)=0.439$, $P=.514$, $\eta^2_p=0.017$). Although PR-P pro-BDNF levels did not significantly differ between groups ($P=.139$), PR-P pro-BDNF levels did significantly decrease in both HOA and na-PD groups after 6 months ($P=.040$; 2 and 7% median percentage decrease, respectively).

The pro-BDNF/BDNF ratio was evaluated in PP-P and PR-P (see **Figure 3.5**).

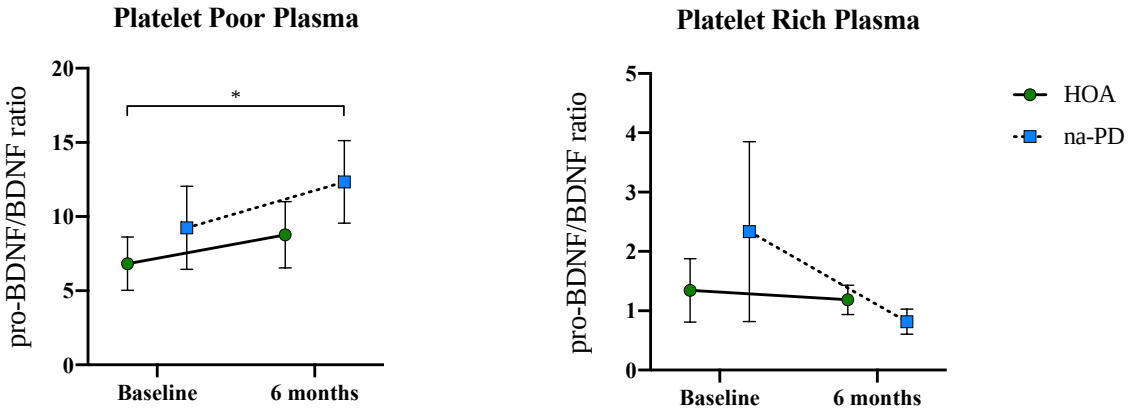

Figure 3.5 Brain-derived neurotrophic factor (BDNF)/pro-BDNF ratio behaviour (log transformed values) over time (6 months). Data are presented as mean and standard error (SE) bars (SE used for clarity of figures). *Significant difference between time points ($p<0.05$).

PP-P data showed that there was not a statistically significant interaction between time and group in explaining the PP-P pro-BDNF/BDNF rate ($F(1,25)=0.258$, $P=.616$, $\eta^2_p=0.010$). However, there was a significant main effect of time showing that the PP-P pro-BDNF/BDNF rate significantly increased in HOA and na-PD groups after a period of 6 months ($P=.036$), whilst there was not a significant main effect of group ($P=.353$).

PR-P data showed that there was not a statistically significant interaction between time and group in explaining the PR-P pro-BDNF/BDNF ratio ($F(1,25)=1.625$, $P=.214$, $\eta^2_p=0.061$). The main effects of time and group were also not significant (Ps=.307 and .857, respectively). See **Figure 3.5**.

3.4.3.3 Serum

Individual levels of serum BDNF at baseline and 6 months later are presented in **Figure 3.6**.

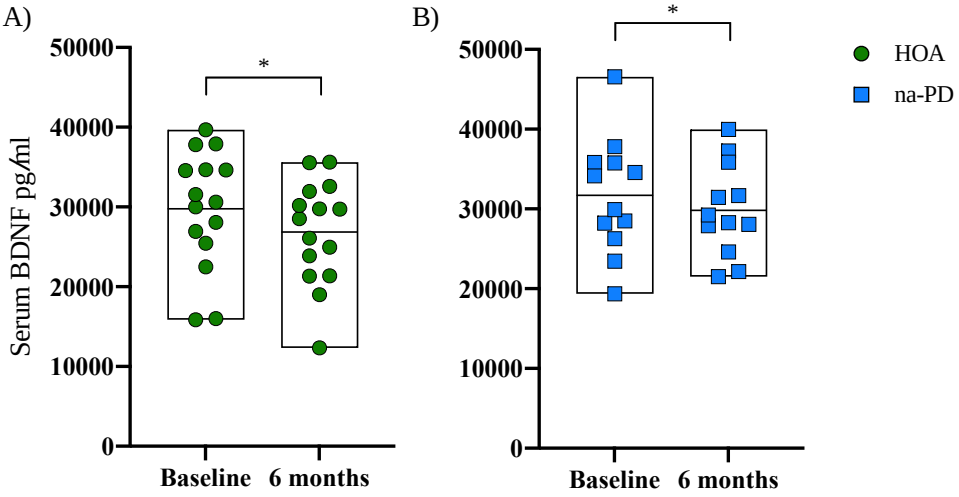

Figure 3.6 Individual responses and changes of serum BDNF over time separated by group. Floating bars represent the maximum (top), the mean (line) and the minimum (bottom) values. A) HOA, healthy older adults (circles). B) Na-PD, non-active people with Parkinson's (squares). *Significant difference between time points ($p<0.05$).

A two-way ANOVA examining serum BDNF differences between HOA and na-PD and over time was conducted. Analyses show that there was not a statistically significant interaction between the effects of group and time on serum BDNF levels ($F(1,25)=0.385$, $P=.540$, $\eta^2_p=.015$). Although serum BDNF levels did not significantly differ between HOA and na-PD ($P=.334$), serum BDNF levels did significantly decrease in both groups after 6 months ($P=.008$; 7 and 10% median percentage decrease, respectively).

3.5 Discussion

The objectives of this study were two-fold. First, in order to ensure the appropriate performance of the immunoassay and account for the complexity of each sample matrix, different concentrations of PBS supplemented with goat serum were tested to optimise the reagent diluent for each sample type. The results demonstrated that the analysis of each sample type required a specific reagent diluent to ensure the accuracy of the ELISA assays. The second aim of the study was to analyse plasma and serum samples of participants with PD and healthy adults. Marked increases in pro-BDNF levels were observed in PwP compared to healthy participants and decreases in platelet-poor plasma and serum BDNF levels were observed after 6 months in both groups of participants.

Although ready-to-use ELISA kits (such as Quantikine ELISA Kits, R&D Systems Europe Ltd., Abingdon Science Park, UK) have been extensively assessed for cross-reactivity and interference against a panel of analyte-related molecules to ensure a specific and accurate detection of the target analyte, they have not been optimised for all analytes or sample types (e.g., saliva and PR-P BDNF), and are expensive. Therefore, DuoSet ELISA Development System (cat #DY248 and cat #DY3175, respectively, R&D Systems Europe Ltd., Abingdon Science Park, UK), which contain matched antibody pairs and the basic components required to develop an immunoassay (i.e., sandwich ELISA), offer a more suitable, flexible, and economical choice to optimise the measurement of proteins of interest (BDNF and pro-BDNF) in several sample types. While Quankitine ELISAs are one of the most published ELISA kits and recognised as the gold standard ELISA for several target analytes (e.g., TNF-α, IFN-γ, IL-6, IL-1β, IL-2, G-CSF, etc.), studies evaluating BDNF measurements have shown poor reproducibility of results and incongruencies, likely because of the variety of methods used for sample collection, but also due to the methodology employed for BDNF analysis. Importantly, although Quantikine ELISAs have been exhaustively tested for superior quality and reproducibility, Polacchini et al. (2016) compared the performance of 5 different ELISA kits and showed that the inter-assay variations of Quantikine ELISAs were higher than those declared by the manufacturers (Polacchini et al., 2016). Hence, suggesting that further assay developments may be required when working with complex sample matrices.

While assay interference is not a general issue with the ready-to-use ELISA kits, components in complex biological matrices (such as serum, plasma, or saliva) may impact BDNF and pro-BDNF measured values. The use of DuoSet ELISA Development System in this study allowed the optimisation of the ELISA method for serum, PP-P, PR-R and saliva, including the verification (with Spike/Recovery assays) that the reagent diluent used for standards and sample dilution is as similar to the sample matrix as possible, optimising the spiking concentration for accurate recovery determinations, as well as selecting the appropriate reagent diluent buffer for subsequent sample analyses. This study findings provide insights into which combination of reagent diluent is the most appropriate for each of the sample types used for BDNF and pro-BDNF analyses. Results suggest that combinations of 40% GS, 40% GS and 30% GS, were optimal as reagent diluent for serum, PR-P and PP-P BDNF, respectively. For pro-BDNF, results suggest that for both PP-P and PR-P, combinations of 10% GS to create optimised reagent diluent are best. Interestingly, an initial optimisation trial for serum pro-BDNF showed favourable recovery results with the 30% GS combination (91% recovery). However, the obtained serum pro-BDNF measurements were below the pro-BDNF standard range and concentrations had to be extrapolated using the 4-PL standards curve-fit. After further analyses it was concluded that serum pro-BDNF was not measurable with the DuoSet ELISA Development System (cat #DY3175, respectively, R&D Systems Europe Ltd., Abingdon Science Park, UK).

In line with our results, measurements of pro-BDNF in serum reported in the literature have been inconsistent and not always measurable in both patients (i.e., patients with MDD) and healthy controls (Yoshida, Ishikawa, Niitsu, et al., 2012). Yoshida et al. (2012) were unable to measure serum levels of pro-BDNF in 29% of MDD participants and 37% of their healthy control group (Yoshida, Ishikawa, Iyo, & Hashimoto, 2012). The authors stated that there were no differences in the frequencies of non-measurable samples between these groups. Moreover, they emphasised that the development of a higher-sensitivity ELISA kit for an accurate measurement of serum pro-BDNF in human samples is needed since the BDNF kits that are available have significantly better sensitivity than pro-BDNF kits. It is necessary here to clarify exactly what is meant by sensitivity: the sensitivity of an assay is the lowest detection level of the analyte of interest that the antibody pair can detect. To improve this, in the present study, different combinations of reagent diluent were optimised for each sample type (including serum) to reduce or correct for matrix effects and improve overall assay accuracy. However, serum pro-BDNF could not be optimised due to the low sensitivity of the pro-BDNF assay that is currently available. Therefore, this study, together with existing research, recognises that a higher-sensitivity ELISA kit for the detection of pro-BDNF in serum is required.

Non-invasive and invasive Measurements of BDNF and pro-BDNF

The measurement of BDNF in samples obtained with non-invasive techniques was of interest. Research has shown that the non-invasive detection of BDNF in other biological fluids other than blood, such as saliva, is possible. Mandel et al. in 2009, were the first to report the presence of BDNF (and also pro-BDNF) in saliva using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western blot analyses (Mandel, Ozdener, & Utermohlen, 2009). In 2011, the same group developed and optimised a sandwich ELISA for salivary BDNF (Mandel et al., 2011). However, they found that salivary concentrations of BDNF were highly variable between individuals, affected by collection method and that their assay could not differentiate between pro-BDNF and BDNF forms (Mandel et al., 2011). Although saliva has been deemed as a reliable and valuable sample matrix in bioanalytical research (e.g., for the measurement of secretory immunoglobulin A [sIgA], monitoring of hormones such as cortisol, among several others), saliva is a difficult matrix to manage and there is, generally, lesser analyte in saliva than blood (except for proteins with known salivary functions) (Chiu et al., 2010; Gröschl, 2017). It is also clear that adequate standardisation of saliva collection methods, sampling and analysis is essential (Gröschl, 2017; Mandel et al., 2011). Bearing all this information in mind, this study sought to improve salivary detection of BDNF by developing an optimised reagent diluent for this biological fluid, which, to our knowledge, has not yet been reported. The optimisation trials for saliva were challenging but, ultimately, the combination of 1%BSA was considered the most appropriate reagent diluent for this sample matrix. However, the recovery proved to be 55%, therefore, for saliva analyses, it is strongly recommended to include a spike recovery test for every sample that would be analysed and only include samples with recovery values within 80% and 120%. Otherwise, saliva BDNF analyses could not be deemed as accurate measurements.

To the best of our knowledge, this study is also the first to report the optimisation steps to develop an optimal reagent diluent for serum, PP-P, PR-R and saliva, to improve assay accuracy and performance. Results suggest that sample matrix interferences behave differently between analytes (i.e., BDNF and pro-BDNF), as well as between sample types (i.e., serum, PP-P, PR-P and saliva). Therefore, suggesting that an optimisation step, to find the appropriate combination of reagent diluent for each sample type and analyte of interest, is key in order to ensure accurate measurements and an optimal assay performance.

Plasma Measurements of BDNF and pro-BDNF and recommendations

The findings in this study were consistent with previous research, and PR-P presented higher levels of both BDNF and pro-BDNF compared to PP-P in both groups of participants (Fujimura et al., 2002; Le Blanc et al., 2020; Lee & Kim, 2009). Platelet activation leads to significant increases in BDNF, which can be observed in the higher BDNF levels presented in serum and PR-P compared to PP-P in both HOA and na-PD. However, contrary to BDNF, pro-BDNF has received less attention and the

contribution of neurons from both the CNS and PNS, immune cells, skeletal muscle and platelets to circulating levels of pro-BDNF remains to be elucidated. Nonetheless, it has been recently discovered that platelets contain pro-BDNF in a 1:5 ratio to BDNF and, although platelet activation does not lead to pro-BDNF secretion, the pool of pro-BDNF stored in platelets represents approximately 10% of total circulating pro-BDNF (Le Blanc et al., 2020). Unfortunately, the study presented in this chapter was not able to compare PP-P pro-BDNF to serum levels of pro-BDNF due to the assay limitations mentioned above. Nonetheless, this study was able to show that both HOA and na-PD presented higher levels of both pro-BDNF and BDNF in PR-P compared to PP-P. Further research is needed to clarify whether the higher levels of pro-BDNF observed in PR-P come from the intraplatelet storage of pro-BDNF or from other cells contributing to increases in pro-BDNF.

BDNF's quantification in PR-P or serum samples are, therefore, affected by platelet activation and clotting (respectively), which complicates the accurate measurement of the free active form of this molecule in peripheral blood (Wessels et al., 2020). Thus, to avoid platelet degranulation, it is recommended to use PP-P as a more accurate representation of the "true" free BDNF and pro-BDNF levels available in peripheral blood. Furthermore, it is important to bear in mind that, in response to exercise, human skeletal muscle is able to express BDNF (Matthews et al., 2009; Rojas Vega et al., 2006). Although neuromuscular activity elicited by physical exercise increases BDNF expression in the skeletal muscle, researchers found that muscle-derived BDNF is not directly released into circulation (Matthews et al., 2009). Instead, muscle-derived BDNF may work in an autocrine and/or paracrine manner within the skeletal muscle bed (i.e., with BDNF signalling acting on nearby cells or binding on receptors on the same secreting cell, respectively) (Matthews et al., 2009). Thus, suggesting that the relationship between skeletal muscle and BDNF-induced improvements in the brain in response to exercise may be indirect. Although it has been subject of debate, research supports the idea that BDNF can cross the BBB bidirectionally (Pan et al., 1998; Poduslo & Curran, 1996). Therefore, BDNF expression and secretion in the CNS would contribute to the levels of circulating BDNF in the periphery. It also occurs in reverse. The pioneering work of Gómez-Pinilla et al. (2002) found that in order to maintain normal levels of BDNF both in the muscles and the CNS, a basal level of neuromuscular activity is required (Gómez-Pinilla et al., 2002). Their results show the potential of voluntary physical activity to upregulate genes associated with neuroplasticity in the CNS and skeletal muscle. Since then, several studies have also presented BDNF as a key driver of exercise-dependent benefits in the CNS (without disregarding its potential effects on the periphery, such as the skeletal muscle). However, BDNF levels in the human brain are difficult to quantify and, often, the measurement of BDNF's levels in peripheral blood is used as a proxy, which also presents some challenges. Since non-neural tissues are also involved in BDNF's secretion to circulation (i.e., platelets), it is difficult to distinguish the source of BDNF. Moreover, the use of different methodologies may introduce bias and affect the results. Between 70% and 99% of circulating BDNF is stored in platelets, which cannot cross the BBB, and only small amounts of free BDNF are present in plasma (Gejl et al., 2019; Le Blanc et al., 2020). Therefore, for peripheral BDNF levels to reflect

fluctuations in BDNF secretion in the brain (and, potentially, from the skeletal muscle), PP-P would be a better source to evaluate acute or chronic exercise-induced changes in BDNF levels.

Pro-BDNF/BDNF ratio

In this study, significant group differences in the PR-P pro-BDNF/BDNF ratio and PR-P pro-BDNF were not observed. The PP-P pro-BDNF/BDNF ratio was not able to highlight significant differences between groups, although PP-P pro-BDNF was significantly higher in na-PD compared to the HOA group, as already mentioned above. Therefore, pro-BDNF measurements may be an important clue to understand and track pathophysiological changes in PD and fluctuations in response to intervention strategies. Nonetheless, in order to ensure accurate measurements, it is imperative to keep in mind the analytical differences observed between analytes (i.e., BDNF and pro-BDNF) and sample types (i.e., serum, PP-P, PR-P and saliva). Moreover, further studies would be required to clarify the role of pro-BDNF/BDNF ratio as a potential biomarker candidate for PD.

The implications of BDNF polymorphisms

To further explore the bioanalytical differences between HOA and na-PD and neurotrophin levels, the potential effect of the $BDNF_{MET}$ polymorphism was also evaluated. Although *in vitro* studies have shown that $BDNF_{MET}$ seems to impair BDNF's secretion (Chen et al., 2008, 2004; de las Heras et al., 2020), we did not observe a clear effect of BDNF genotype on HOA or na-PD participants' levels of BDNF and pro-BDNF across sample types. Genetic studies evaluating the association of this polymorphism with PD are inconsistent and literature on how it affects BDNF levels is scarce. Overall, research agrees that the association between $BDNF_{MET}$ and PD and the polymorphism implications in PD pathogenesis are not clear (Lee & Song, 2014).

BDNF and pro-BDNF levels in HOA and na-PD

Another objective of the project was to evaluate plasma (PP-P and PR-P) and serum levels of BDNF and pro-BDNF in na-PD and HOA and how those change over time. BDNF levels across all the sample types evaluated did not present any significant differences between groups. However, there was a significant decrease in PP-P BDNF after 6 months for both groups that was more accentuated in the na-PD group (2 vs 48% percentage decrease). This was reflected in a significantly increased PP-P pro-BDNF/BDNF ratio for both groups and highlights the need to develop strategies for both the healthy older and non-active PwP to maintain or improve their PP-P BDNF levels over time. Group differences were only observed for PP-P pro-BDNF, where the na-PD group presented significantly higher levels of pro-BDNF compared to healthy controls. This significant observation suggests that analytical differences between na-PD and HOA might not be observed in BDNF measurements alone or using sample types such as PR-P or serum. These findings are particularly important to help in identifying and developing reliable biomarkers that could be relevant not only

for the early detection of PD or to follow PD's progression, but also to accurately track the effects of potential therapies (e.g., exercise) aimed at delaying, stopping, or reversing the degeneration process that takes place in PD. A recently published article by Yi et al. (2021) also provides evidence that complementing BDNF data with pro-BDNF measurements may help in drawing more convincing conclusions, and has better diagnostic value, than performing BDNF measurements alone (Yi et al., 2021). Researchers found that participants that were diagnosed with PD presented significantly higher levels of pro-BDNF compared to a control group of participants that were not diagnosed with PD but presented with Parkinsonism (a clinical syndrome that includes the presence of tremor and bradykinesia) (Yi et al., 2021). It is unfortunate that Yi et al. (2021) study did not evaluate BDNF and pro-BDNF levels in PP-P, which would avoid platelet degranulation and release of BDNF. Nonetheless, their outcomes, which are in line with the results obtained in the present study, provide valuable information. However, the contribution of pro-BDNF levels to PD pathology remains poorly understood. It is known that the effects of pro-BDNF, opposed to BDNF, facilitate neuronal death and other proapoptotic functions negatively affecting synaptic plasticity. Nonetheless, future research should investigate the molecular implications of presenting higher peripheral levels of pro-BDNF on the CNS and, more specifically, on dopaminergic neurons.

3.6 Conclusions

Taken together, this study identifies which reagent diluent combination provides the most accurate measurement of BDNF (for serum, PR-P, PP-P and saliva) and pro-BDNF (serum, PR-P and PP-P), and the difficulties encountered when working with complex sample matrices. Future studies involving different laboratories validating the proposed methodological steps to develop optimised reagent diluents for each sample type are recommended to contrast the obtained results. To control for the effects that sample collection methodology can cause on BDNF and pro-BDNF measurements, strict and standardised sample collection protocols were followed throughout the study. In order to ensure accurate BDNF and pro-BDNF measurements in future studies, it is recommended to take into consideration the following points: there are components in a sample matrix that can interfere with the assay and need to be accounted for, the standard curve of the analyte of interest should be created in a matrix that closely resembles the matrix of the samples being analysed, when sample dilutions are required, the diluent being used must be optimised for each sample type (and will be the same solution used to create the standard curve), and Spike/Recovery assays should be performed to determine the suitability of a suggested matrix (i.e., optimised reagent diluent) for each of the sample types included in the study. Not accounting for these factors can affect the accuracy and reproducibility of BDNF measurements, which is currently hindering the validation of BDNF for clinical purposes.

Chapter 4. Study 2 – The Therapeutic Effects of Multi-modal Exercise for People with Parkinson's: A Longitudinal Community-based study

4.1 Abstract

Introduction: Parkinson's Disease (PD) is a complex and variable neurodegenerative condition. Due to its progressive nature and lack of effective treatments, a range of motor and non-motor symptoms develop. Exercise interventions have the potential of improving and sustaining physical and cognitive function in PD. Multi-modal (MM) exercise, that includes cognitive challenges, may be more beneficial than single modalities. However, research should investigate those outcomes concurrently and assess the long-term and underlying neuroprotective effects of MM exercise. **Aim:** to evaluate the effects of a weekly community-based MM exercise session (for up to 3 years) on physical function, cognition, and wellbeing outcomes in people with PD (PwP). Furthermore, these outcomes and brain-derived neurotrophic factor (BDNF) levels were compared to an aged-matched group of non-active people with PD (na-PD, n = 16) and healthy older adults (HOA, n = 18) to evaluate potential differences and the rate of functional and cognitive decline in these groups. **Methods**: 30 participants (MM-EX group; age 65 ± 9 years; Hoehn and Yahr (H&Y) scale I to IV) attended a once-a-week MM group exercise session (60 min). Saliva and finger prick samples, and a battery of health, functional and cognitive assessments were completed every four months for one (n = 27), two (n = 20) and three years (n = 15). Results were compared to na-PD (age 68 ± 7 years; H&Y scale ≤ III) and HOA (age 61 ± 6 years). **Results**: exercise attendees significantly improved walking capacity (5% improvement after 8 months), functional mobility (11% improvement after 4 months), lower extremity strength (15% increase after 4 months) and bilateral grip strength (9% increase after 28 months) and maintained function across 1, 2 or 3 years. Group comparisons show that only the MM-EX significantly improved their mobility, lower extremity strength, cognition and BDNF levels. Moreover, a potential effect of BDNF genotype was observed. **Conclusion**: weekly attendance to a community-based MM exercise session can improve and maintain physical and cognitive function in PwP, with the potential to modify the progressive nature of PD, slow down progression and provide neuroprotection.

4.2 Introduction

Parkinson's is a progressive neurological condition that can present functional and cognitive deficits. In those with PD, there is a loss of dopaminergic neurons in the substantia nigra, region of the midbrain, along with damage to other neuronal systems (Braak, Tredici, et al., 2003). Currently, there is no cure and pharmacologic management is usually the first-line strategy to treat PD's symptomatology. The choice of medication type and dose, depends on many variables (e.g., symptoms, severity, comorbidities, interaction with other medication, etc.). Although the drugs that are available have good symptomatic effect (especially at the initial stages of the condition), none of them has yet shown the ability to halt or reverse PD's progression and their efficacy wanes over time. Moreover, the long-term use of some medications (such as L-DOPA) often leads to problems associated with the appearance of dyskinesias, unpredictable "on" and "off" fluctuations and freezing episodes. There are also important non-motor symptoms of PD that do not respond to dopaminomimetic drugs, such as autonomic dysfunction (e.g., constipation), sleep problems, affective disorders (e.g., depression) or cognitive decline (Korczyn, 2004). As the disease progresses, therapy for PD at more advanced stages becomes scant and, both, PD features worsen and medication managing becomes more difficult. Therefore, more work is needed to gain a better understanding of the mechanisms underlying PD, which will allow the development of more effective treatments. For instance, non-pharmacological approaches, such as exercise, should be further evaluated. Several exercise modalities are presently under investigation, nevertheless, the literature on exercise prescription for PwP still lacks clarity regarding the dose (namely type, frequency and intensity) that is required to elicit a therapeutic response. Moreover, despite the rapid accumulation of positive evidence hailing the role of exercise as medicine for PwP, individuals with PD are 30% less active than aged matched healthy controls, even in the early stages of the disease where the ability to perform exercise is still comparable to that of healthy individuals (Lord et al., 2013; Nimwegen et al., 2011). Thus, further work is required to create motivating, evidence-based, and innovative training approaches for PwP.

Exercise has proven to be a valuable and helpful non-pharmacological approach to improve the pathognomonic signs of PD, such as gait impairments, bradykinesia, non-motor symptoms and QoL. In addition to the functional, cognitive and behavioural benefits associated with exercise, recently, considerable literature has emerged around the theme of neuroprotection induced by exercise-enhanced neuroplasticity, which, could have the potential of slowing down the development of PD (Alonso-Frech et al., 2011; Muresanu, 2007; Oguh et al., 2014). However, the biological mechanisms that cause these effects are still poorly understood. Recently, it has been suggested that the link between exercise, cognitive improvement and neuroplasticity relies on the enhancement of trophic factor signalling (Monteiro-Junior et al., 2015). Trophic factors, such as the neurotrophin BDNF (and its precursor, pro-BDNF, with diametrically opposed functions), are important for brain neuroplasticity, survival, differentiation and neuronal growth, and they have been shown to play an important role in exercise-induced cognitive enhancement (Campos et al., 2016; Ferris, Williams, &

Shen, 2007; Monteiro-Junior et al., 2015). The effects of exercise on trophic factors have been reported numerous times in many independent studies evaluating healthy adults, but there is limited work on older people and scant work in PwP. Nonetheless, it is possible that the benefits of exercise for PwP are related to the acute and/or chronic effects on neurotrophic factors (Coelho et al., 2013).

BDNF obtained from venous blood sampling and gene expression analysis has been proposed as a promising biomarker for cognitive reserve against different neurodegenerative diseases, such as PD and AD (Beeri & Sonnen, 2016; Coelho et al., 2013; Costa et al., 2015). Although there is still a lack of consensus within published data, a number of studies have reported low levels of neurotrophic factors in PD (Parain et al., 1999). Thus, the use of exercise to induce neurobiological adaptations is particularly relevant for PwP and the measurement of systemic concentrations of BDNF may provide valuable information about the underlying mechanisms by which exercise elicits a beneficial effect on PwP. Additionally, non-invasive detection of BDNF in other biological fluids, such as saliva, are of interest. The major advantages for using saliva in diagnosis relative to blood-based assays are that collection procedures are non-invasive, painless and convenient. Moreover, saliva collection may be performed several times a day and can be easily collected in field-based settings (Papacosta & Nassis, 2011). Nevertheless, a definitive clinical validation is still lacking, and a better understanding of exercise interventions aimed at increasing BDNF levels in different body fluids is of clinical relevance. Thus, the development of biomarkers related with performance, maintenance and plasticity of brain function are important to provide insight into the relevant mechanisms of the neurodegenerative process that takes place in PD, as well as its progression, and helping to improve and develop effective future treatment strategies for PwP.

There are key factors that could play an important role to enhance neuroplasticity within an exercise setting: intensity, duration, frequency, specificity, difficulty, complexity of practice and length of the intervention, and, currently, there is little agreement on what type, intensity or duration of exercise is the most effective in significantly improving functional performance as well as enhancing brain plasticity and cognition in PwP, which makes it difficult to optimise exercise interventions. There has been increasing evidence showing that it might be intensity and not duration, the factor that plays a crucial role in enhancing the biological mechanisms that underly neuroplasticity and neuroprotection. For instance, when duration is held constant, research shows that high-intensity exercise, as opposed to low-intensity exercise, is able to induce neuroplasticity at both molecular and behavioural levels (i.e., controlled aspects of human action). High-intensity exercise resulted in more pronounced BDNF increases and showed greater improvements in motor skill retention (Ferris et al., 2007; Schmidt-Kassow et al., 2012; Thomas et al., 2016). On the contrary, when intensity is held constant, an increase in duration does not lead to additional benefits (Schmidt-Kassow et al., 2012). Schmidt-Kassow and colleagues (2012) showed that exercising at high-intensity for 20 min was sufficient to increase BDNF concentrations, and additional benefits were not observed by continuing the intervention for a total of 30 min (Schmidt-Kassow et al., 2012).

Due to Parkinson's symptomatology and its higher prevalence amongst the elderly population, it could be conceivably hypothesised that high-intensity exercise bouts would be too challenging and not appropriate for rehabilitation purposes of this specific population. Nonetheless, the feasibility, tolerability and cardiorespiratory benefits for PwP engaging with more intense exercise have been investigated and research has provided evidence that high-intensity interval training is feasible and acceptable for PwP with early or mid-stage PD (Harvey et al., 2019; Uhrbrand, Stenager, Pedersen, & Dalgas, 2015). However, in real life settings (e.g., community-based classes) and conventional rehabilitation, exercise regimes tend to be of reduced intensity (Abbruzzese, Marchese, Avanzino, & Pelosin, 2016). Afshari and colleagues (2017) analysed exercise habits, perceptions about exercise, and barriers to exercise in 'low' and 'high' exercisers with PD, and observed that overall, although participants engaged in several exercise modalities, the most common were those considered low-intensity (Afshari, Yang, & Bega, 2017). It remains unclear whether low-intensity programmes are more common due to a scarce availability of more intense exercising options. Investigating and designing appropriate exercise interventions is important to meet the needs of PwP, increase the likelihood of compliance and help PwP overcome their barriers to exercise (e.g., their ideal exercise conditions, beliefs about exercise, lack of motivating factors, etc.). Thus, emphasising the need to develop and implement exercise interventions where participants can safely work at higher intensities in order to induce neuroplasticity and that, importantly, can succeed in real-life settings (e.g., community-based).

Exercise programmes and interventions including aerobic and/or resistance training have traditionally been the most commonly delivered forms of exercise by professionals. However, participants perspectives and experiences gathered from an existing group exercise programme for PwP were in support of interventions that address several components of fitness (i.e., aerobic, flexibility, resistance, and neuromotor) (Rossi, Torres-Panchame, Gallo, Marcus, & States, 2018). Participants preferred the comprehensive multi-modal (MM) nature of the programme presented in States et al. (2017), which was aligned with current recommendations (ACSM, 2017; Rossi, Torres-Panchame, et al., 2018; States et al., 2017). Additionally, participants reported particularly enjoying the 'intensity and novelty' and the notion that the MM programme "*really was targeted on the issues that- that Parkinson's people have*" (Rossi et al. 2018, p. 4; States et al. 2017). Thus, to address the multifaceted impairments presented in PD, holistic approaches might be better. For instance, MM programmes incorporating aerobic, strength, balance, flexibility, coordination, and cognitive activities.

There is published evidence suggesting that MM programmes are possibly the most effective interventions to improve musculoskeletal and functional outcomes in older adults (Gianoudis et al., 2014). Moreover, significant improvements in balance, mobility, lower extremity function, falls risk and exercise capacity, were observed after engaging with a 60 min MM class twice a week for 16-weeks (Vaughan et al., 2014). Overall, proposing that MM exercise may offer greater benefits than single-mode exercises (i.e., cardiovascular exercise alone). The findings observed in interventions

performed in older adults can be extended to the Parkinson's community since research has shown that engaging with MM programmes from 6 months up to 5 years are safe for PwP and provide improvements in mobility, gait parameters (i.e., stride length and velocity), balance and cognition (i.e., executive functions) (Pereira et al., 2012; States et al., 2017; Tanaka et al., 2009; Vaughan et al., 2014; Vitório et al., 2011). Hence, evidence from a number of experimental studies have paved the way for developing a programme with exercises that train neuromotor function during exercises relatable to ADLs, aims to improve trunk stabilisation, work beyond voluntary thresholds, practice correction techniques to improve asymmetries, and intends to work at high intensity. Altogether, in an overall attempt to stimulate both physical and mental faculties to improve functional capacity, cognition and slow down the rate of decline of PD. In this regard, for this study, a MM exercise intervention was designed to address the following domains: physical function (e.g., postural control, range of motion, coordination, balance, strength and aerobic capacity) and cognition (e.g., executive function, dual tasks [which require the simultaneous performance of a motor and cognitive task]).

As mentioned above, it has been widely studied and proved that experience-dependent neuroplasticity is largely dependent on intensity, repetition, specificity, difficulty, and complexity of practice. 85% of the research studies investigating the effects of exercise on the brain have provided evidence that brain function can change following exercise training (Chen et al., 2020). In older adults, both the duration of the intervention and the exercise session can modify brain structure (e.g., reduce white matter volume atrophy) and increase brain volume. More specifically, interventions lasting >48 weeks using sessions of 45 to 60 min are required to elicit those benefits (Chen et al., 2020). However, PwP might need more time to achieve effective learning and automatization (Abbruzzese et al., 2016). Although there is evidence suggesting that both intervention length and session duration may influence brain function and structure, which can be related to regular training, the vast majority of studies in PwP have focused on shorter interventions (<12 weeks). Therefore, there is clearly a need to develop long-term interventions, particularly for PwP, due to the chronic progressive nature of this condition.

Another important part of exercising in a community-based class is the beneficial effects of combining a philosophy of inclusiveness with a programme that seeks to enhance physical activity and social engagement. Research suggests that, once involved with community-based exercise programmes, participants perceive a sense of social belonging and feel less isolated and lonely (Barragan, 2021). This is particularly important for PwP, who might use exercise settings as social networks (Hirsch, Iyer, Englert, & Sanjak, 2011). Additionally, it has been suggested that the impact of social interactions could be a critical factor of cognitive reserve and have beneficial effects for cognitive aging, for instance, reducing the risk of developing dementia (Amieva et al., 2010). Taking part in the community-based MM exercise programme designed for this study in a group setting includes social facets (e.g., participants and their partners have a separate room where they can socialise and regularly meet outside of the exercise class hours) with the aim to provide emotional and psychological benefits for PwP and help to build a social network.

Taken together, the initial purpose of this study was to evaluate the feasibility of running a community-based exercise class for PwP tightly linked with research. Class participants who agreed to take part, participated in regular assessments of physical function, cognition and biomarkers, with the aim to investigate the efficacy of a community-based multi-modal exercise in the improvement of functional (physical and cognitive) and analytical parameters (i.e., blood and saliva BDNF). Secondly, another aim of this study was to the assess the chronic effects of engaging with the MM exercise programme on functional, cognitive, neurobiological, mood and QoL parameters after attending for 1, 2 and 3 years in the community-based exercise class. In combination with the data on session effort and attendance, this will help to advance scientific knowledge and understanding on the effects of MM exercise on neurotrophic factors and see if there are any relationships between changes in these measures and other parameters currently being taken as part of the study (such as functional capacity or cognition).

Finally, the quantification of motor and cognitive deterioration during the development of PD is imperative in order to develop interventions aiming at modifying and reducing functional decline. Nevertheless, the normal aging process also tends to reduce physical and cognitive function in the healthy older adult (HOA) (Harada, Natelson Love, & Triebel, 2013; Milanović et al., 2013). Thus, monitoring biomarkers levels, cognitive and physical function changes in both HOA and non-active PwP (na-PD) will help to better understand the rate of PD's progression and the impact that the MM exercise intervention might have on the MM exercising group (MM-EX). Furthermore, the completion of physical activity questionnaires on a regular basis will provide additional information about the amount of physical activity that all participants perform on a daily basis and how this might affect deterioration over time.

Overall, this study is designed to provide an in-depth understanding of the rate of motor and cognitive changes during PD's progression, as well as bringing insight into the disease clinical context by measuring the levels of endogenous production of specific molecules, such as BDNF, in different body fluids.

4.3 Hypothesis

It is hypothesised that na-PD would present a higher rate of decline in the study outcomes over time compared to HOA. Moreover, it is expected that the individual's physical activity levels have an impact on the rate of progression (i.e., people engaging with higher levels of regular physical activity would present a lower functional decline). Higher scores in physical and cognitive tests, as well as in biomarkers levels such as BDNF, are anticipated in HOA and PwP that are more active (hence, the MM-EX group as opposed to the na-PD group). At the same time, HOA were expected to present higher scores and biomarkers levels than the na-PD, as well as a slower decline rate over time. Additionally, MM-EX is expected to reduce the decline of the outcome measures over time in order to maintain or even improve function, reaching levels similar to those of HOA.

4.4 Methods

4.4.1 Study Design

In October 2016 researchers at the university of Kent, in collaboration with two charities (the Medway Working Age Group and Parkinson's Equip) set up a MM community-based exercise class specific for PwP.

Initially designed as a pilot study following an observational opportunistic study design, the aim of this cohort-study was to assess the feasibility of operating a community clinical exercise group for PwP as well as measuring the impact of MM exercise on health parameters, functional capacity and BDNF levels, with the intention to follow a community-based participatory research (CBPR) approach (Hirsch et al., 2011). Measurements were taken on four occasions over the period of approximately 1 year and provided enough evidence to confirm the feasibility of continuing with a bigger single arm observational study assessing the functional longer-term effects of the MM class and exploring additional parameters of relevance for PwP, such as non-motor symptoms (e.g., cognitive function) and QoL. Thus, using circuit training as a multi-component exercise intervention (namely MM), the aim of the whole project was to assess the impact of MM exercise on physical and cognitive function, as well as BDNF levels, in a community-based group of PwP.

With the establishment of the MM-EX, it was decided two more experimental groups were required as comparison groups; HOA and na-PD. With the introduction of both comparison groups, the study followed a quasi-experimental design.

4.4.2 Participants

Participants' inclusion criteria are described in **Chapter 2** (see **section 2.2**). Participants joined the MM exercise class on a rolling basis, starting sometime between the end of 2016 and the beginning of 2020. In total, 39 participants were recruited with the assistance of a community support group, via PD organisations (e.g., Parkinson's UK) and word of mouth. Participants were free to withdraw from the study without any obligation and continue engaging with the MM class if they wished (although no participants requested this). Since the MM exercise class was available both to continuing and new participants, many participants attendance was consistent throughout 1 year, and some for up to 3 years. For an overview of the participant numbers progression throughout the study please refer to **Figure 4.2** and **Appendix G.**

Comparison group participants included HOA (i.e., adult in good physical and mental function responsible for one's own health and well-being as well as managing or coping with disease symptoms and functional limitations [Skelton et al. 2018]) between the ages of 50 and 85, and PwP of similar age and Hoehn & Yahr stage I, II or III (na-PD group).

Table 4.1 Demographic data of MM exercise class participants (MM-EX) and comparison groups; non-active people with Parkinson's (na-PD) and Healthy Older Adults (HOA). For continuous variables, one way ANOVA and Mann-Whitney U tests were conducted between HOA, na-PD and MM-EX ½ year groups, and the mean values ± standard deviations are listed. For all other variables, chi-square and Fisher's exact tests were calculated and frequency counts indicate the number of participants in each category relative to their grouping followed by the proportion of the sample in parenthesis [a]Significant differences between HOA and na-PD. [b]Significant differences between HOA and MM-EX ½ year. [c]Significant differences between MM-EX ½ year and na-PD. [d]No significant differences observed after Bonferroni adjustments were applied.

Group n	**HOA** (n=18)	**na-PD** (n=16)	**MM-EX** (n=30)	***P***
Gender				
Female	11	5	5	
Male	7	11	25	
Age (years)	61 ± 6	68 ± 7	65 ± 9	0.040[a]
BMI (kg/m^2)	27.9 ± 4.6	29.0 ± 3.9	28.5 ± 3.2	0.719
Years since PD diagnosis	N/A	4 ± 2	5 ± 6	0.933
Hoehn and Yahr Stage	N/A			0.728 Fisher's Exact Test
Stage 1		10 (63%)	13 (43%)	
Stage 2		2 (13%)	5 (17%)	
Stage 3		4 (25%)	11(37%)	
Stage 4		0	1 (3%)	
PD Staging	N/A			1.000
Early		7 (44%)	13 (43%)	Fisher's exact test
Moderate		9 (56%)	16 (53%)	
Advanced		0	1 (3%)	
LEDD	N/A	494 ± 233)	581 ± 483	0.501
Comorbidities				
Hypertension	2 (11%)	0	8 (28%)	
Hypotension	0	0	3 (10%)	
Arthritis	0	1 (6%)	6 (21%)	
Joint replacement	0	0	1 (3%)	
Cancer	0	1 (6%)	2 (7%)	
Epilepsy	0	0	1 (3%)	
Heart Disease	0	0	4 (14%)	
Regular exerciser at baseline?				.024[d] Fisher's exact test
Yes	17 (94%)	13 (81%)	18 (60%)	
No	1 (6%)	3 (19%)	12 (40%)	
Side Affected	N/A			.024[d]
Left		10 (63%)	7 (23%)	Fisher's exact test
Right		3 (19%)	11 (37%)	
Both		3 (19%)	5 (17%)	
N/A		0	7 (23%)	
Employment Status				0.066
Retired	7 (39%)	12 (75%)	13 (43%)	
Employed	11 (61%)	4 (25%)	17 (57%)	

4.4.3 Assessments

The study was set up alongside the MM exercise class with the intention to establish it on a longer-term basis. Therefore, participants' enrolment was allowed throughout the length of the intervention, meaning that participants started the study at different times (apart from the initial cohort of participants that were recruited to originally set up the intervention [n=17]). This can be observed in the flow chart presented in **Figure 4.2** (also see **Appendix G**). That is, as the study progresses, there are less participants that have completed the later assessments, mainly because they started the intervention at a later time. Thus, MM-EX participants were assessed every 4 months (every 17 weeks on average) for up to 3-years. It is worth noting that the MM intervention was delivered for 3 months on a weekly basis. After that period, the assessments were completed, and participants had a break from the MM programme for approximately 4 weeks. MM-EX assessments were organised around the university semester holidays and the breaks took place at easter (April), summer (August) and winter (December) vacation periods. During this time, participants were advised to stay active and keep practicing the exercises at home, however, their daily levels of physical activity were not tracked. See **Figure 4.1** for schematic of the study timeline and assessments schedule.

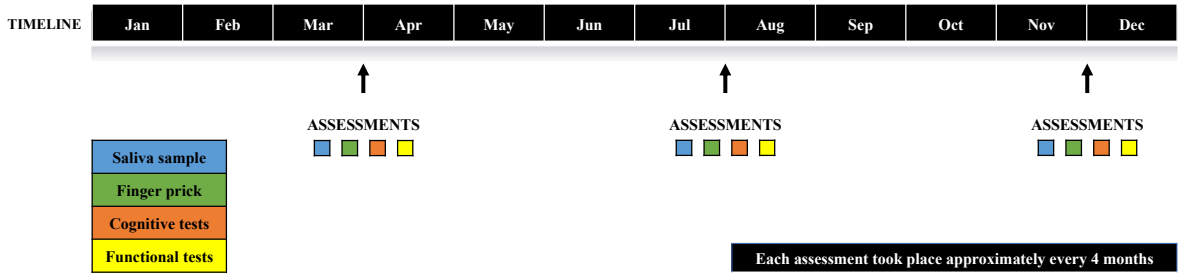

Figure 4.1 Timeline and assessments schematic. MM-EX participants were consolidated in testing sessions over one or two consecutive days every 4 months. HOA and na-PD participants completed the study assessments in different occasions, each, approximately, 3 months apart.

Altogether, 30 participants completed 3 assessments, 27 participants completed 4 assessments (equivalent to 1 year), 20 participants completed 7 assessments equivalent to 2 years and 15 participants completed the last assessment after 3-years of engaging with the MM intervention (10 assessments in total). No comparisons between the 1-, 2-, and 3-year groups were performed because of the overlap in participants among the groups (i.e., the 3-year group was a subset of the 2-year group, and the 2-year group was a subset of the 1-year group).

Both comparison groups (HOA and na-PD) were evaluated approximately every 13 weeks (i.e., 3 months), as a review of how the participants were progressing over time. Thus, their progress was recorded at regular intervals after this initial assessment at least 4 more times to evaluate progression for one year (see **Figure 4.1**).

For an overview of the testing schedule of the study please see **Figure 4.1** and **Figure 4.2** (also see **Appendix G** for an extended version of **Figure 4.2**).

For the group comparison analyses, the first 3 assessments of MM-EX, HOA and na-PD groups were included, which had equivalent time intervals between assessments. The 1st assessment is equivalent to baseline, the 2nd assessment was completed 3 months later, and the 3rd assessment concluded approximately 6 months later.

After the initial year of the study, the time intervals between MM-EX's group assessments were of approximately 4 months and a total of 4 assessments were completed within 1 year.

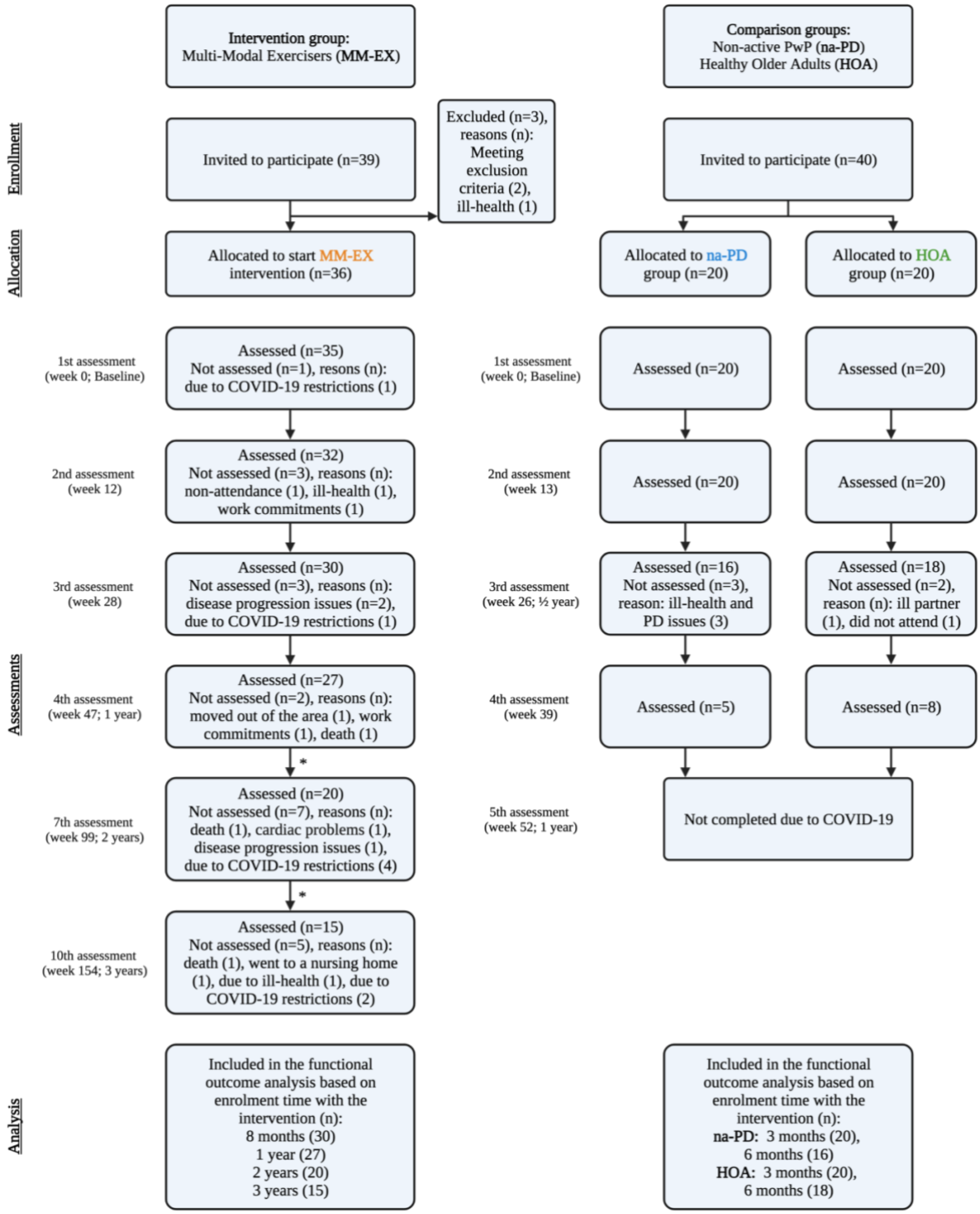

Figure 4.2 Participant's flow chart. As the study progresses, there are less participants completing each assessment, because enrolment with the study occurred on a rolling basis. Altogether, for the MM-EX group, 27 participants completed 4 assessments (equivalent to 1 year), 20 participants completed an assessment after 2 years (equivalent to taking part in 7 assessments) and 15 participants completed the last assessment after 3 years of engaging with the MM intervention (10 assessments in total). For the na-PD group, 16 participants completed 3 assessments (6 months). For the HOA group, 18 participants completed 3 assessments (6 months). *A full version of the flow chart including all the information between assessments 4th, 7th and 10th can be found in the **Appendix G.** Flow chart created with BioRender.com.

4.4.3.1 Measurements

Initially, for the MM-EX group, health measures and functional assessments (described in **Chapter 2**) were evaluated at baseline and compared with subsequent assessments completed at the time points described in **section 4.4.3 Assessments**.

In order to evaluate the underlying biological mechanisms that MM exercise could induce, it was decided to add finger prick and saliva samples to the array of tests that EX-MM completed. Finger prick and saliva samples, which are less invasive than venepuncture and can be rapidly and easily collected in field-based settings, were used to investigate exercise-related changes in BDNF. Additionally, the cognitive and mood measures described in **Chapter 2** were also added to evaluate whether MM exercise could also induce cognitive changes and improvements in QoL and mood.

Participants completed all measurements that were performed during the assessment days following the same order: saliva and finger prick samples, and cognitive tests were completed before the functional assessment.

During the MM exercise sessions, the HR of the MM-EX group participants was measured on two occasions as a screening measure to check health status for cardiovascular diseases (CVD) or autonomic dysfunction. First, HR was taken at the beginning of the class and, second, at the end after finishing the cool-down and having rested for several minutes. If participants' HR did not return to values approaching pre-exercise HR measurements, participants were asked to walk around the class for several minutes followed by another seated resting period. Their HR was measured until it reached appropriate values.

At the end of the MM exercise sessions, participants were asked to provide a rating of perceived exertion (RPE) based on their perception of the exercise session as a whole (i.e., session RPE, which is a method that quantifies the exercise intensity component with single number representative of the combined intensity and duration of the exercise session), measured by the 6-20 Borg rating of perceived exertion scale (Borg, 1998; Foster et al., 2001). Participants were encouraged to work "as hard as they can".

Both comparison groups were evaluated with the same battery of assessments that the MM-EX group completed and, additionally, venous blood samples were collected from both HOA and na-PD in order to assess biomarker (BDNF and pro-BDNF) changes over time in serum and plasma (these results are part of study 1 and are presented in Chapter 3).

Feedback from the assessments was provided to all participants.

4.4.3.2 Community-based exercise class

The MM programme was initially designed for PwP that were part of a local support group. Thus, thanks to the close coordination and collaboration with this active and growing community support group (named the Medway Working Age Group [MWAG]), the MM exercise programme was set using an approach similar to States et al. and Hirsch's et al. community-based participatory research model (Hirsch et al., 2011; States et al., 2017). All participants that took part in the MM exercise class also decided to enrol as research participants. External participants (i.e., PwP that were not part of the local support group) were also interested in joining the MM class thanks to members of the support group that discussed the MM exercise programme with colleagues, through exercise referral from a Parkinson's nurse and through word of mouth. Details of the MM exercise programme and the research study were explained to potential participants once they contacted a study investigator, and when they decided to take part, new participants were invited to complete the baseline assessments. Participants enrolment with the study occurred on a rolling basis.

4.4.4 Multi-Modal Exercise Class

The circuit-based MM programme, completed on a weekly basis, lasted for one hour. The use of different methodologies in exercise testing makes the comparison between studies a challenging task. In order to facilitate the comparison, in the study presented in this chapter, the standardising recommendations proposed by Basso and Suzuki were followed (Basso & Suzuki, 2017). Therefore, the duration of the multi-modal exercise class was considered long under their premises of short duration exercise lasting 1 to 15 min, moderate duration exercise lasting 16 to 45 min, and long duration exercise lasting 46 min or longer.

Starting with an extensive and progressive warm-up covering all main body parts and components that would be included in the circuit, participants then engaged with a 23-station circuit before ending with a cool-down and full body stretches (see **Table 4.2**). All participants exercised together as a group. In both sections, the warm-up and the cool-down, participants were distributed in a circle around one of the instructors and followed the commands for each exercise in a "do as I do" approach. In the MM circuit, each participant started on a different station. Participants completed the exercises at an intensity corresponding to the "work as hard as you can" command for 1 min. before moving onto the next station. Participants had a 30 sec transition time to move between stations which was classified as 'active rest' (i.e., stay gently marching).

Participants were encouraged to work at increased frequency, intensity and difficulty during practice, which leads to more learning and greater structural changes in the brain (Petzinger et al., 2013). There is mounting evidence that intensive and challenging goal-based exercises (e.g., task-oriented training) combined with aerobic components can enhance neuroplasticity (Petzinger et al., 2013). The exercises organised in stations were specifically designed to tackle and improve Parkinson's

specific characteristics, such as gait impairments, balance problems, rigidity or bradykinesia, amongst others (see **Table 4.2**; training components). Accordingly, the aim was to work beyond voluntary thresholds, which means that participants were encouraged to move through a full range of motion and work at higher intensity levels beyond their voluntary limits (Lockwich, Schwartzkopf-Phifer, Skubik-Peplaski, Andreatta, & Kitzman, 2021). Also, the exercises were meaningful (transferable to ADLs), goal related, with combined movement and cognitive challenges. Emphasis was put on repetition and the movements were big global and multidirectional. Overall, combined movements (arms and legs) within aerobic and strength challenges were included throughout the circuit. The order of the stations was carefully selected and organised not only based on the symptoms that were being addressed with each exercise but also mixing cardiovascular exercises with strength whilst trying to alternate muscle groups used.

Throughout the class, participants were encouraged to work at moderate-to-high intensity (expected to work at a sufficient threshold to promote neuroplasticity). To define exercise intensity, based on the American College of Sports Medicine guidelines, it is suggested to use percentages of VO_2 max. in the following way: low-intensity exercise as ≤39% of VO_2 max., moderate-intensity exercise between 40% to 59% of VO_2 max., and high-intensity exercise as ≥60% VO_2 max (Garber et al., 2011; Pollock et al., 1998). Due to the practicalities of the study (initially set up as pilot study) and the setting of the exercise group (community-based), VO_2 max. measurements were not obtained. Instead, levels of perceived exertion of the session measured with the session RPE, which has been validated for PwP, were obtained at the end of each exercise session and used as a valid measure of intensity (Basso & Suzuki, 2017; Penko, Barkley, Koop, & Alberts, 2017). Participants were educated in how to use this scale. Verbal feedback, cues that drew attention to the tasks and encouragement were provided to all participants, which are suggested to modify and strengthen existing motor circuits that help consolidate a learned behaviour (Petzinger et al., 2013).

Understanding the complexity of PD when designing exercise interventions is vitally important. The heterogeneity of the group participants makes it considerably difficult to ensure that all receive an optimal exercise dose. To overcome this, several exercises were included to cover a diverse array of functions throughout the MM circuit. Moreover, for some of the exercises, different options were offered to participants to individualise within the same group, to add a progression or a regression to accommodate individual differences and variations (also important from a safety point of view).

Over time, the structure of the programme was maintained, as well as the majority of the exercises. However, the order of the stations, some particularities of the exercises and the cognitive challenges proposed to be completed alongside the physical exercises, were occasionally changed to increase complexity and enhance environmental enrichment, which improves neuroplasticity (Baroncelli et al., 2010; Fox et al., 2006). Nonetheless, the fundamentals of each exercise were maintained, as well as the required cognitive domains that each exercise demands. As mentioned above, individual and specific adjustments of overload were added for the class participants when necessary.

All sessions were monitored by at least two exercise professionals that also invited students from the School of Sport and Exercise Sciences to the class. Students were previously introduced to the particularities of this programme and paired to those participants that required further assistance.

During the length of the study, there was an average of 17 participants per session (range: 8 – 24).

4.4.4.1 Students' assistance

A key part of this research project was the involvement of student volunteers from the School of Sport and Exercise Sciences assisting in the supervision and administration of the MM exercise class as part of their placement or dissertation project. Students' initially received training on the programme and were instructed to initially assist those participants that required closer supervision as well as motivating the overall class to exercise at moderate-high intensities, maintaining big range of motion and completing the exercises with the appropriate technique. In total, the ratio of exercise instructors and students ranged from 1:10 down to 1:2 at various times.

Throughout this project, many participants expressed their gratitude towards the instructors and student helpers and both PD participants and their partners highlighted that exercise attendees enjoyed the social interaction with the students, which contributed to the supportive nature of the exercise program. Participants' perspectives are collected in a research article that is pending publication (title: "*Exercise is part of my whole medication regime' – people with Parkinson's and their partners' participation experiences with a community-based group exercise class*"). From the perspective of the students, taking part in this exercise class was both an excellent learning experience and socially rewarding, a unique opportunity to gain a better understanding of PD and learn how to better manage persons with PD and similar conditions.

Table 4.2 Structure of the multi-modal exercise class and details of the different modules of the session. Each station of the circuit was completed during 1 min under the instruction to work "as hard as you can" followed by 30 sec of active rest allowing enough time to transition to the next station. Progressions and regressions were provided according to each individuals' capacity and constant encouragement was given to all participants throughout the class. The exercises listed as example are not exclusive for the training component and usually impact more than one element.

Warm-up (15 min)					
Components	General stretching Muscle activation from head to toes, whole body mobility Walking in different directions, increasing step and stride length, engaging with arm movements, coordination between upper body and lower body, etc.				
Multi-Modal Circuit (35 min)					
Training Components	***Muscular Strength*** *(core stability, strengthening, posture, reduce rigidity)*	***Aerobic fitness*** *(cardiovascular conditioning, exercise capacity)*	***Coordination and Balance*** *(multi-directional exercises, working on range of motion, dual tasks)*	***Gait impairments*** *(step and stride length, postural control whilst moving, bradykinesia, freezing)*	***Cognitive Tasks whilst doing physical exercises*** *(processing speed, cognitive flexibility, memory)*
Example of exercises	Overhead ball throw Squats with arm row One step forward with wide arms using a thera-band (or other light-weight materials) Wall press-ups Arm rows (using a band) Bell ringing (arm swing and squat)	Step-ups/astride jumps High knee marching Jogging on the spot Half star jump/jumping jacks Climb the ladder (knee lift with opposite arm raise)	Heel to toe walking Reach and Twist Punching and marching Step back and lift opposite arm forwards High knee lift with finger clicks or clap Throw and catch a scarf Arm rolling with steps	Step across the river Fast shuttle walk with big arm swing, turnings and stops Box step to coloured cones 2 side steps and clap Sideways walking crossing feet over each other	Saying the months of the year in alphabetical order/reverse alphabetical order Stroop colour-word test Voice projection Looking around the class, try to find 5 red things
Cool-Down (10 min)					
Components	Gentle walking Static balance exercises and proprioception Posture control General stretching (aimed at improving mobility and range of motion)				

4.4.5 Attendance

Attendance to the MM exercise class was monitored and used to examine adherence to the programme. Participants' attendance to the class was recorded at each session and summed at the end of each block of sessions between assessments. For participants who stopped their participation to the MM programme, the timing of withdrawal was noted, and the reasons documented whenever possible. Attendance rate was defined as: (total number of attended MM exercise sessions)/(total number of offered MM exercise sessions). Both average attendance rate and attendance rate for the 4-weeks previous to assessment sessions were calculated.

4.4.6 Sample size and Statistical Analysis

Due to the opportunistic nature of this study, a priori calculations of sample size could not be performed at the beginning. Seventeen MM-EX participants initially enrolled with an initial pilot study that involved 4 assessments throughout 1 year. In total, 39 participants were recruited throughout the duration of the study. However, 27 of them completed the 4 visits included in 1 year. Post hoc power analysis calculations were performed retrospectively to ensure that the study sample size was suitable to detect the effect of the exercise intervention at a level of significance equal to 0.05. Thus, power calculations were based on the results obtained from repeated measures assessments of one of the primary functional outcome measures, which is mobility (assessed with the TUG). At an alpha level set at 0.05, an effect size equal to 0.574 (η^2_p=0.248) and a sample size of 27, the probability of detecting a "true" effect when it exists was 0.992 (i.e., >99% power) (G*Power software, version 3.1.9.6). Based on these results, it was also possible to estimate the sample size required to obtain sufficient power for the study for subsequent years (i.e., up to 3 years study continuation). This revealed that a sample size of 14 would be required to provide, at least, 80% power to detect a true effect, should it exist.

As explained in the **section 4.4.2 Participants**, two comparison groups were later added: na-PD and HOA. Sample size was estimated using preliminary data that evaluated TUG as an outcome measure in a repeated measures (within-between interactions) study design. At an alpha level and power set at 0.05 and 0.80, respectively, the total sample size required to determine significant changes over 5 different time points was estimated to be 28; 14 participants in each group (G*Power software, version 3.1.9). To account for a drop-out rate of at least 20%, 20 participants were recruited. As mentioned in the **COVID-19 Mitigation Statement**, due to COVID-19 pandemic, the intervention had to be suddenly interrupted, which implied that the 5th time point could not be completed for any participant. All recruited participants were able to complete at least the first 3 months assessment of the study (which corresponded to the 2nd time point), 18 HOA and 16 na-PD participants completed the 6 months assessment (3rd time point) and 8 HOA and 5 na-PD completed the 9 months assessment

(4th time point). The flow chart presented in **Figure 4.2** depicts the longitudinal pathway of the participants as they completed each time point or withdrew from the study.

Baseline demographic and medical information were compared between groups. One-way ANOVA tests were conducted to assess differences between groups for continuous variables (age and BMI). Mann-Whitney *U* test was used to assess differences between MM-EX and na-PD groups for continuous variables (years since diagnosis and LED) and ordinal variables (the Hoehn and Yahr scale stage and overall Parkinson's Staging). Chi-square tests, and Fisher's exact test were used to compare the proportions in each category across the 3 groups (HOA, na-PD and MM-EX). Pearson's correlation coefficient (r) was used to study the strength of the association between attendance, IPAQ scores and different outcomes.

Physical function was evaluated by comparing baseline data with the outcomes completed on the different timepoints presented in **Figure 4.1** and **Figure 4.2**. Moreover, to determine changes from baseline and over time, linear mixed-effects models were built, as described below. Significant interactions or main effects were analysed post-hoc using Bonferroni-corrected t-tests where appropriate. All data were analysed on an intention-to-treat basis similar to what Gianoudis and colleagues performed (Gianoudis et al., 2014). Therefore, data from all participants were included in the analyses regardless of compliance and no data was imputed for the few participants with missing data. Participants who discontinued the MM exercise were measured at subsequent assessments if they agreed to return for testing. Additional analyses were performed to evaluate the effects of attendance on the study outcomes.

Linear mixed-effects models for repeated measures were used to analyse changes in physical function cognition and mood over time; being the 6MWT, TUG, 1-STS and GS the functional outcome measures, the MMP, TMT-A and B and CDT the cognitive function outcome measures, and finally, BRUMS and OPQOL-Short. Age, disease duration (i.e., months since diagnosis), H&Y scale stage and LEDD were evaluated as covariates.

Model fitting and selection, and the analyses for this study were conducted in a stepwise fashion. First of all, the effects of MM exercise over time were assessed using a fixed effects model to evaluate repeated measures (i.e., repeated measures ANOVA modelled with a fixed slope and intercept). This model has traditionally been used to analyse longitudinal data and is often considered the most preferable option (Vaisey & Miles, 2017). However, it presents important limitations that restrict its usefulness. For instance, its flexibility is minimal (e.g., it does not allow missing observations), only accounts for one source of non-independence at a time (e.g., repeated measurements in individuals) and does not control for differences between individuals (Schober & Vetter, 2018).

An example with the 6MWT outcome measure will be presented to explain the model fitting process that was repeated for each of the outcome measures stated. A fixed effects analysis of repeated measures data was initially performed to evaluate the results of the 6MWT over time. However, participants differed in the 6MWT results obtained leading to differences between participants in their average results over time. This variation, as mentioned above, is not accounted for in this fixed effects analysis of repeated measures model, which presented a residual error of 133.572 and a coefficient of determination (R^2) of 0.015. Therefore, it was decided to use a different analytical model namely linear mixed model, also known as linear mixed-effects model, which is more appropriate when the focus is on within-subject change (Bates, Mächler, Bolker, & Walker, 2015; Schober & Vetter, 2018). The first mixed-effects model built to evaluate each functional outcome included time as a fixed variable (using total time points completed over the course of 1 yr, 2 yr and 3 yr on separate models). To account for the dependency of repeated measures (i.e., within-subject nature), the random variance in intercepts due to unexplained differences between participants was modelled as a random intercept for each participant (Bates et al., 2015). Additionally, restricted maximum likelihood (REML) was used as the estimation method since it can produce unbiased estimates of variance and covariance parameters. Comparing the residual error and R^2 between the fixed repeated measures and the mixed-effects model, both parameters improved for the mixed-effects model; with the residual error being 30.346 as opposed to the initial value of 133.572, and R^2 being 0.818 compared to 0.015. Thus, meaning that, with the mixed-effects model, more variation was captured in the response variable (i.e., dependent variable) compared to a fixed effects model. For these reasons, mixed-effects modelling was the preferred statistical approach to evaluate the longitudinal data collected in the present study. Moreover, the model improvement was statistically evaluated with the function lmerTest::ranova() in R, which removes the random-effect term of the improved model and presents Likelihood Ratio Tests (LRTs) comparing the reduced model to the improved model (Kuznetsova, Brockhoff, & Christensen, 2017). The results showed that adding random effects significantly improved the model ($P<.001$). Subsequently, age, disease duration (i.e., months since diagnosis), H&Y scale stage and LEDD were evaluated as covariates. If the final model resulted in a significantly better model fit, the covariate was kept in the model. This was assessed using Akaike Information Criteria (AIC) and LRTs. When comparing nested models with LRTs, models were refitted using maximum likelihood (ML) to allow the comparison of nested models that only differ in their fixed effects (in this case, the random effect variable 'subject' was kept in all model comparisons) (Meteyard & Davies, 2020; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Thus, the final mixed-effects models to evaluate functional outcome changes over time were built with time as a fixed effect variable, controlling for covariates (if appropriate), while leaving the random-intercept (i.e., subject as random effects) and estimation method unchanged.

To test for the effects of MM exercise to the MM-EX group on specific functional and cognitive outcomes compared to na-PD and HOA, linear mixed-effects models were built using group (MM-EX, na-PD and HOA) and time (baseline 1st assessment, 2nd assessment and 3rd assessment [i.e., total

number of time points completed over the course of approximately 6 months]) as fixed factors and individuals as random factors.

Assumptions of the built linear mixed-effects models were analysed by investigating the residual versus predicted values, as well as the distribution of the residuals of the outcome variables, which was expected to follow a normal distribution. When the assumption of normality of the residuals was violated, data was log-transformed (i.e., for the TUG). The significance level was set at 0.05. Statistical analyses and graphs illustrating the fitted values resulting from the mixed models were performed with R, version 4 [www.r-project.org]).

Finally, given the nature of the study (i.e., opportunistic), its long duration and the symptomatology of PwP which can affect the ability to maintain functional behaviour on a routine schedule, there are anomalies present in our data. One participant missed one of the assessments for unknown reasons, and, on a different occasion, the assessment results were misplaced and could not be inputted. For this reason and in order to include the information captured with repeated measurements whilst considering the pattern of outcomes that each participant experienced in reaching their results, an approach using mixed-effects methods was chosen to analyse the longitudinal data. Mixed-effects models can accommodate unbalanced data patterns and use all available participants and observations in the analysis whilst providing unbiased results and valid estimates of the intervention effects in the presence of missing data (Detry & Ma, 2016). Additionally, the first measures of salivary and blood BDNF as well as cognitive function for 15 of the 24 participants that were evaluated, came from assessments completed 1 year after starting the MM exercise programme. The reason why 15 participants do not have true baseline measurements for those tests is that those measures were not administered during the initial evaluations in 2016. Therefore, in order to run appropriate comparison analyses between MM-EX, HOA and na-PD groups and over time, only those MM-EX participants that completed their baseline assessments for all tests before starting the MM exercise intervention were included in the group comparison analyses. That is, a total of 9 MM-EX participants that engaged with the MM exercise intervention for up to 3 assessments (i.e., 8 months approximately). BDNF group comparisons were completed using a one-way analysis of covariance (ANCOVA), treating the baseline measurement as covariate. Preliminary checks were completed to assess the assumptions of normality, linearity, homogeneity of regression slopes, and homogeneity of variance.

All MM-EX participants were evaluated on regular basis, including MM-EX participants without a true baseline, and individual feedback with the outcomes each assessment was provided to all participants after every evaluation they took part in. That is, a total of 252 individual assessments. However, to keep a consistent and comparable methodology within the study groups, which allowed the completion of accurate statistical analysis, only the cognitive and BDNF data from participants with a true baseline was included in the study presented in this chapter.

In order to study cognition and mood in those participants that joined the MM study before cognitive and mood measurements were included, linear mixed-effects models with a random-intercept (i.e., subject as random effects) were performed in order to evaluate changes over time in those participants that had joined the MM exercise class for at least 1 year. After this period of 1 year, the first measurement for the tests presented below were obtained. These participants were divided into two subgroups: 15 participants completed assessments for 1 year (a total of 4 assessments) and 11 participants completed the assessments for 2 years (a total of 7 assessments). Changes over time were evaluated.

4.5 Results

Participants' demographic information is summarised in **Table 4.1** and showed no differences between MM-EX and both comparison groups across all clinical and sociodemographic variables. The na-PD group, however, were significantly older than the HOA group, though they were similar on all other demographic variables (see **Table 4.1**).

4.5.1 Attendance

The flow of participants through the MM programme sessions is illustrated in **Figure 4.2**. All participants attended all the assessment sessions whilst being enrolled with the study apart from one participant that missed the second assessment but continued attending the MM class and the following assessments.

Thirty-five participants completed at least the first block of MM exercise sessions, named as block A in **Table 4.3**, which corresponds to all the sessions encompassed between the 1st assessment (i.e., baseline) and the 2nd assessment (i.e., approximately at week 12, which corresponds to a period of approximately 3 months). At the end of each block, there was a short break from the MM exercise class of 3 to 4 weeks before starting the following block. Block B was completed by 30 participants and corresponds to the period between the 2nd and 3rd assessments (i.e., approximately week 28, shortly after 6 months of engagement with the MM programme). Twenty-seven participants completed block C, which corresponds to the period between the 3rd and 4th assessments (i.e., approximately at week 47, after 1 year of engaging with the MM class). Block D was completed by 20 participants and corresponds at the time period between the 4th and 7th assessments (i.e., approximately at week 99, after 2 years of engaging with the MM programme). Finally, 15 participants completed block E, which corresponds to the period between the 7th and 10th assessments (i.e., approximately at week 154, after 3 years of engaging with the MM programme).

The average overall attendance was high (79%; see **Table 4.3**) and showed moderate-to-low variability across blocks of sessions. Overall attendance between the 3rd and 4th assessments was strongly correlated with the last 4-weeks attendance before the 4th assessment, $r_s(25)=.810$, $P<.001$.

Nonetheless, results of the Spearman correlation suggest that participants with higher attendance rate after 1 yr of engaging with the MM programme do not strictly experience higher improvements in the 6MWT, TUG or 1-STS ($r_s(24)=-.132$, P=.519; $r_s(25)=.209$, P=.295; $r(25)=-.001$, P=.995, respectively). Therefore, a significant association between attendance rate and functional improvement was not observed.

To further investigate whether there was a relationship between attendance and functional improvement, a chi-square test of independence was performed to evaluate overall attendance between assessment 3 and 4, and the last 4-weeeks attendance prior to the 4th assessment (i.e., 1 yr). Firstly, the potential effect of overall attendance was evaluated and the criterion for high attendance was set at 75% (Hicks et al., 2012; Nascimento et al., 2015; Shubert, Altpeter, & Busby-Whitehead, 2011; Tunur, DeBlois, Yates-Horton, Rickford, & Columna, 2020). Fifteen participants completed more than 75% of the sessions, with an average attendance of 85%, and 12 participants attended less than 75% of the sessions, with an average attendance of 57%. There were no significant differences between the proportion of participants with less that 75% attendance whose 6MWT, TUG and 1-STS scores improved between assessments 3 and 4, and those who attended more than 75% of the sessions ($\chi^2(1)=1.688$, P=.194, $\chi^2(1)=1.330$, P=.249, and $\chi^2(1)=1.080$, P=.299, respectively). Whilst overall attendance is important in order to guarantee a minimum weekly practice, participants' attendance prior to the assessments might be of relevance to elicit the functional, cognitive and neuroplastic benefits expected. Thus, attendance throughout the 4-week period prior to the regular assessments was evaluated. On average, eighteen participants attended more than 75% of the last 4 sessions, with an 85% attendance, and 9 participants attended less than 75% of the last 4 sessions, with an average of 33% attendance. A similar set of results for the 6MWT, TUG and 1-STS were observed when evaluating the attendance rate for the last 4-weeks attendance prior to the 4th assessment ($\chi^2(1)=0.675$, P=.411, $\chi^2(1)=0.490$, P=.484, and $\chi^2(1)=0.675$, P=.411, respectively).

IPAQ measurements were added to have an insight into the amount of physical activity that participants were engaging with outside of the exercise class and evaluate whether participants' attendance rate would correlate with their measures of habitual practice of physical activities. IPAQ values and attendance rates (both the whole block of MM exercise sessions between assessments and last 4-week attendance) of three different study timepoints were used and none of the correlations showed significance. Additionally, the correlation coefficient (r) for each comparison was lower than 0.174 and close to 0, showing the absence of any linear relationship between attendance and overall activity levels.

The relationship between IPAQ and outcome scores (6MWT, TUG and 1-STS) was evaluated for all three comparison groups including only participants that completed IPAQ measurements from their 1st assessment (baseline). HOA's IPAQ measures did not significantly correlate at any timepoint with the values of the 6MWT, TUG and 1-STS test. Similarly, na-PD's IPAQ values did not correlate

with any of the outcome measures apart from the 6MWT at the 3rd assessment ($r_s(14)=.600$, P=.014). Interestingly, MM-EX group's results showed a significant correlation between IPAQ and outcome scores at the 3rd assessment for the 6MWT, TUG and 1-STS tests ($r_s(6)=.833$, P<.001; $r_s(6)=-.786$, P=.021; $r_s(6)=.738$, P=.037, respectively).

Over the more than 3 years that the MM exercise community-based programme has run and more than 2200 person-hours of participation in the programme, no injuries or other adverse events were reported by participants. At the start and throughout the study, participants were asked to report any previous or prevalent injuries and the exercises were appropriately adapted or changed, if needed, to allow their completion. Some participants reported fatigue or transient muscle soreness as a result of their participation (information reflected in the qualitative study presented in **Chapter 5**). Four participants reduced their participation for a few weeks due to different causes not related with their involvement with the MM class; one of them suffered from sciatica, another participant presented a blood clot on one leg that caused pain and required surgery, and two participants had a urine infection that affected their capacity to exercise. Nonetheless, none of these events arose as a direct result of their participation in the class. The risk of falling during the MM class was controlled by eliminating potential tripping hazards, providing safe transitions between exercise stations, and procuring support from the exercise instructors and students to participants, particularly to those that were more prone to falling. Only one fall did occur during one session that did not lead to an injury (this participant had experienced falling before and learnt how to fall to avoid injuring themselves). Other mitigating measures used to reduce any incidence of adverse effects of exercise were regular checks of cardiovascular health status before and after the MM exercise class. Participant's vital signs were regularly checked (i.e., HR) as well as any changes in PD's symptomatology and their medication.

Table 4.3 Participants' attendance rates across the duration of the multi-modal exercise programme expressed as percentage of the total number of classes attended within each block. Blocks A, B, C, D and E represent the percentage attendance between baseline (i.e., 1st assessment) and 2nd assessment (Block A), 2nd and 3rd (Block B), 3rd and 4th (Block C), 4th and 7th (Block D), and 7th and 10th (Block E), respectively. The following letters indicate the reasons for not attending the assessments: W = work commitments; A = non-attendance (reason not provided); D = died, P = disease progression issues, R = due to COVID-19 restrictions, M = moved out of the area, N = went to a nursing home, C = cardiac problems, I = ill-health. [a]PS indicates participants who were part of the initial pilot study. A) Shows the attendance rates over the whole duration of the study. B) Shows the attendance rates during the 4-weeks prior to an assessment.

A) ATTENDANCE RATES BETWEEN BLOCKS OF SESSIONS AND ASSESSMENTS

PARTICIPANTS[a]	week 12 **Block A** n=32	week 28 **Block B** n=30	1 year **Block C** n=27	2 years **Block D** n=20	3 years **Block E** n=15	**Overall mean**
1 (PS)	90%	75%	71%	66%	73%	**75%**
2 (PS)	70%	100%	79%	76%	78%	**81%**
3 (PS)	A	58%	57%	33%	67%	**54%**
4 (PS)	90%	75%	64%	69%	74%	**74%**
5 (PS)	80%	100%	86%	86%	72%	**85%**
6 (PS)	60%	83%	W			**72%**
7 (PS)	90%	100%	100%	89%	83%	**92%**
8 (PS)	100%	67%	79%	86%	N	**83%**
9 (PS)	60%	92%	43%	C		**65%**
10 (PS)	90%	75%	79%	P		**81%**
11 (PS)	90%	100%	79%	88%	81%	**87%**
12 (PS)	100%	92%	100%	77%	D	**92%**
13 (PS)	70%	92%	50%	46%	60%	**63%**
14 (PS)	60%	67%	79%	67%	66%	**68%**
15 (PS)	80%	75%	79%	61%	61%	**71%**
16 (PS)	70%	83%	86%	67%	53%	**72%**
17 (PS)	80%	P				**80%**
18	75%	79%	53%	79%	I	**71%**
19	83%	93%	80%	84%	80%	**84%**
20	80%	93%	87%	82%	92%	**87%**
21	80%	64%	60%	66%	60%	**66%**
22	100%	79%	47%	79%	75%	**76%**
23	79%	60%	70%	D		**70%**
24	80%	80%	53%	57%	R	**68%**
25	100%	60%	100%	70%	R	**82%**
26	100%	67%	67%	R		**78%**
27	93%	93%	M			**93%**
28	100%	92%	86%	R		**93%**
29	100%	85%	D			**92%**
30	100%	62%	46%	R		**69%**
31	80%	92%	86%	R		**86%**
32	83%	P				**83%**
33	100%	R				**100%**
MEAN	**85%**	**81%**	**74%**	**71%**	**72%**	**79%**
SD	13%	14%	17%	14%	11%	11%

B) ATTENDANCE RATES FOR THE 4-WEEKS PREVIOUS TO AN ASSESSMENT

PARTICIPANTS[a]	~week 12 **Block A** n=32	~week 28 **Block B** n=30	1 year **Block C** n=27	2 years **Block D** n=20	3 years **Block E** n=15	**Overall mean**
1 (PS)	75%	75%	50%	58%	92%	70%
2 (PS)	75%	100%	75%	100%	58%	82%
3 (PS)	A	50%	25%	42%	58%	44%
4 (PS)	100%	75%	50%	83%	83%	78%
5 (PS)	100%	100%	75%	83%	67%	85%
6 (PS)	0%	100%	W			50%
7 (PS)	100%	100%	100%	92%	83%	95%
8 (PS)	100%	50%	75%	75%	N	75%
9 (PS)	100%	75%	75%	C		83%
10 (PS)	100%	50%	75%	P		75%
11 (PS)	100%	100%	100%	92%	67%	92%
12 (PS)	100%	100%	100%	58%	D	90%
13 (PS)	50%	100%	75%	50%	58%	67%
14 (PS)	75%	50%	75%	67%	75%	68%
15 (PS)	75%	100%	75%	67%	50%	73%
16 (PS)	75%	100%	75%	75%	42%	73%
17 (PS)	100%	P				100%
18	75%	75%	50%	75%	I	69%
19	50%	100%	75%	83%	92%	80%
20	75%	100%	100%	75%	100%	90%
21	75%	100%	50%	83%	67%	75%
22	100%	100%	0%	58%	75%	67%
23	75%	25%	75%	D		58%
24	75%	75%	25%	50%	R	56%
25	100%	25%	100%	58%	R	71%
26	75%	50%	50%	R		58%
27	100%	100%	M			100%
28	100%	100%	100%	R		100%
29	100%	75%	D			88%
30	100%	50%	0%	R		50%
31	75%	100%	100%	R		92%
32	100%	P				100%
33	100%	R				100%
MEAN	**84%**	**80%**	**68%**	**71%**	**71%**	**77%**
SD	22%	25%	29%	16%	17%	16%

4.5.2 Intensity (RPE)

Participants were always encouraged to exercise on a "as hard as you can" basis. Due to the group nature of the MM programme, the core of the class content and intensity of the circuit did not change notably across sessions (see **Table 4.4**). Training intensity was monitored using the RPE scale (scale of 6 to 20) (Borg, 1998), and, on average, participants exercised at RPE 13.3, which corresponds to a "somewhat hard" effort. More specifically, 3% of participants exercised at RPE 11, 18% of participants exercised at RPE 12, 42% of participants exercised at RPE 13, 24% of participants exercised at RPE 14, and 9% and 3% of participants exercised at RPEs of 15 and 16, respectively.

Table 4.4 Participants' rate of perceived exertion (RPE) across the whole duration of the multi-modal exercise programme within each block. Blocks A, B, C, D and E represent the time period between baseline (i.e., 1st assessment) and 2nd assessment (Block A), 2nd and 3rd (Block B), 3rd and 4th (Block C), 4th and 7th (Block D), and 7th and 10th (Block E), respectively.

A) RATES OF PERCEIVED EXERTION DURING THE EXERCISE BLOCKS OF SESSIONS BETWEEN ASSESSMENTS

PARTICIPANTS[a]	~week 17 **Block A** n=32	~week 34 **Block B** n=30	1 year **Block C** n=27	2 years **Block D** n=20	3 years **Block E** n=15	**Overall mean**
1 (PS)	12	13	13	13	13	**13**
2 (PS)	12	12	12	12	13	**12**
3 (PS)		14	13	13	13	**13**
4 (PS)	13	13	13	13	13	**13**
5 (PS)	16	15	14	14	14	**14**
6 (PS)	13	13				**13**
7 (PS)	15	15	16	15	14	**15**
8 (PS)	13	13	13	13		**13**
9 (PS)	14	13	11			**13**
10 (PS)	18	16	14			**16**
11 (PS)	15	15	14	13	14	**14**
12 (PS)	13	13	14	16		**14**
13 (PS)	14	14	15	15	13	**14**
14 (PS)	15	15	13	14	14	**14**
15 (PS)	13	14	14	13	13	**13**
16 (PS)	13	13	13	13	13	**13**
17 (PS)	15					**15**
18	13	13	12	13		**13**
19	12	12	13	13	13	**13**
20	12	12	12	12	12	**12**
21	13	13	14	13	13	**13**
22	12	12	12	13	13	**13**
23	13	13	15			**14**
24	12	11	12	12		**12**
25	14	15	15	13		**14**
26	13	12	12			**12**
27	15	15				**15**
28	13	13	13			**13**
29	13	13				**13**
30	11	11	11			**11**
31	16	14	13			**14**
32	12					**12**
33	12					**12**
MEAN	**13.4**	**13.4**	**13.2**	**13.3**	**13.3**	**13.3**
RANGE	(11-18)	(11-16)	(11-16)	(12-16)	(12-14)	(11-16)
SD	1.4	1.2	1.2	0.9	0.5	1.0

4.5.3 Health Measurements

4.5.3.1 MM-EX assessments

BMI and WC were evaluated periodically (see **Table 4.5** and **Table 4.6**). Linear mixed-effects models assessing yearly values of BMI and WC showed a lack of significant changes over time, which represented a maintenance of those anthropometric measurements (all comparisons presented P>.05).

Table 4.5 Mean for BMI (kg/m^2) throughout the assessments and its respective 95% confidence levels.

	1st assessment	*4th assessment*	*7th assessment*	*10th assessment*
BMI 1 yr (kg/m^2) *(n=27)*	28.6 (27.4-29.9)	28.3 (27.0-29.5)		
BMI 2 yr (kg/m^2) *(n=20)*	28.5 (27.0-29.9)	28.0 (26.6-29.5)	28.1 (26.6-29.5)	
BMI 3 yr (kg/m^2) *(n=15)*	29.0 (27.5-30.5)	29.4 (27.9-30.9)	28.8 (27.4-30.3)	29.4 (27.2-30.1)

Table 4.6 Overall mean for WC (cm), and separated by gender, throughout the assessments and its respective 95% confidence levels.

	1st assessment	*4th assessment*	*7th assessment*	*10th assessment*
WC 1 yr (cm) *(n=27)*	97.9 (94.0-102.0)	97.4 (93.5-101.0)		
Females *(n=5)*	94.9 (79.8-110.0)	91.4 (76.3-107.0)		
Males *(n=22)*	99.2 (95.2-103.0)	99.2 (95.2-103.0)		
WC 2 yr (cm) *(n=20)*	93.1 (85.7-100.0)	97.2 (89.8-105.0)	97.8 (90.4-105.0)	
Females *(n=3)*	95.3 (58.5-132.0)	90.3 (53.5-127.0)	91.0 (54.2-128.0)	
Males *(n=17)*	92.7 (84.5-101.0)	98.4 (90.2-107.0)	99.0 (90.8-107.0)	
WC 3 yr (cm) *(n=15)*	91.9 (83.0-101.0)	99.1 (90.3-108.0)	100.4 (91.5-109.0)	99.6 (90.7-108.0)
Females *(n=1)*	75 (-)	75 (-)	74 (-)	77 (-)
Males *(n=14)*	93.1 (84.3-102.0)	100.9 (92.1-110.0)	102.2 (93.5-111.0)	101.2 (92.4-110.0)

4.5.3.2 Group comparisons

A total of 64 participants were included in the analyses (30 participants in the MM-EX group, 16 participants in the na-PD group and 18 participants in the HOA group). For the mixed-effects model analysis, HOA's group was used as a reference variable to make the group comparisons against. Baseline results for all three comparison groups are presented in **Table 4.1**.

The mixed-effects model that evaluated BMI changes over time and between groups showed a non-significant main effect of time (F(2,121.010)=1.993, P=.141) and group (F(2,61.003)=0.434, P=.650), as well as their interaction (F(4,121.011)=1.020, P=.400).

The mixed-effects model that evaluated WC changes over time and between groups showed a significant main effect of group (F(2,61.045)=3.786, P=.028), and time (F(2,199.099)=6.856, P=.002) with a non-significant interaction (F(4,199.099)=1.464, P=.217). These results were reflected in a significant decrease of WC over time for HOA. That is, HOA's WC decreased in both the 2nd and 3rd assessments compared to the 1st (*b*=-2.033, *t*(119)=-2.475, P=.044, and *b*=-2.528, *t*(119)=3.077, P=.008, respectively). Moreover, the HOA's decrease at the 3rd assessment made HOA's WC differ from the Parkinson's comparison groups (MM-EX: *b*=9.078, *t*(65.5)=-2.875, P=.016, na-PD: *b*=8.419, *t*(65.5)=-2.313, P=.072).

4.5.4 Functional Outcomes

An overview of the estimated marginal means and confidence intervals for the functional outcome measures is presented in **Table 4.23**. The covariates evaluated were age, disease duration (i.e., months since diagnosis), H&Y scale stage and LEDD. No extreme outliers were detected in our data.

4.5.4.1 MM-EX 1 yr analyses

A total of 27 participants completed the MM programme for 1 yr and were included in the analyses.

4.5.4.1.1 6MWT

First, walking capacity was assessed by measuring the distance covered with the 6MWT on 4 occasions over the course of 1 yr. Results of the linear mixed-effect model examining the effect of MM exercise over time on walking capacity are presented in **Table 4.7**. The effect and significance of adding covariates in the model was evaluated beforehand. Analyses revealed that LEDD significantly predicted 6MWT measures over time (F(1,25.064)=4.982, P=.035, η^2_p=0.170). Subsequently, the value of adding LEDD as a covariate was evaluated and results confirmed that controlling for LEDD significantly improved the model (χ^2(1)=4.906, P=.027). Significant differences in walking distance were observed between time points that resulted in a significantly

higher walking capacity at the 3rd assessment (approximately 6 to 8 months after starting the MM exercise programme) of almost 22-m compared to baseline (see **Table 4.7**; *b*=21.959, *t*(76.248)=2.622, P=.011). Importantly, after 1 yr of engaging with MM exercise, walking capacity was maintained and no declines in 6MWD were observed compared to baseline (see **Table 4.7**; *b*=7.259, *t*(76.096)=0.879, P=.382).

Table 4.7 Results of linear mixed-effects model evaluating walking capacity changes measured as the distance covered during the 6MWT over time (measured in meters).

	b	**SE_b**	**95% *CI***	***P***
6MWD at 1st assessment (Intercept)	456.473	18.987	419.471, 493.466	**<.001**
6MWD at 2nd assessment	0.958	8.373	-15.327, 17.259	.909
6MWD at 3rd assessment	21.958	8.373	5.673, 38.259	**.011**
6MWD at 4th assessment	7.259	8.259	-8.813, 23.331	.382
LEDD	-0.056	0.025	-0.104, -0.007	**.035**

An overview of the estimated marginal means for each time point with time as fixed effect and subject as random effect is presented in **Table 4.8**. LEDD is not included as covariate in the results presented in **Table 4.8** to allow comparison with the results presented in the Physical Outcome Measures table presented in **Table 4.23**.

Table 4.8 Estimated marginal means and their respective 95% confidence levels across four assessments evaluating the 6MWT over 1 yr. Numbers in bold represent significant improvements compared to the 1st assessment (P<.05).

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*
6MWT 1 yr (m)	425 (399-451)	426 (400-452)	**447 (421-473)**	432 (406-458)

4.5.4.1.2 TUG

Mobility was assessed as the time taken to complete the TUG test. A linear mixed-effect model was used to assess mobility after engaging with MM-EX for 1 yr on 4 different assessments. Age, LEDD, H&Y scale stage and disease duration were assessed as possible covariates but none of them significantly predicted the response variable or improved the model fit. Results of the linear mixed-effect model examining the effect of MM exercise on the TUG showed significant differences in mobility across time points over 1 yr; F(3,76.179)=3.319, P=.024, η^2_p=0.120). Therefore, resulting in an improvement of mobility (see **Table 4.9** for the results of the mixed-effects model).

Table 4.9 Results of linear mixed-effects model evaluating mobility changes measured with the TUG over time (measured in seconds).

	b	**SE*b***	**95% *CI***	***P***
TUG at 1st assessment (Intercept)	8.851	1.045	8.122, 9.645	**<.001**
TUG at 2nd assessment	-1.099	1.032	-1.169, -1.032	**.004**
TUG at 3rd assessment	-1.080	1.032	-1.149, -1.016	**.017**
TUG at 4th assessment	-1.055	1.032	-1.123, 1.008	.097

An overview of the estimated marginal means for each time point with time as fixed effect and subject as random effect is presented in **Table 4.10**.

Table 4.10 Estimated marginal means and their respective 95% confidence levels evaluating the TUG over 1-yr (four assessment points). Numbers in bold represent significant improvements compared to the 1st assessment (P<.05).

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*
TUG 1 yr (sec)	8.9 (8.1-9.1)	**8.1 (7.4-8.8)**	**8.2 (7.5-9.0)**	8.4 (7.7-9.2)

4.5.4.1.3 1-STS

Functional lower extremity strength was assessed with the 1-STS task on 4 different occasions over the period of 1 yr. Age, LEDD, H&Y scale stage and disease duration were assessed as possible covariates but none of them significantly predicted the response variable or improved the model fit. Results of the linear mixed-effect model examining the effect of MM exercise on the 1-STS showed significant differences in functional lower extremity strength across time points over 1 yr; $F(3,76.121)=4.192$, $P=.008$, $\eta^2_p=0.140$). Therefore, resulting in a significant improvement of the 1-STS (see **Table 4.11** for the results of the mixed-effects model).

Table 4.11 Results of linear mixed-effects model evaluating functional lower extremity strength changes measured with the 1-STS over time (total number of repetitions measured).

	b	**SE*b***	**95% *CI***	***P***
1-STS at 1st assessment (Intercept)	20.296	1.153	18.024, 22.569	**<.001**
1-STS at 2nd assessment	2.553	0.812	0.974, 4.132	**.002**
1-STS at 3rd assessment	2.370	0.802	0.811, 3.930	**.004**
1-STS at 4th assessment	1.447	0.812	-0.131, 3.028	.079

An overview of the estimated marginal means for each time point with time as fixed effect and subject as random effect is presented in **Table 4.12**.

Table 4.12 Estimated marginal means and their respective 95% confidence levels across four assessments evaluating the 1-STS over 1 yr. Numbers in bold represent significant improvements compared to the 1st assessment (P<.05).

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*
1-STS 1 yr (rep)	20.3 (18.0-22.6)	**22.8 (20.5-25.2)**	**22.7 (20.3-25.0)**	21.7 (19.4-24.1)

4.5.4.1.4 GS

Bilateral GS was evaluated on 4 different occasions over the course of 1 yr. The effect and significance of adding covariates in the models was evaluated beforehand and analyses revealed that disease duration significantly predicted left GS (L-GS) measurements over time (*b*=-0.056, *t*(25.007)=-2.093, P=.047). Therefore, the covariate was added to the model, which significantly improved over the former one ($\chi^2(1)=4.359$, P=.037). Disease duration did not significantly predict right GS (R-GS) and it was not included in its model (F(1,24.977)=3.025, P=.094). The results from both linear mixed-effect models showed that bilateral grip strength did not significantly change over time and, therefore, function was maintained (L-GS: F(3,77.028)=0.154, P=.927, η^2_p=0.006; R-GS: F(3,76.988)=0.574, P=.634, η^2_p=0.020).

An overview of the estimated marginal means for each time point with time as fixed effect and subject as random effect is presented in **Table 4.13**.

Table 4.13 Estimated marginal means and their respective 95% confidence levels across four assessments evaluating the bilateral GS over 1 yr.

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*
L-GS 1 yr (kg)	32.8 (29.0-36.6)	33.3 (29.5-37.1)	32.8 (29.0-36.6)	33.0 (29.2-36.8)
R-GS 1 yr (kg)	31.1 (27.9-34.4)	31.5 (28.2-34.8)	30.9 (27.6-34.2)	30.6 (27.3-33.8)

4.5.4.2 MM-EX 2 yr analyses

A total of 20 participants completed the MM programme for 2 yr and were included in the analyses. The reasons for participant loss to follow-up are presented in the participants' flow chart (see **Figure 4.2**).

4.5.4.2.1 6MWT

The effect and significance of adding covariates was evaluated. Analyses revealed that controlling for H&Y scale stage significantly improved the model ($\chi^2(3)=8.005$, P=.046). Although the main effect of H&Y was not significant (F(3,16.012)=2.625, P=.086), interestingly, scoring 3 at the H&Y scale, significantly predicted 6MWT scores over time (*b*=-79.957, *t*(16.034)=-2.629, P=.018). Thus, compared to those participants with a H&Y score of 1, participants with H&Y scale 3 presented lower 6MWD over time. However, these results should be interpreted with caution. Out of the 20 participants that completed 2 yr of MM-EX, 8 participants were at H&Y scale 1, 8 at H&Y scale 3, only 3 participants scored 2 at the H&Y scale and 1 participant presented a H&Y scale of 4. Having insufficient participants within the levels of the H&Y scale variable increases the standard error (SE_b, see **Table 4.14**). Additionally, the small sample size limits the possibility of exploring further interactions. Therefore, caution must be applied when interpreting the significance of the estimates. It is known that larger degrees of covariate imbalance produce less efficient estimates of the effect of MM exercise over time, and, consequently, lower power levels (Moerbeek & Van Schie, 2016). Presenting balanced levels of participants across the H&Y scale was not a recruitment requirement for the MM-EX group. Taking this information into consideration, no covariates were included in this model and results followed a similar pattern. Results of the linear mixed-effect model examining the effect of MM exercise on the 6MWT showed that there were not significant differences across time points in walking capacity over 2 yr; F(6,112.050)=1.500, P=.185, η^2_p=0.07. Therefore, resulting in a maintenance of walking capacity.

Table 4.14 Parameter information for the levels of the covariate H&Y scale stage evaluating walking capacity changes measured as the distance covered during the 6MWT over time (measured in meters).

	b	**SE_b**	**95% *CI***	***P***
(Intercept)	453.999	22.599	412.424, 495.574	**<.001**
H&Y 2	-8.403	41.164	-84.173, 67.368	.841
H&Y 3	-79.957	30.410	-135.931, -23.982	**.018**
H&Y 4	-2.117	64.480	-120.805, 116.572	.974

An overview of the estimated marginal means for each time point with time as fixed effect and subject as random effect is presented in **Table 4.15**.

Table 4.15 Estimated marginal means and their respective 95% confidence levels across seven assessments evaluating the 6MWT over 2 yr.

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*
6MWT 2 yr (m)	421 (386-455)	412 (378-447)	436 (402-471)	418 (383-452)	415 (381-450)	426 (392-460)	408 (374-442)

4.5.4.2.2 TUG

The effect and significance of adding covariates was evaluated and revealed that H&Y significantly predicted TUG measures over time (F(3,16.018)= 3.626, P=.036). Additionally, controlling for H&Y scale stage significantly improved the model (χ^2(3)= 10.377, P=. 016). Similar to the above **section 4.5.4.2.1 6MWT**, participants with H&Y scale 3 presented higher TUG measures over time compared to those participants with a H&Y score of 1 (*b*=1.300, *t*(16.048)=3.213, P=.005). However, these results should be also interpreted with caution due to the small sample size and unbalanced levels of the covariate H&Y, which increases the standard error, lessens the efficiency of the estimates and can lower power levels (Moerbeek & Van Schie, 2016). Therefore, for the final model, covariates were not included, and a similar pattern of results were obtained. That is, the linear mixed-effect model shows significant improvements in the TUG (i.e., reduction in the time to complete the test) compared to baseline that were maintained after 2 yr of engaging with the MM exercise (results presented in **Table 4.16**). An overview of the estimated marginal means for each time point with time as fixed effect and subject as random effect is presented in **Table 4.17**.

Table 4.16 Results of linear mixed-effects model evaluating mobility over 2 yr with the TUG measured as the time that takes to stand up from a chair, walk 3 m, turn, walk back and sit down (measured in seconds).

Variable	***b***	**SE*b***	**95% *CI***	***P***
TUG at 1st assessment (Intercept)	9.093	1.051	8.247, 10.025	**<.001**
TUG at 2nd assessment	-1.122	1.038	-1.205, -1.044	**.002**
TUG at 3rd assessment	-1.088	1.037	-1.167, -1.013	**.024**
TUG at 4th assessment	-1.064	1.038	-1.143, 1.009	.097
TUG at 5th assessment	-1.087	1.037	-1.167, -1.013	**.024**
TUG at 6th assessment	-1.074	1.037	-1.153, -1.001	.054
TUG at 7th assessment	-1.082	1.037	-1.161, -1.008	**.034**

Table 4.17 Estimated marginal means and their respective 95% confidence levels across seven assessments evaluating the TUG over 2 yr. Numbers in bold represent significant improvements compared to the 1st assessment (P<.05).

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*
TUG 2 yr (sec)	9.1 (8.2-10.1)	**8.1 (7.3-9.0)**	**8.4 (7.6-9.2)**	8.6 (7.7-9.5)	**8.4 (7.6-9.2)**	8.5 (7.7-9.4)	**8.4 (7.6-9.3)**

4.5.4.2.3 1-STS

None of the evaluated covariates significantly predicted the outcome variable, therefore, they were not added to the final model.

Results of the linear mixed-effect model examining the effect of MM exercise on the 1-STS test show significant improvements at the 2nd and 3rd assessments in functional lower extremity strength compared to baseline (see **Table 4.18**). Additionally, functional lower extremity strength was maintained after 2 yr, without any significant decreases (see **Table 4.19**).

Table 4.18 Results of linear mixed-effects model evaluating functional lower extremity strength changes over 2 yr measured with the 1-STS over time (total number of repetitions measured).

Variable	***b***	**SE*b***	**95% *CI***	***P***
1-STS at 1st assessment (Intercept)	20.400	1.268	17.910, 22.890	**<.001**
1-STS at 2nd assessment	2.917	1.017	0.960, 4.874	**.005**
1-STS at 3rd assessment	2.050	1.002	0.122, 3.978	**.043**
1-STS at 4th assessment	1.757	1.017	-0.200, 3.715	.087
1-STS at 5th assessment	1.150	1.002	-0.778, 3.078	.254
1-STS at 6th assessment	0.800	1.002	-1.128, 2.728	.426
1-STS at 7th assessment	1.725	1.034	-0.265, 3.714	.098

Table 4.19 Estimated marginal means and their respective 95% confidence levels across seven assessments evaluating the 1-STS over 2 yr. Numbers in bold represent significant improvements compared to the 1st assessment (P<.05).

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*
1-STS 2 yr (rep)	20.4 (17.8-23.0)	**23.3 (20.7-25.9)**	**22.4 (19.9-25.0)**	22.2 (19.6-24.8)	21.6 (19.0-24.1)	21.2 (18.6-23.8)	22.1 (19.5-24.7)

4.5.4.2.4 Bilateral GS

None of the evaluated covariates significantly predicted the outcome variable, therefore, they were not added to the final model evaluating left and right grip strength measures over 2 yr.

Results of the linear mixed-effect model examining the effect of MM exercise on grip strength show that both L and R grip strength did not significantly change over time ($F(6,113.010)=1.024$, $P=.414$, $\eta^2_p=0.050$, and $F(6,113)=1.546$, $P=.170$, $\eta^2_p=0.080$, respectively). Therefore, bilateral grip strength was maintained over two years without any significant decreases (see **Table 4.20**).

Table 4.20 Estimated marginal means and their respective 95% confidence levels across seven assessments evaluating the bilateral GS over 2 yr.

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*
L-GS 2 yr (kg)	33.0 (28.9-37.9)	32.3 (28.2-36.4)	31.6 (27.5-35.6)	32.4 (28.4-36.5)	32.3 (28.1-36.3)	33.0 (29.0-37.1)	33.6 (29.5-37.7)
R-GS 2 yr (kg)	32.0 (28.5-35.4)	31.4 (28.0-32.9)	30.8 (27.4-34.3)	30.3 (26.9-33.8)	32.3 (28.9-35.8)	31.8 (28.3-35.2)	30.4 (27.0-33.9)

4.5.4.3 MM-EX 3 yr analyses

A total of 15 participants completed the MM programme for 2 yr and were included in the analyses. The reasons to loss to follow-up are presented in the participants' flow chart (see **Figure 4.2**).

4.5.4.3.1 6MWT

None of the evaluated covariates significantly predicted the outcome variable nor significantly improved the model fit. Therefore, covariates were not added to the final model. Results of the linear mixed-effect model examining the effect of MM exercise on the 6MWT showed that there were not significant differences across time points in walking capacity over 3 yr; $F(9,124.010)=1.009$, $P=.437$, $\eta^2_p=0.07$. Therefore, resulting in a maintenance of walking capacity (see **Table 4.23**).

4.5.4.3.2 TUG

The covariate H&Y significantly predicted the response variable ($F(2,12.004)=4.711$, $P=.031$) and controlling for H&Y scale stage significantly improved the model ($\chi^2(2)=8.693$, $P=.013$). More specifically, in comparison with those with H&Y scale 1, participants presenting H&Y scale 3 were approximately 1.4 sec slower completing the TUG task across all time points (b=1.392,

t(12.006)=3.027, P=.011). Results of the linear mixed-effect model without including the covariate showed a similar pattern, that is, after 3 yr of engaging with MM exercise, TUG measures significantly improved in all time points compared to baseline (see **Table 4.21**); overall main effect of time: F(9,125.010)=2.092, P=.035, η^2_p=0.130. Therefore, resulting in a significant improvement of mobility across the different assessments completed for up to 3 yr (see **Table 4.23**).

Table 4.21 Results of linear mixed-effects model evaluating mobility over 3 yr. With the TUG measured as the time that takes to stand up from a chair, walk 3 m, turn, walk back and sit down (measured in seconds).

Variable	***b***	**SE*b***	**95% *CI***	***P***
TUG at 1st assessment (Intercept)	9.150	1.068	8.035, 10.420	**<.001**
TUG at 2nd assessment	-1.132	1.042	-1.224, -1.047	**.003**
TUG at 3rd assessment	-1.122	1.041	-1.211, -1.039	**.005**
TUG at 4th assessment	-1.085	1.041	-1.172, 1.005	**.044**
TUG at 5th assessment	-1.126	1.041	-1.215, -1.042	**.004**
TUG at 6th assessment	-1.108	1.041	-1.197, -1.026	**.012**
TUG at 7th assessment	-1.135	1.041	-1.226, -1.051	**.002**
TUG at 8th assessment	-1.169	1.041	-1.262, -1.083	**<.001**
TUG at 9th assessment	-1.111	1.041	-1.200, -1.029	**.010**
TUG at 10th assessment	-1.106	1.041	-1.194, -1.024	**.014**

4.5.4.3.3 1-STS

The covariate LEDD significantly predicted 1-STS measures over time (F(1,13.006)=5.453, P=.036) and controlling for LEDD significantly improved the model (χ^2(1)=5.254, P=.022). Participants with higher LEDD presented lower 1-STS measures over time compared to those participants taking less medication (*b*=-0.005, *t*(13.006)=-2.335, P=.036). Whilst accounting for LEDD, the linear mixed-effect model shows significant improvements in the 1-STS compared to baseline that were observed and maintained after 3 yr of engaging with the MM exercise (results presented in **Table 4.22**). An overview of the estimated marginal means for each time point with time as fixed effects and subject as random effect is presented in **Table 4.23**.

Table 4.22 Results of linear mixed-effects model evaluating mobility over 3 yr with the 1-STS measured as the amount of sit to stand repetitions that participants can complete in 1 min (total number of repetitions measured).

Variable	***b***	**SE_b**	**95% *CI***	***P***
1-STS at 1st assessment (Intercept)	24.004	1.827	20.482, 27.526	**<.001**
1-STS at 2nd assessment	2.667	1.023	0.718, 4.617	**.010**
1-STS at 3rd assessment	3.200	1.003	1.288, 5.112	**.002**
1-STS at 4th assessment	1.600	1.003	-0.312, 3.512	.113
1-STS at 5th assessment	2.200	1.003	0.288, 4.112	**.030**
1-STS at 6th assessment	0.200	1.003	-1.712, 2.112	.843
1-STS at 7th assessment	1.600	1.003	-0.312, 3.512	.113
1-STS at 8th assessment	1.867	1.003	-0.046, 3.779	.065
1-STS at 9th assessment	1.000	1.003	-0.912, 2.912	.321
1-STS at 10th assessment	1.200	1.003	-0.712, 3.112	.234
LEDD	-0.005	0.002	-0.009, -0.001	**.036**

4.5.4.3.4 Bilateral GS

The effect and significance of adding covariates was evaluated. Analyses revealed that controlling for H&Y scale stage significantly improved the models for L-GS ($\chi^2(2)=6.342$, P=.042) and R-GS ($\chi^2(2)=8.071$, P=.018). Although the main effect of H&Y was only significant for R-GS (F(2,11.990)=4.275, P=.040) and not significant for L-GS (F(2,12.003)=3.157, P=.079), interestingly, scoring 3 at the H&Y scale, significantly predicted L-GS (*b*=-8.854, *t*(12.005)=-2.511, P=.027) and R-GS (*b*=-8.199, *t*(11.992)=-2.831, P=.015) scores over time. Thus, compared to those participants with a H&Y score of 1, participants with H&Y scale 3 presented lower bilateral GS over time. However, these results should be interpreted with caution as described in the above sections. In order to keep consistency within sections and analyses performed, the covariate H&Y was not included in the final models for GS. Nonetheless, the models presented the same pattern of results even without accounting for the covariate. The linear mixed-effect model for L-GS show significant improvements in L-GS at the 8th assessment compared to baseline (*b*=3.044, *t*(125.003)=2.554, P=.012), and overall L-GS function was maintained after 3 yr of engaging with the MM exercise (see **Table 4.23** for an overview of the estimated marginal means for all measurements). A similar but not significant trend was observed at the 8th time point for R-GS (*b*=1.878, *t*(124.988)=1.666, P=.098), whilst overall R-GS function was also maintained throughout the 3 yr.

Table 4.23 Estimated marginal means for each physical function measurement throughout the assessments and its respective 95% confidence levels. Numbers in bold represent significant improvements compared to the 1st assessment (P<.05).

Physical Outcome Measure	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*	*8th*	*9th*	*10th*
6MWT 1 yr (m)	425 (399-451)	426 (400-452)	**447 (421-473)**	432 (406-458)						
6MWT 2 yr (m)	421 (386-455)	412 (378-447)	436 (402-471)	418 (383-452)	415 (381-450)	426 (392-460)	408 (374-442)			
6MWT 3 yr (m)	423 (378-468)	415 (370-460)	437 (392-482)	428 (384-473)	424 (379-469)	434 (389-479)	416 (371-461)	415 (370-460)	421 (376-466)	418 (373-463)
TUG 1 yr (sec)	8.9 (8.1-9.1)	**8.1 (7.4-8.8)**	**8.2 (7.5-9.0)**	8.4 (7.7-9.2)						
TUG 2 yr (sec)	9.1 (8.2-10.1)	**8.1 (7.3-9.0)**	**8.4 (7.6-9.2)**	8.6 (7.7-9.5)	**8.4 (7.6-9.2)**	8.5 (7.7-9.4)	**8.4 (7.6-9.3)**			
TUG 3 yr (sec)	9.1 (8.0-10.5)	**8.1 (7.0-9.3)**	**8.1 (7.1-9.4)**	**8.4 (7.4-9.7)**	**8.1 (7.1-9.3)**	**8.3 (7.2-9.5)**	**8.1 (7.0-9.2)**	**7.8 (6.8-9.0)**	**8.2 (7.2-9.4)**	**8.3 (7.2-9.5)**
1-STS 1 yr (rep)	20.3 (18.0-22.6)	**22.8 (20.5-25.2)**	**22.7 (20.3-25.0)**	21.7 (19.4-24.1)						
1-STS 2 yr (rep)	20.4 (17.8-23.0)	**23.3 (20.7-25.9)**	**22.4 (19.9-25.0)**	22.2 (19.6-24.8)	21.6 (19.0-24.1)	21.2 (18.6-23.8)	22.1 (19.5-24.7)			
1-STS 3 yr (rep)	20.9 (18.3-23.4)	**23.5 (20.9-26.1)**	**24.1 (21.5-26.6)**	22.5 (19.9-25.0)	**23.1 (20.5-25.6)**	21.1 (18.5-23.6)	22.5 (19.9-25.0)	22.7 (20.2-25.3)	21.9 (19.3-24.4)	22.1 (19.5-24.6)
L-GS 1 yr (kg)	32.8 (29.0-36.6)	33.3 (29.5-37.1)	32.8 (29.0-36.6)	33.0 (29.2-36.8)						
L-GS 2 yr (kg)	33.0 (28.9-37.9)	32.3 (28.2-36.4)	31.6 (27.5-35.6)	32.4 (28.4-36.5)	32.3 (28.1-36.3)	33.0 (29.0-37.1)	33.6 (29.5-37.7)			
L-GS 3 yr (kg)	35.7 (31.6-39.8)	34.9 (30.9-39.0)	34.0 (29.9-38.1)	35.0 (31.0-39.1)	35.1 (31.0-39.1)	35.7 (31.7-39.8)	36.8 (32.7-40.8)	**38.8 (34.7-42.8)**	36.5 (32.4-40.5)	34.8 (30.7-38.8)
R-GS 1 yr (kg)	31.1 (27.9-34.4)	31.5 (28.2-34.8)	30.9 (27.6-34.2)	30.6 (27.3-33.8)						
R-GS 2 yr (kg)	32.0 (28.5-35.4)	31.4 (28.0-32.9)	30.8 (27.4-34.3)	30.3 (26.9-33.8)	32.3 (28.9-35.8)	31.8 (28.3-35.2)	30.4 (27.0-33.9)			
R-GS 3 yr (kg)	33.7 (30.1-37.3)	32.2 (28.6-35.8)	32.2 (28.6-35.8)	32.0 (28.4-35.6)	33.5 (30.0-37.1)	33.1 (29.5-36.7)	32.5 (29.0-36.1)	35.6 (32.0-39.1)	33.7 (30.1-37.3)	32.1 (28.6-35.7)

4.5.4.4 Group comparisons

A total of 64 participants were included in the analyses (30 participants in the MM-EX group, 16 participants in the na-PD group and 18 participants in the HOA group). For the mixed-effects model analysis, HOA's group was used as a reference variable to make the group comparisons against. All results are presented in **Table 4.24**.

4.5.4.4.1 6MWT

In the mixed-effect model, the inclusion of age as a covariate significantly predicted the outcome measure 6MWD ($F(1,59)=22.088$, $P<.001$; b=-6.063, $t(59)=-4.700$, $P<.001$) and significantly improved the model ($\chi^2(1)=20.034$, $P<.001$). However, the assumption of independence between the covariate and group effect was not met. Therefore, adding age into the model could obscure or cause spurious group effects and compromise the interpretation of the analysis. Nonetheless, it is worth noting that the results from the model including the covariate age and the model not including age as covariate were equivalent. Therefore, age was not included into the final linear mixed-effects models for any of the physical function outcome analyses.

In this instance, the final model showed a significant main effect of time ($F(2,120)=5.315$, $P=.006$, $\eta^2_p=0.080$) and group ($F(2,60)=15.100$, $P<.001$, $\eta^2_p=0.330$), being reflected in HOA significantly scoring better than both MM-EX and na-PD in the 6MWT across all time points, and presenting a significant improvement in 6MWT after 6 months compared to baseline (i.e., at the 3rd assessment). None of the Parkinson's groups (MM-EX and na-PD) presented significant differences across time or between them, nor was there an interaction between time and group ($F(4,120)=0.797$, $P=.530$, $\eta^2_p=0.030$).

4.5.4.4.2 TUG

The linear mixed-effects model showed a significant main effect of time ($F(2,121.086)=4.627$, $P=.012$, $\eta^2_p=0.070$) and group ($F(2,61.032)=9.020$, $P<.001$, $\eta^2_p=0.230$). Although the overall effect of the interaction was not significant ($F(4,121.092)=1.889$, $P=.117$, $\eta^2_p=0.060$), the interaction coefficient for the MM-EX group at the 2nd assessment had a significant effect (b=-1.130, $t(121.128)=-2.664$, $P=.009$). Post hoc analyses showed that both the HOA and na-PD groups' TUG scores, did not significantly change over time. However, for the MM-EX group, TUG scores significantly improved at the 2nd and 3rd assessments compared to baseline after engaging with the MM programme ($P=.005$, and $P=.011$, respectively).

4.5.4.4.3 1-STS

The linear mixed-effects model shows that there was a significant main effect of time ($F(2,120.215)=6.738$, $P=.002$, $\eta^2_p=0.100$), as well as a main effect of group ($F(2,61.088)=8.512$, $P<.001$, $\eta^2_p=0.220$). Importantly, the time by group interaction was also significant ($F(4,120.209)=2.714$, $P=.033$, $\eta^2_p=0.080$); meaning that the MM-EX group significantly improved their 1-STS scores at the 2nd and 3rd assessments compared to baseline ($P<.001$, and $P<.001$, respectively), whilst the HOA group only showed significant improvements at the 3rd assessment ($P=.031$), and the na-PD group's 1-STS scores did not significantly change across time points. As expected, overall, the HOA significantly outperformed the MM-EX and na-PD groups. However, after the MM-EX groups' improvement at the 2nd assessment, the significant differences in 1-STS scores between MM-EX and HOA disappeared ($P=.101$) and HOA scores remained significantly different from the na-PD group ($P=.005$).

4.5.4.4.4 Bilateral GS

Bilateral GS analyses did not present any significant main effects of time (L-GS: $F(2,120.970)=0.303$, $P=.739$, $\eta^2_p=0.005$; R-GS: $F(2,121.016)=1.245$, $P=.292$, $\eta^2_p=0.020$), group (L-GS: $F(2,60.950)=0.545$, $P=.583$, $\eta^2_p=0.020$; R-GS: $F(2,60.998)=0.782$, $P=.462$, $\eta^2_p=0.020$), nor was there an interaction between the two (L-GS: $F(2,121.980)=0.968$, $P=.428$, $\eta^2_p=0.030$; R-GS: $F(2,121.018)=0.470$, $P=.758$, $\eta^2_p=0.020$).

Table 4.24 Mean for each groups' physical function measurement throughout the assessments, adjusted for any other variables in the models (i.e., estimated marginal means), and its respective 95% confidence levels. Numbers in bold represent significant improvements compared to: [a]1st assessment, [b]HOA group, [c]MM-EX group or [d]na-PD group ($P<.05$).

Measure	*Group*	*1st Assessment (Baseline)*	*2nd Assessment*	*3rd Assessment*
6MWT (m)	**HOA**	**560 (516-604)[c,d]**	**569 (525-613)[c,d]**	**589 (545-633)[a,c,d]**
	MM-EX	430 (396-465)	428 (393-463)	443 (408-478)
	na-PD	427 (380-474)	437 (390-483)	438 (392-485)
TUG (sec)	**HOA**	**6.6 (5.8-7.4) [c,d]**	**6.8 (6.0-7.7) [c,d]**	**6.4 (5.6-7.2) [c,d]**
	MM-EX	9.1 (8.2-10.0)	**8.3 (7.5-9.1)[a]**	**8.3 (7.6-9.2)[a]**
	na-PD	9.5 (8.3-10.8)	9.3 (8.1-10.6)	8.9 (7.8-10.1)
1-STS (rep)	**HOA**	**27.3 (24.2-30.5)[c,d]**	**27.3 (24.2-30.5)[d]**	**29.8 (26.7-33.0)[c,d]**
	MM-EX	20.1 (17.6-22.5)	**23.0 (20.5-25.4)[a]**	**22.8 (20.3-25.2)[a]**
	na-PD	19.5 (16.1-22.9)	19.8 (16.4-23.1)	20.1 (16.7-23.5)
L-GS (kg)	**HOA**	31.2 (26.9-35.5)	30.9 (26.6-35.2)	32.0 (27.7-36.3)
	MM-EX	31.2 (28.4-35.1)	30.8 (27.4-34.2)	30.8 (27.4-34.2)
	na-PD	28.2 (23.6-32.8)	29.0 (24.4-33.6)	28.5 (23.9-33.1)
R-GS (kg)	**HOA**	34.6 (29.8-39.3)	36.0 (31.3-40.8)	35.6 (30.9-40.3)
	MM-EX	32.8 (29.2-36.5)	33.4 (29.7-37.0)	32.9 (29.2-36.6)
	na-PD	31.3 (26.2-36.3)	31.4 (26.4-36.4)	30.9 (25.9-36.0)

4.5.5 Cognitive and Mood Outcomes

A total of 43 participants were included in the analyses (9 participants in the MM-EX group, 16 participants in the na-PD group and 18 participants in the HOA group). MM-EX group presents a lower sample size due to the particularities described in **section 4.4.6 Sample size and Statistical Analysis**. For the mixed-effects model analysis, HOA's group was used as a reference variable to make the group comparisons against.

In addition to the group comparisons presented below (see **section 4.5.5.1**), a subgroup of MM-EX participants that engaged with the MM class for up to 3 years were also monitored. As mentioned in **section 4.4.2,** cognitive measures were added at a later stage of this research, therefore, this subgroup did not hold a true baseline and their results were not included in the analyses presented below in **section 4.5.5.1**. However, their cognitive function was also assessed on a regular basis and this periodic evaluation presented important results. Their data was analysed using linear mixed-models and participants that joined the MM exercise class for at least 1 year were included. After this period of 1 year, the first measurement for the tests presented below was obtained. These participants were divided in two subgroups: 15 participants completed assessments during 1 year (a total of 4 assessments) and 11 participants completed the assessments for 2 years (a total of 7 assessments).

First, these participants showed an initial significant improvement in the MMP scores that was maintained throughout all timepoints for up to 1 year ($P<.001$). Results behaved in a similar fashion for those participants that completed the cognitive tests for up to two years, where MMP scores at all timepoints were better than the first assessment ($P<.012$).

TMT-A scores decreased over time but that improvement in the results did not reach significant levels for those that completed the assessment for 1 year. Nevertheless, participants that completed the assessments for up to two years, showed a significant improvement in their results 1 year after the first assessment was completed ($P=.033$). In regard to TMT-B, scores did not significantly change for those that completed the assessments for up to 1 year. Therefore, function was maintained. Participants that completed the assessments for up to 2 years, showed significant improvements at their 3rd and 5th assessments compared to baseline ($P=.024$ and .045, respectively).

CDT scores remained constant for both subgroups and, interestingly, those participants that presented lower scores were later clinically diagnosed with dementia (n=4).

OPQOL-Brief questionnaire scores did not present any significant changes across timepoints for any of the subgroups.

The regular completion of BRUMS questionnaire revealed no significant changes in anger, confusion, depression, after 1 and 2 years of completing the assessments. Participants that completed

the assessments for up to 2 years felt significantly less fatigued, in general terms, after 1 year compared to baseline (P=.018) and their perception of tension was also reduced in the 2nd and 7th assessments (P=.011 and .033, respectively). Their vigour showed a significant improvement at the 5th assessment (P=.024).

4.5.5.1 Cognitive Function (MMP)

4.5.5.1.1 Group comparison

The main effect of time yielded an F ratio of F(2,80)=2.771, P=.069, η^2_p=0.060, showing a trend towards the predicted direction but, overall, indicating that the MMP change score did not significantly change over time. The main effect of group (F(2,40)=1.855, P=.170, η^2_p=0.080) and the interaction (F(4,80)=1.160, P=.335, η^2_p=0.050) were not significant. These results could be a consequence of the small sample size of the MM-EX group, since, when MM-EX results were further evaluated with pairwise comparisons, a significant improvement in MMP scores at the 3rd assessment was observed for the MM-EX group only (P=.037, see **Figure 4.3**).

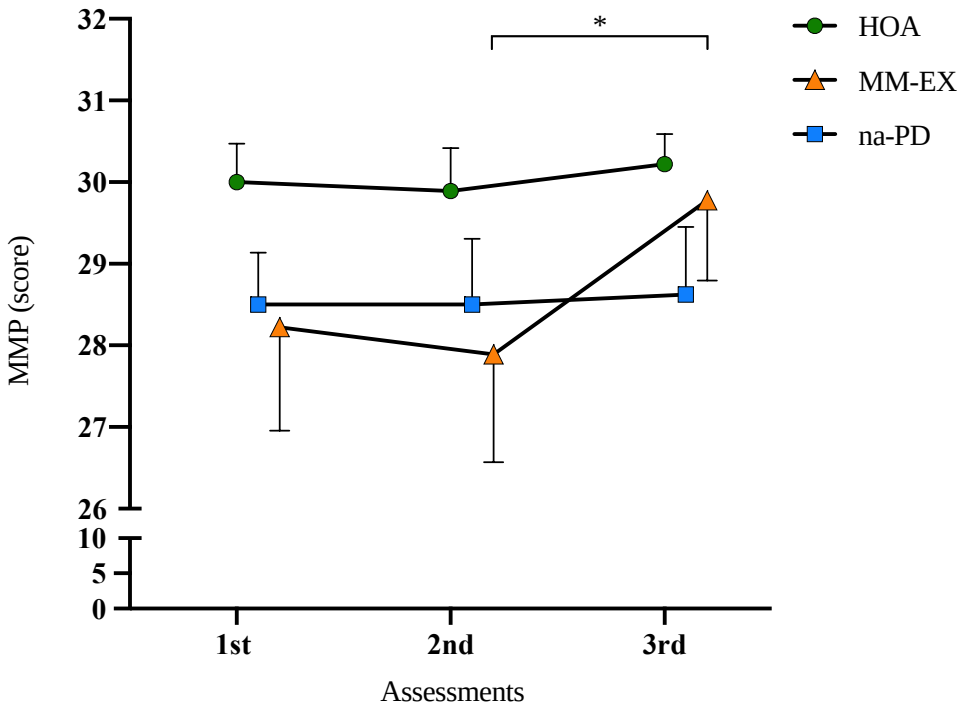

Figure 4.3 The Mini-Mental Parkinson's (MMP) is a cognitive with a maximum score of 32. Data are presented as mean and standard error (SE) bars (SE used for clarity of figures). *Significant difference between time points for the MM-EX group (P=.037).

4.5.5.1.2 Correlations

Most of the results reported for the MM-EX, showed improvements at the 3rd assessment. The existence of any relationship between the cognitive function scores measured with the MMP test, and physical function was evaluated. Analyses showed that, at baseline (1st assessment) there was a significant correlation between MMP scores and both 6MWT and TUG measures (r(7)=.789, P=.012, and r(7)=-.929, P<.001, respectively). Interestingly, the strength of the relationship was improved after engaging with the MM-EX for more than half a year (3rd assessment) (r(6)=.918, P=.001, and r(6)=-.933, P=.001).

However, the analyses investigating the relationship between the change in scores (between the 1st and 3rd assessments) of the MMP and the physical outcome measures, showed that there were not any significant correlations.

4.5.5.2 TMT-A and TMT-B

The linear mixed-effects model showed that the interaction between group and time was not significant, although post-hoc comparisons showed that both MM-EX and na-PD groups presented worse TMT results compared to HOA in both parts of the test, predominantly in the TMT-B (see **Table 4.25**). Part A is a measure of rote memory and part B is a measure of executive function. It is then important to mention that 1 participant from the MM-EX group and 1 participant from the na-PD were unable to complete TMT-B on one occasion, and 1 participant from the MM-EX group could not complete the TMT-B on any of the assessments. Therefore, this last participant could not be included in the analyses.

Table 4.25 Overall cognitive function, attention, visual screening ability and processing speed, was measured with the TMT-A and TMT-B. The score on each part of the TMT (A or B) represents the amount of time required to complete the task. Data are presented as estimated marginal means and its respective 95% confidence levels. [a]Significant differences between HOA and na-PD. [b]Significant differences between HOA and MM-EX. [c]Significant differences between MM-EX and na-PD. *Significant differences compared to baseline.

Parts of the TMT	***Group***	***1st assessment (Baseline)***	***2nd assessment***	**3rd assessment**
TMT-A (sec)	HOA	24.3 (18.1-30.5)	23.2 (16.9-29.4)	23.1 (16.9-29.3)
	MM-EX	**39.7 (30.3-49.2)[b]**	33.8 (24.5-43.2)	34.9 (25.6-44.2)
	na-PD	33.1 (26.5-39.7)	**34.5 (27.9-41.1)[a]**	31.8 (25.2-38.4)
TMT-B (sec)	HOA	46.3 (23.2-69.4)	43.5 (20.4-66.5)	42.8 (19.7-65.8)
	MM-EX	79.4 (43.8-114.9)	71.7 (37.1-106.3)	**96.3 (61.7-130.9)[b]**
	na-PD	70.6 (46.0-95.2)	69.5 (45.0-93.9)	75.3 (50.8-99.7)

4.5.5.3 CDT

Due to the nature of the methodology followed for this test, descriptive statistics and a qualitative assessment of the results were used to analyse this data. All the participants in the HOA group obtained the higher scores possible (i.e., a score of 4). Most of the participants from the groups MM-EX and na-PD obtained the maximum score. However, it was observed that those who obtained lower results, were the same participants with higher (i.e., worse) scores in the TMT (see **Table 4.26**). As a note of interest, in the MM-EX group, those who obtained low results in the CDT were later diagnosed with dementia (n=4).

Table 4.26 The CDT was used as a measure of spatial dysfunction and neglect, as well as a screening method for detecting MCI. Data are presented as estimated marginal means ± SE.

	Group	***1st assessment (Baseline)***	***2nd assessment***	**3rd assessment**
CDT (score)	HOA	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	MM-EX	3.89 ± 0.11	3.78 ± 0.11	3.56 ± 0.11
	na-PD	3.88 ± 0.08	4.00 ± 0.00	3.88 ± 0.08

4.5.5.4 Quality of Life

A linear mixed-effect model examined the scores of the OPQOL-Brief questionnaire. The model showed a significant time by group interaction ($F(4,80)=3.134$, $P=.019$); meaning that the na-PD group significantly improved their QoL scores at the 2nd assessment compared to baseline ($P=.017$), whilst the HOA and MM-EX groups QoL scores did not significantly change across time points and remained constant. No significant differences were observed between groups (see **Table 4.27**).

A preliminary single item on global QoL not included in the total score was also evaluated. Participants were asked to answer the following question: "*Thinking about both the good and bad things that make up your quality of life, how would you rate the quality of your life as a whole?*", which was coded from "very bad" (=1) to "very good" (=5). No significant interaction or differences between groups and across time points were observed, and participants from all three groups' average response was either "good" (=4) or "very good" (=5) (see **Table 4.28**).

Table 4.27 The OPQOL-Brief questionnaire was used as a measure of QoL in an older population. Data are presented as estimated marginal means and its respective 95% confidence levels. *Significant differences compared to baseline.

	Group	***1st assessment (Baseline)***	***2nd assessment***	**3rd assessment**
OPQOL-Short (score)	HOA	59.9 (57.5-62.3)	58.6 (56.2-61.0)	58.9 (56.5-61.4)
	MM-EX	57.4 (54.0-60.9)	54.9 (51.4-58.3)	55.6 (52.1-59.0)
	na-PD	56.7 (54.1-59.3)	**60.1 (57.5-62.7)***	59.2 (56.6-61.8)

Table 4.28 Single item on global QoL coded as "very bad" (=1), "bad" (=2), "alright" (=3), "good" (=4) and "very good" (=5). Data are presented as estimated marginal means ± SE levels.

	Group	***1st assessment (Baseline)***	***2nd assessment***	**3rd assessment**
Global QoL (score)	HOA	4.72 ± 0.15	4.48 ± 0.15	4.56 ± 0.15
	MM-EX	4.22 ± 0.21	4.00 ± 0.21	4.11 ± 0.21
	na-PD	4.25 ± 0.16	4.25 ± 0.16	4.38 ± 0.16

4.5.5.5 Mood

None of the linear mixed-effects models showed a significant interaction between group and time (baseline, 2nd and 3rd assessments) for any subscale of the affective states included in the BRUMS questionnaire. The only trend observed, present in the subscale "confusion", showed a significant pairwise comparison at the 2nd assessment which informed that na-PD had significantly higher levels of confusion compared to HOA (P=.008). All results are presented in **Table 4.29** below.

Table 4.29 Mood measurements using the BRUMS questionnaire. Identifiable affective states, with a maximum score of 16, were evaluated throughout 3 assessments and compared between 3 groups. Data are presented as estimated marginal means ± SE. [a]Significant differences between HOA and na-PD. [b]Significant differences between HOA and MM-EX. [c]Significant differences between MM-EX and na-PD. *Significant differences compared to baseline.

Subscale	***Group***	***1st assessment (Baseline)***	***2nd assessment***	**3rd assessment**
Anger	HOA	3.11 ± 0.69	2.94 ± 0.69	3.44 ± 0.69
	MM-EX	2.22 ± 0.98	1.67 ± 0.98	2.22 ± 0.98
	na-PD	2.12 ± 0.73	2.31 ± 0.73	2.75 ± 0.73
Confusion	HOA	1.00 ± 0.50	1.00 ± 0.50	2.11 ± 0.50
	MM-EX	2.56 ± 0.71	1.89 ± 0.71	2.00 ± 0.71
	na-PD	2.69 ± 0.53	**3.25 ± 0.53[a]**	2.56 ± 0.53
Depression	HOA	1.22 ± 0.67	2.22 ± 0.67	2.28 ± 0.67
	MM-EX	2.56 ± 0.94	2.22 ± 0.94	1.56 ± 0.94
	na-PD	2.12 ± 0.71	2.06 ± 0.71	2.06 ± 0.71
Fatigue	HOA	5.78 ± 0.87	5.83 ± 0.87	5.83 ± 0.83
	MM-EX	4.11 ± 1.23	5.67 ± 1.23	4.89 ± 1.23
	na-PD	7.38 ± 0.92	6.62 ± 0.92	6.38 ± 0.92
Tension	HOA	1.83 ± 0.66	3.06 ± 0.66	3.11 ± 0.66
	MM-EX	4.00 ± 0.93	3.67 ± 0.93	2.56 ± 0.93
	na-PD	3.31 ± 0.70	3.19 ± 0.70	2.81 ± 0.70
Vigour	HOA	11.17 ± 0.78	10.44 ± 0.78	11.06 ± 0.78
	MM-EX	8.67 ± 1.11	8.78 ± 1.11	7.89 ± 1.11
	na-PD	9.50 ± 0.83	8.75 ± 0.83	9.50 ± 0.83

4.5.6 Samples Analyses

4.5.6.1 Saliva BDNF

It is crucial to assess the efficiency of the ELISA in detecting all of the analyte present (i.e., BDNF). Before running final sample analyses, optimisation and spike recovery trials were performed in order to investigate which reagent diluent was the most appropriate to dilute saliva samples and run the assay. As explained in **Chapter 3** (**section 3.4.1**), saliva samples did not provide acceptable recovery results. Therefore, it was decided to add a spike recovery test for each sample being analysed and only samples with recovery values between 80% and 120% were included.

Only 11, out of a total of 53 samples that were analysed across 2 years, presented acceptable recovery results (see **Table 4.30**). Thus, highlighting the importance of evaluating the accuracy of analytical methods in sample types such as saliva.

Table 4.30 Participant's saliva samples collected at different timepoints were analysed for BDNF. Additionally, a spike-recovery assessment was completed for each sample. A high number of samples presented undetectable levels of BDNF.

	Baseline	**1 year**	**2 years**
Total nº of participants	22	17	14
Nº of detectable /undetectable BDNF samples	12/10	8/9	9/5
Nº of samples at 120% ≤ recovery ≥ 80%	5	3	3
Nº of samples at 80% < recovery ≥ 70%	2	2	1
Nº of samples with non-acceptable recoveries (≤ 80% or ≥ 120%)	3	3	3
Samples without recovery data	2	-	2

Due to being a significantly small sample size, only descriptive statistics were performed. Individuals' data is presented in **Table 4.31**.

Table 4.31 Saliva BDNF values in pg/ml including samples with recovery values between 80 and 120%. Numbers in italics represent samples with a recovery value between 70 and 80%.

	Baseline	**1 year**	**2 years**
Participant 1	4.9	144.9	-
Participant 2	92.7	6.9	90.3
Participant 3	*40.2*	64.7	38.5
Participant 4	81.2	*58.5*	-
Participant 5	36.1	-	38.9
Participant 6	21.7	-	-

4.5.6.2 Blood BDNF

4.5.6.2.1 BDNF Genotyping

A total of 64 participants were included in the analyses (24 participants in the MM-EX group, 18 participants in the na-PD group and 14 participants in the HOA group). After the analyses, a total of

4 samples were deemed unknown (1, 1 and 2, from the MM-EX, na-PD and HOA groups, respectively). Therefore, 23 participants in the MM-EX group, 13 participants in the na-PD group and 16 participants in the HOA group were finally included.

The BDNF genotypes distribution did not significantly differ between groups (P=.394). However, a significant association between allele frequency and group was found ($\chi^2(2)=11.504$, P=.003). Post hoc analyses revealed that the allele distribution for HOA is significantly different than the MM-EX and na-PD allele distributions. Thus, there is a significant excess of the Val allele in both MM-EX and na-PD groups compared to HOA (P=.007, see **Table 4.32**).

Table 4.32 BDNF rs6265 genotype distributions and allele frequency. The combination of Val and Met alleles results in three different Val66Met genotypes: GG (Val/Val), GA (Val/Met) and AA (Met/Met). Chi-square and Fisher's exact tests were calculated. Values are absolute (relative frequencies in parenthesis). *Significant result (P<.05).

Group	***n***	***Genotype***			***P***	**Allele frequency**		***P***
		GG (%)	**GA (%)**	**AA (%)**		**Val (%)**	**Met (%)**	
HOA	16	7 (44)	4 (25)	5 (31)	.394	18 (56)	14 (44)	.003*
MM-EX	23	16 (69)	5 (22)	2 (9)		37 (80)	9 (20)	
na-PD	13	8 (62)	2 (15)	3 (23)		18 (69)	8 (31)	

In subsequent analyses, individuals with Val/Met or Met/Met genotypes were combined (Met carriers; $BDNF_{MET}$) and compared with individuals with the Val/Val genotype ($BDNF_{VAL}$).

The possible effect of BDNF genotype on the rate of change in the scores of the primary functional measures (6MWT, TUG and 1-STS) between the 3rd assessment and baseline across the study groups was also evaluated with a two-way ANOVA. Simple main effects analysis showed that group and BDNF genotype did not significantly affect the change in 6MWT scores between the 3rd assessment and baseline (P=.129 and .085, respectively), nor was there an interaction between group and genotype (F(2,46)=.564, P=.573). The same pattern of results were obtained for the TUG test and 1-STS results, where main effects and the interaction between group and genotype were not significant (TUG: F(2,47)=2.777, P=.072; 1-STS: F(2,47)=.982, P=.382). Although the differences observed did not reach significance, it is worth noting that $BDNF_{VAL}$ carriers in the na-PD group presented worse results over time than na-PD participants with the $BDNF_{MET}$ genotype. Interestingly, the opposite was observed in the MM-EX group. After engaging with the MM intervention, participants with the $BDNF_{VAL}$ genotype, obtained better scores in the evaluated tests than $BDNF_{MET}$ carriers. Regarding cognitive function, the change in scores in the MMP test was evaluated. The main effects of group and genotype were not significant, however, the results followed a similar distribution to the above-mentioned outcomes. That is, MM-EX participants with the $BDNF_{VAL}$ genotype improved

their MMP scores after the MM intervention, whilst $BDNF_{MET}$ carriers did not present any change in MMP scores. On the contrary, participants in the na-PD group presented the opposite results distribution – participants with the $BDNF_{MET}$ polymorphism presented better MMP scores.

4.5.6.2.2 Finger Prick BDNF

Sample analysis methodology is described in **Chapter 2**.

A total of 43 participants were included in the analyses (9 participants in the MM-EX group, 12 participants in the na-PD group and 16 participants in the HOA group). MM-EX group presents a lower sample size due to the particularities described in **section 4.4.6 Sample size and Statistical Analysis.** Two samples from the HOA and 4 samples from the na-PD group could not be analysed due to presenting volumes that were too low to run the analysis. It is worth noting that a small number of samples exceeded the standards range and the concentration was extrapolated from the standards curve (5 HOA samples, 5 MM-EX samples and 3 na-PD samples).

An ANCOVA was run to examine whether BDNF concentrations differed between the MM-EX and comparison groups after engaging with the MM exercise intervention (i.e., concentrations from the 3rd assessment), while controlling for their baseline levels (i.e., 1st assessment).

After adjustment for baseline BDNF levels, there was a statistically significant difference between the groups in BDNF levels measured in the 3rd assessment ($F(2,33)=7.899$, $P=.002$, $\eta^2_p=0.320$). That is, the mean concentration of BDNF was significantly greater in MM-EX compared to HOA and na-PD (see **Table 4.33**).

Table 4.33 Mean value for each groups' BDNF measurement at the 3rd assessment, adjusted for their baseline BDNF levels (i.e., estimated marginal means [emmean]), and its respective 95% confidence levels (CL). BDNF is measured in pg/mL.

Group	***emmean***	**SE**	**95% *CL***
HOA	1479	1.164	1096, 2042
MM-EX	3890	1.228	2570, 5888
na-PD	1549	1.191	1096, 2239

Next, finger prick BDNF values were evaluated whilst taking into account participants BDNF genotype. A total of 33 participants were included in the analyses (7 participants in the MM-EX group, 11 participants in the na-PD group and 15 participants in the HOA group). Not all participants' genotype could be analysed due to limitations in sample volume.

Due to the small sample size within the MM-EX group and the skewed nature of BDNF data, the geometric mean was used to report the BDNF results for each group separated by their BDNF genotype (see **Table 4.33**).

Non-parametric Mann-Whitney U tests showed that, at baseline (1st assessment), MM-EX, na-PD and HOA participants did not present significantly different levels of BDNF based on their BDNF genotype (all P>.05). These results' distribution was maintained at the 3rd assessments, meaning that after more than half a year, BDNF levels of participants with the $BDNF_{MET}$ genotype did not significantly differ from those of participants with the $BDNF_{VAL}$ genotype.

Moreover, non-parametric Wilcoxon Signed-Rank Tests indicated that none of the BDNF genotype subgroups ($BDNF_{MET}$ and $BDNF_{VAL}$) within each of the comparison groups (MM-EX, na-PD and HOA) presented significantly different levels of BDNF between the 1st and 3rd assessment (see **Table 4.34**). However, a trend was observed, that is, na-PD and HOA participants with the $BDNF_{VAL}$ genotype presented a decline trend in their BDNF levels (Z=-1.690, P=.091, and Z=-1.782, P=.075, respectively). As presented in **Table 4.34**, BDNF levels of na-PD and HOA participants with the $BDNF_{MET}$ genotype also presented a decline over time, however, results were not significant (Z=-.730, P=.465, and Z=-1.599, P=.110, respectively).

Table 4.34 Geometric mean (Geometric Standard Deviation) for each group's BDNF measurement (in pg/mL) at the 1st and 3rd assessments separated by BDNF genotype.

Group	***BDNF genotype***	***n***	***Baseline (1st assessment)***	***3rd assessment***
HOA	$BDNF_{MET}$	9	4601 (4)	1714 (2)
	$BDNF_{VAL}$	6	3642 (2)	1326 (2)
MM-EX	$BDNF_{MET}$	2	926 (9)	2688 (9)
	$BDNF_{VAL}$	5	2361 (3)	4574 (2)
na-PD	$BDNF_{MET}$	4	4600 (4)	1659 (3)
	$BDNF_{VAL}$	7	4489 (2)	1852 (2)

4.5.7 IPAQ Short form

IPAQ short form can be used to estimate the levels of total physical activity in MET-min/week. This measure was included at a later basis as described for the cognitive and finger prick measurements. MET-min. represent the amount of energy expended whilst carrying out physical activity. Dividing the values obtained with IPAQ Short by 60 transforms the units to MET-hours/week.

Linear mixed-effects model evaluating MM-EX group IPAQ changes in 22 participants, showed that IPAQ levels did not significantly change over time (F(2,41.311)=0.945, P=.397). Group comparisons

were also evaluated and only MM-EX that completed IPAQ at their 1st assessments were included (see **Table 4.35**). A total of 42 participants were included in the analyses (8 participants in the MM-EX group, 16 participants in the na-PD group and 18 participants in the HOA group). The mixed-effects model that evaluated IPAQ Short form changes over time and between groups showed a non-significant main effect of time ($F(2,78)=1.208$, $P=.304$) and group ($F(2,39)=2.982$, $P=.062$), as well as their interaction ($F(4,78)=1.226$, $P=.307$). Overall, the na-PD presented lower values of physical activity compared to both the HOA and MM-EX groups.

Table 4.35 The IPAQ Short form was used as a measure of total physical activity in MET-hours/week. Data are presented as estimated marginal means and its respective 95% confidence levels.

	Group	***1st assessment (Baseline)***	***2nd assessment***	**3rd assessment**
IPAQ Short form (MET-hours/week)	HOA	71 (52-90)	46 (27-65)	39 (20-57)
	MM-EX	54 (26-82)	45 (17-73)	46 (18-74)
	na-PD	28 (8-48)	29 (9-49)	33 (13-53)

4.5.8 Medication changes (LEDD)

Medication changes were recorded throughout the study and the effect of LEDD on the outcome variables was evaluated as a covariate and accounted for in all the analyses where needed. Furthermore, there were no significant changes in LEDD throughout the course of the study in any of the groups involved compared to baseline (see **Table 4.1**).

4.6 Discussion

The present study shows that a weekly, supervised, structured, community-based MM group exercise programme is feasible, safe and able to provide significant improvements in walking capacity, mobility and functional lower extremity strength, and maintain bilateral GS, after 1, 2 and 3-years. Importantly, no significant declines were observed for any of the outcomes being measured, which is particularly relevant for PwP due to the chronic progressive nature of PD.

In accordance with the present results, previous studies have demonstrated that regular structured exercise is beneficial for PwP. Different measures widely used in Parkinson's research and clinical settings were chosen to evaluate physical function in this study (Tomlinson et al., 2013). Although statistical hypothesis testing is an important approach to investigate the effects of an intervention such as MM exercise, we are aware that statistical significance does not always imply clinical importance and, on the other hand, the lack of statistical significance may not indicate the absence of clinical importance. The existing literature has provided useful Minimal Clinically Important

Difference (MCID; often also referred to as minimal detectable change [MDC], reliable change index [RCI] and minimal important difference [MID]) reference values for the functional outcomes measured in this study (Haley & Fragala-Pinkham, 2006).

In participants that engaged with MM exercise for 1-yr, the longitudinal evaluation of the MM-EX showed significant improvements in walking capacity after 8 months. This improvement resulted in participants being able to walk 22 metres further during the 6MWT, which may be considered clinically meaningful (i.e., for adults with pathology, a change of 14.0 to 30.5 metres holds practical importance) (Bohannon & Crouch, 2017). Further improvements in the 6MWT were also observed in the MM-EX compared with the na-PD (15 metres, which is within the MCID range). For the TUG, results from published studies revealed wide variations and MCIDs of 1.63 sec and 3.5 sec have been reported as approximate thresholds (Huang et al., 2011; Lim et al., 2005). Although our results did not meet these suggested MCIDs, MM-EX participants were able to significantly improve their TUG (functional mobility) even after 3-years. This observation is important, since previous research evaluating aerobic exercise and sensory attention focused exercise interventions could not show significant improvements in participants' TUG (Sage & Almeida, 2009). Moreover, compared to the MM-EX, the na-PD group TUG scores were slower (i.e., worse) and did not significantly change from baseline. Regarding 1-STS, further studies are required to establish the MCID for the PwP. Published results for the 30 sec STS test suggest that improvements of more than 3 repetitions are needed but caution must be applied should this information be extrapolated to the 1-STS (Petersen et al., 2017). In our study, the MM-EX group was able to significantly improve their lower extremity strength across 1, 2 and 3-years of MM exercise. When comparing their results to na-PD, the MM-EX were able to significantly improve their number of repetitions by 3, whilst the na-PD scores did not change after 6 months. For GS measurements, very small MCID have been reported in PwP (Villafañe et al., 2016). However, a recent review of articles that incorporated adults with different pathologies or disorders suggests that changes of 5.0 to 6.5 kg could be a better estimate of meaningful changes in GS (Bohannon, 2019). The GS results of this study remained consistent over time for HOA and na-PD, and the 3-year MM-EX was the only group that, after 8 assessments significantly improved their left GS, which has been suggested to improve ADLs performance and overall health (Villafañe et al., 2016). In all the other study lengths for up to 3-years function was maintained and, therefore, there were not any declines in walking capacity, functional mobility, grip or leg strength. It is worth noting that different MCID have also been suggested for PwP, some of them being substantial due to the amount of variability in the dataset (caused by a wide range of disease severity) (Steffen & Seney, 2008). If a better homogeneity of participants reduces variability, it would suggest that individual MCIDs would be required for each H&Y stage of PD. Thus, data must be interpreted cautiously and further research on MCIDs with a greater number of participants in each H&Y stage is required.

Alongside the abovementioned measures of physical function, the current study also evaluated cognition and was able to show the potential of MM exercise to improve cognitive function. That is, only the MM-EX group significantly improved their cognitive levels measured with the MMP, whilst the HOA and na-PD did not present any changes after 6 months. The cognitive improvements observed in the MM-EX group were the reflection of an improved mental imagery, visuospatial abilities and use of internal cues (abilities that were tested in the memory subsection of the MMP), orientation (temporal and spatial), attention and mental control (frontal abilities), verbal fluency and concept processing (Mahieux et al., 1995). The lack of a differential effect of group on the CDT could be limited by a ceiling effect, with most participants obtaining the maximum score for this test. To prevent any learning effects on the performance of cognitive tests due to exposure to them at baseline, a relative long duration between assessments (3 to 4 months) was set. The fact that none of the study groups significantly improved their scores at the first assessment after baseline suggests that any possible learning effect were minimised after baseline. These results add to the growing body of literature that suggests that physical activity may enhance cognitive function and provides specific evidence about the beneficial effect that MM exercise have on PwP's cognition (Cheng & Su, 2020; Petzinger et al., 2013).

The measurement of systemic concentrations of BDNF can provide valuable information about the underlying mechanisms by which exercise elicits a beneficial effect on PD. Therefore, the development of biomarkers related with brain plasticity is important to provide insight into the neurodegenerative process that takes place in PD, as well as its progression. It has been well accepted that there is a reduced expression of BDNF in PD (Parain et al., 1999). Given the protective and neuroplastic role of this neurotrophin, BDNF measurements have been proposed as a biomarker for cognitive reserve against different neurodegenerative diseases, such as PD and AD (Beeri & Sonnen, 2016; Costa et al., 2015). Although blood has been the primary fluid used for the assessment of BDNF, the non-invasive detection of BDNF in other biological fluids is of interest. It has been confirmed that neurotrophic factors, such as BDNF, can be detected in saliva, however, the degree of agreement with blood samples has not been fully investigated (Mandel et al., 2011). Thus, the efficiency of the optimised ELISA in detecting all of the analyte present (i.e., BDNF) in saliva was initially evaluated. Unfortunately, only 54.72% of the samples that were collected provided measurable BDNF results and only 20.75% of the measured samples provided acceptable recovery results, meaning that in 79.25% of the measured samples there may be some factor in the saliva matrix causing a falsely elevated/depressed BDNF value. This is an important observation. Due to the high percentage of samples presenting not acceptable recovery results, future studies intending to measure BDNF in saliva should always run a spike recovery test for each sample being analysed and only samples with recovery values between 80% and 120% should be included in their final analyses. Otherwise, the accuracy of the results may be questionable. Importantly, the interpretation of previous studies that have used BDNF measurements in saliva without evaluating the performance and accuracy of the assay may not be reliable (Vrijen, Schenk, Hartman, & Oldehinkel, 2017).

Regarding plasma BDNF, the intervention was able to increase basal levels of BDNF in response to the regular engagement with the MM class, whilst BDNF levels of HOA and na-PD decreased after 6 months. These results are in accord with previous studies that found that circulating levels of BDNF decline over time (Erickson et al., 2010). Thus, the MM intervention successfully increased the levels of the important neuroprotective factor, BDNF.

The effect of BDNF polymorphism on BDNF levels and function has been discussed in the literature. It is suggested that presenting the Met variant of Val66Met polymorphism ($BDNF_{MET}$) could be related with the prevalence of depression, anxiety and poor cognitive performance in healthy individuals, however, it's role with neurodegenerative diseases is not very clear (Egan et al., 2003; Hariri et al., 2003; Notaras et al., 2015). We observed that there were similar distributions of BDNF genotype (GG, GA, AA) across the study groups. However, when looking at the allele distribution, we observed that both MM-EX and na-PD (PD groups) presented a different distribution compared to the HOA group. That is, there was a significant excess of the Val allele in both MM-EX and na-PD groups compared to HOA. *In vitro* studies suggest that $BDNF_{MET}$ impairs BDNF's regulated secretion and distribution to neurons (Chen et al., 2008, 2004; de las Heras et al., 2020). However, BDNF levels of participants with the $BDNF_{MET}$ genotype did not significantly differ from those of participants with the $BDNF_{VAL}$ genotype. Interestingly, when the effects of BDNF genotype on the rate of change of the primary functional measures TUG, STS and 6MWT were evaluated, although results did not reach statistical significance, na-PD participants with the Val/Val genotype ($BDNF_{VAL}$) presented worse results on the primary functional measures compared to Met carriers. But, importantly, for the MM-EX group, the opposite was observed, that is, $BDNF_{VAL}$ participants presented better cognitive and functional scores after engaging with the intervention compared to Met carriers. Different hypotheses have been proposed to describe the role of BDNF genotype on BDNF levels or moderating the response to exercise, however, to date, published results do not consistently support any of the proposed hypotheses (de las Heras et al., 2020). Several other factors (e.g., age, biological sex, etc.) could be influencing the effect of BDNF genotype, nonetheless, could the present research outcomes suggest that physical inactivity might be more detrimental for $BDNF_{VAL}$ individuals? Or would $BDNF_{VAL}$ participants be better responders to exercise? This would be a fruitful area for further work.

Taking all the objective results together, it was observed that the HOA generally outperformed the MM-EX and na-PD. However, it is important to emphasise that, in contrast to the na-PD, only the MM-EX group was able to significantly improve both their physical and cognitive function, which was also reflected in an improvement in BDNF levels. The observed improvements in different functional domains and biomarkers of neuroplasticity support the notion that MM exercise can improve and help restoring many aspects of motor and cognitive dysfunction present in PD.

PD is a very diverse condition where the progression and severity of symptoms can significantly differ from person to person, which has been recently recognised as different clinical phenotypes (Armstrong & Okun, 2020). These different representations of PD are caused by many genotypes and multiple molecular mechanisms that make the development of interventions and other potential treatments a challenging task (Sarkar et al., 2016). Research studies have revealed that exercise therapy can improve QoL, muscle strength, cardiorespiratory fitness, balance and walking capacity, and that the maintenance of high regular physical activity levels and exercise habits are associated with a better clinical course of PD in the long-term (Tsukita, Sakamaki-Tsukita, & Takahashi, 2022). However, a comprehensive review of the literature has commented that the prescription of isolated intensive exercise modalities (e.g., resistance training, endurance training and other intensive training modalities closely related to the former ones) may not be an optimal approach for PwP. Instead, researchers suggest that an ideal programme for PwP should combine these elements (strength, endurance) (Uhrbrand et al., 2015). Due to the MM nature of the training approach presented in the current study, the exercise programme was able to combine these key elements and evaluate not only physical function but also changes in cognition and biomarker levels in response to MM exercise. Overall, the long-term evaluation of the present study provides evidence that weekly engagement with MM exercise is effective to improve walking capacity, lower body strength and endurance, and cognition. Moreover, no deterioration on any of the evaluated outcomes was observed following MM exercise for up to 3 years. Importantly, when compared to a non-active group of PwP, in order to see improvements, engaging with the MM exercise was key. Thus, overall, MM exercise showed clinical implications and the potential of modifying Parkinson's progression.

The intervention length is important. A long-term study design was followed under the premise that since PD is a chronic progressive condition, longer interventions should be evaluated. Moreover, short interventions (<8-12 weeks) have not been able to show the improvements in function that are seen with longer interventions (Tidman & Skotzke, 2020; Uhrbrand et al., 2015). However, apart from the literature that has evaluated the effects of longer versus short interventions on physical or cognitive function, no studies have directly evaluated the effects of the length of an exercise intervention on neuroplasticity in PwP, which complicates the ability to determine an optimal study length as a reference to induce neuroplastic events (El-Sayes, Harasym, Turco, Locke, & Nelson, 2019). Although the present study does not directly compare different groups with different intervention lengths, it evaluated outcomes across different lengths of engagement with the MM programme and, regarding the evaluation of neuroplastic events, it was able to show improvements in BNDF levels (biomarker of neuroplasticity) after 8 months of engaging with the MM intervention. As a strength of the study, the long-term duration of the MM community-based exercise class (up to 3 years, 154 weeks), and the multidimensional assessment of participants outcomes (biomarker levels, physical function, cognition, mood and QoL) can be highlighted. However, given the fact that PD is a slow progressive condition and that age-related declines in function take time to develop, the length of the intervention for the comparison groups (HOA and na-PD) may have been insufficient

in duration to observe any significant declines in function over time in these groups (Cavanaugh et al., 2015; Milanović et al., 2013). Nonetheless, as mentioned above, the length of the intervention (for the comparison groups) was sufficient to show differences in capillary BDNF levels. Another strength of the study was that medication dosage (and other adjuvant treatments) was controlled during the length of the project, allowing the distinction of medication effects (which remained constant throughout the study) from the benefits observed in response to the exercise intervention.

A major reason for the shortened length of the intervention for the comparison groups was the COVID-19 pandemic. When it struck, the intervention had to suddenly stop as well as any data collection. Furthermore, all social meetings including exercising groups and group assessments were prohibited throughout the whole year of 2020, meaning that the intervention had to be immediately interrupted and no further assessments could be carried out. This meant that not all participants were able to engage in all the required data points to complete the full period of 1, 2 or 3 years (which affected sample size), and that the comparison groups could only complete 3 of the 5 assessments that were scheduled (which affected sample size and the study design).

Certain limitations of this project must be acknowledged. Although the design of the present study, being quasi-experimental, may have higher external validity than most true experiments because it involves a real-world intervention instead of artificial laboratory settings, it lacks random assignment of the participants to the intervention group (White & Sabarwal, 2014). For the MM-EX group, most recruited participants were members of an active PD's Local Support Group that were pro-actively seeking to engage with a specific exercise class for PwP. Therefore, this sample may not be representative of the whole PD's community. Also, a substantial amount of research participants across the comparison groups included in this study were self-selected members actively involved with community-based support groups or volunteering work. Therefore, it would have also been of great interest to include a comparison group of PwP or healthy adults that do not engage with support groups and are at risk of being disenfranchised from exercise or social gatherings (due to having social anxiety, fear to publicly exercise, or other reasons).

Another important point to consider is participant's activity levels. Although IPAQ measurements were completed, objective measures of activity (e.g., using activity trackers or accelerometers) were not obtained. This, limited the researcher's abilities to accurately control what participants engaged with, exercise wise, outside of the MM class. Trying to account for this, changes over time were evaluated and participant's data showed that estimated levels of total physical activity in MET-min/week remained constant throughout the study. Furthermore, HOA and na-PD participants were also asked whether they regularly engaged with physical activities. Although overall levels of physical activity of the na-PD were lower than the other comparison groups, some participants from this group reported engaging with activities such as cycling, walking or swimming, mostly in form of unstructured exercise. However, none of them took part in structured MM exercise. This was also

shared amongst the HOA group, who engaged with activities such as cycling, running, Zumba, Pilates and badminton, amongst others, but none regularly performed activities with the characteristics of the MM programme presented here, which was organised following the current recommendations for PwP on exercise prescription and provides optimal exercise advice for PwP based on current leading authorities (Martignon et al., 2020; McDonnell et al., 2018; Parkinson's Foundation, 2020; Radder et al., 2020). It is important to note that any structured exercise intervention could be of potential benefit for PwP, however, MM exercise provides a unique experience to include traditional fitness components (strength, aerobic, balance, flexibility) and specifically address the deficits presented in PD by working on posture control, symmetry, full range of motion, high intensity exercises, complex and combined movements, goal-related exercises and cognitive load with exercises that demand mental effort (Lockwich et al., 2021; Pereira et al., 2012; Petzinger et al., 2013; States et al., 2017; Tanaka et al., 2009; Vaughan et al., 2014; Vitório et al., 2011). Moreover, the MM programme was delivered in a specific social context (i.e., group setting) that provided a safe space, camaraderie, psychosocial benefits and more symptomatic improvements than exercising alone (Raje et al., 2019; Sheehy, McDonough, & Zauber, 2017). Thus, community-based MM exercise for PwP is a structured, feasible, safe, highly reproducible strategy that requires minimal equipment and has the potential of arresting PD's progression by eliciting neuroprotective, functional, and cognitive improvements that were not observed in PwP that did not complete this exercise modality. These findings contribute to the understanding of structured MM exercise as a neuroprotective and disease-modifying approach to slow down PD progression.

Participants' outcome data was also evaluated based on their attendance. Adherence to the class is important to guarantee a minimum weekly practice and elicit potential benefits, however, no differences in the measured outcomes were observed between the low- and high-attendance participants. The cut-off for high-attendance was set at 75%, however, due to the overall attendance being high, the low-attendance group might have not been a good representation of poor attendees. The low-attendance group had an average attendance of 68%, which was very close to the minimum required attendance value (70%) widely used in research studies (Gobbi et al., 2021). Nonetheless, a direct effect of % attendance on the outcomes was not observed, which might suggest that the consistent attendance of participants throughout the study elicited similar effects on all the participants. Evaluating the results using a different cut-off might provide more insights, however, in this study, using a lower attendance threshold would have compromised the sample size and power of the analysis due to the overall high adherence of participants.

Finally, due to not having a comparison group performing an exercise modality that was not MM exercise, it is not possible to corroborate whether the observed benefits arise due to the aerobic component or other characteristics of the MM programme (i.e., synergistic effects of the combination of strength, aerobic and cognitive components). Silveira and colleagues, demonstrated that aerobic exercise was more effective than goal-based exercise in improving cognition in PwP (Silveira, Roy, Intzandt, & Almeida, 2018). However, we have observed improvements in both physical and

cognitive function, which could be enhanced by the MM nature of the exercise programme. Moreover, a MM programme including an aerobic component as well as goal-based and cognitive exercises might be able to improve both physical and cognitive function better than practicing those modalities alone, where goal-based modalities have been successful in improving motor symptoms and aerobic exercise promoted cognitive benefits (Petzinger et al., 2013; Silveira et al., 2018). In the present MM study improvement in both domains were observed and our results are in line with research undertaken in institutionalised older adults that has shown that a MM intervention is able to elicit improvements in mobility, functional capacity and cognitive performance (Fraga et al., 2021; Vaughan et al., 2014). Nonetheless, further research is required to clarify the origin of the beneficial effects elicited with the evaluated MM intervention.

There are two noteworthy elements that contributed to the success of the long-term MM programme. First, the involvement of student volunteers assisting in the supervision and administration of the class, which was hugely appreciated by the class participants. Students' involvement provided participants with close supervision and motivation to work at moderate-to-high intensities, whilst maintaining a large range of motion and exercising with the appropriate technique. Finally, this study could have not been completed without the industrious involvement of a local support group for PwP to develop and engage in a PD specific exercise class. Their enthusiasm for the programme and determination to collaborate with the researchers, who were also the exercise instructors, was key. Using an approach similar to the CBPR suggested by Hirsch et al. (2011), which was also similarly employed by States et al. (2017), participants became research advocates and were involved in different steps of the research project (from generating the MM intervention to dissemination of the results) (Hirsch et al., 2011; States et al., 2017). Participants' opinions and inputs were considered at all stages of this project and, additionally, focus group meetings were used to gather class participants' and their partners' thoughts and perceptions of the MM class (see study 3, **Chapter 5**).

4.7 Conclusions

The results of this study show that once a week attendance to a community-based MM exercise class for 1, 2 or up to 3 years, can maintain or significantly improve physical and cognitive performance in PwP. Moreover, results show that basal levels of BDNF can increase in response to the regular engagement with the MM class. It is worth highlighting that these results were observed with a session running only once a week (i.e., as a stand-alone intervention, does not meet the overall levels of physical activity recommended by exercise guidelines [ACSM, 2016, 2017; Davies, Atherton, McBride, & Calderwood, 2019], however, it might increment participants retention and better attendance), and improvements were most likely seen after 4 to 8 months of engaging with the MM exercise intervention. Taken together, the community-based MM programme described in this study presents a specific, supervised, safe, reproducible, low-cost programme with clinical applicability that has the potential to slow down PD progression and provide improvements in motor and cognitive function.

Chapter 5. Study 3 – 'Exercise is Part of my Whole Medication Regime': People with Parkinson's and their Partners' Experiences with a Community-based Group Exercise Class

5.1 Abstract

Introduction: Exercise interventions can improve both physical and cognitive performance in people with Parkinson's (PwP). However, many studies report structured exercise interventions that are of short duration (<6 months) and located in highly controlled laboratory environments. Community-based interventions present a viable approach to providing PwP opportunities to exercise and offer several ancillary therapeutic benefits but need to be rigorously evaluated. Qualitative research methods (e.g., focus groups) can provide unique insights into the effectiveness of real-world exercise interventions and can capture information about the participants' experiences and perspectives. **Aim:** to gain an understanding of the underlying reasons, opinions, and motivations influencing participation and adherence in a community-based exercise class for PwP from the perspective of affected persons with PD (i.e., class participants) and partners of PwP. A particular focus is also given to the perceived role that volunteers play in supporting the delivery of the class. **Methods:** Two separate focus group interviews were conducted: one with class participants (PwP: n = 7, H&Y scale I to III); the other with partners of PwP (n = 4). Participants were recruited by purposive sampling from a community-based exercise class for PwP in the area of Medway (Kent, UK). **Results:** Using a reflexive thematic analysis framework, five main themes were created: 'Rallying to the challenge', 'Exercise as therapy', 'Perceptions of healthcare professionals' attitudes', 'Perceived beneficial and adverse effects of exercise', and 'Suggestions for improving the exercise class'. **Conclusions:** findings add qualitative insight into existing literature highlighting the beneficial role that community-based exercise interventions can play in the management of Parkinson's. They can bring together participants, family, clinicians, and researchers to provide viable exercise opportunities. Participants and partners felt that the group class provided PwP with an opportunity to proactively self-manage their health and benefit from membership of the group through connections with class participants, family, student volunteers and class instructors.

5.2 Introduction

It is now accepted that exercise training as a therapeutic intervention can play an important part in overall disease management for PwP by helping them maintain functional independence and quality of life (Chen et al., 2020; Lawson et al., 2016; Martinez-Martin, 2017). Moreover, it has been shown to help delay or reverse functional decline (e.g., gait speed, strength, balance) and can also facilitate neuroplasticity and brain repair (i.e., neurogenesis) in PwP (Da Silva, Domingues, De Carvalho, Allodi, & Correa, 2016; Gobbi et al., 2021; Mak et al., 2017; Petzinger et al., 2013). To date, however, reviews have tended to only discuss the short-term benefits of exercise training for PwP (Goodwin, Richards, Taylor, Taylor, & Campbell, 2008; Shu et al., 2014), with most interventions typically lasting for 12 weeks (Mak et al., 2017). In their review, Mak et al. (2017) reported that exercise training can modify long-term motor symptoms and physical functioning, with a training period of at least 6 months being effective for providing clinically meaningful changes in motor symptoms (as assessed by the UPDRS Part III [UPDRS-III]) (Mak et al., 2017). However, only four studies in their review lasted for more than 6 months (Corcos et al., 2013; Duncan & Earhart, 2012; Prodoehl et al., 2015; Schenkman et al., 2012).

Exercise interventions for PwP are typically delivered in health (led by a clinical physiotherapist), group (community class) or home (individual) settings but most take place in highly controlled laboratory settings (e.g., university research centres and clinical research units). Such interventions may not always be viable, and researchers cannot expect to produce the same findings outside of these settings. More recently, it has been demonstrated that home-based programs with minimal supervision can produce similar results to supervised programs (Flynn, Preston, Dennis, Canning, & Allen, 2020; van der Kolk et al., 2019). What remains unknown, however, is how feasible, safe, and/or effective these interventions are in the long-term, or how to deal with factors such as adherence and adverse effects. A recent qualitative review of different exercise interventions by Hunter et al. (2019) highlighted how such programs are perceived and experienced (Hunter, Lovegrove, Haas, Freeman, & Gunn, 2019). Generally, PwP perceive exercise as an enjoyable experience that is beneficial for their symptoms, wellbeing and quality of life, however, their preference for exercise setting (either supervised or home-based) appears to be highly individualised, with some PwP preferring programs at the hospital and others at home (Hunter et al., 2019; Khalil, Nazzal, & Al-Sheyab, 2016).

Group-based programs, in particular, appear to offer psychosocial benefits beyond functional benefits gained from exercise programs delivered by instructors in clinical settings and individualised home-based programs (Claesson, Ståhle, & Johansson, 2020). Specifically, group-based exercise brings PwP together, which may be an important factor for sustaining participation. Group-based exercise that enables individuals to exercise with others at a similar stage of disease, provides participants with positive reinforcement feedback from the instructor, can maintain or improve physical function, and practises motor skill training (e.g., walking, sitting and standing) are perceived

positively (O'Brien, Clemson, & Canning, 2016; O'Brien, Dodd, & Bilney, 2008). However, delivering group-based exercise programs can be challenging. For example, Quinn et al. (2010) reported that, whilst there are important social factors linked to group-based exercise, therapists felt that grouping people together with different levels disability could generate different feelings (e.g., whilst integrating people from all stages is important, some participants have reported that they value being with people who are at a similar stage and might find seeing PwP in advance stages upsetting [Charlton & Barrow, 2002]). Moreover, although safety was not mentioned as a concern by PwP, it was an important factor for therapists, who are aware that the setting of a particular exercise programme may compromise the ability of someone with PD to complete a sufficiently challenging exercise program in a safe manner (Quinn et al., 2010). Despite this, therapists have recognised that regular physiotherapy or one-to-one exercise instruction may not be cost-effective strategies for PwP due to the long-time course of the condition, and the cost of service delivery could affect engagement (Quinn et al., 2010).

In a novel approach to develop sustainable exercise programs for PwP, Rossi et al. (2018) created a tailored exercise program for PwP in a group-based setting in a university wellness, recreation, and athletic centre (Rossi, Torres-Panchame, et al., 2018; States et al., 2011, 2017). Impressively, the researchers were able to sustain the program for up to 10 years. They also used student assistants to supervise the sessions (in a similar way to the student helpers involved in the present study), although this was only for the last 2 years of the whole study duration, by which time the program was already well established. The researchers interviewed fourteen participants who completed their exercise program and highlighted tailored and varied exercise content and social cohesion with a positive and nurturing environment as important factors supporting adherence. However, their program may be difficult to replicate in other community settings because of the equipment (weight training and dual-action exercise machines) that were required to complete some of their sessions. Multi-modal exercise, comprising circuit-style training, may, therefore, be a more inclusive form of group exercise that can easily be replicated in other community centres.

There is substantial evidence from both quantitative and qualitative studies suggesting that exercise can not only positively impact the symptomatology of PD, but that those measurable beneficial effects are also perceived by PwP in forms of enjoyment, sense of improvement and psychosocial qualities such as being part of a group, which has been described as one of 'the most prominent way of coping' with the limitations that PwP may present (Charlton & Barrow, 2002; Claesson et al., 2020). Thus, the development and evaluation of easily accessible (e.g., community-based) long-term exercise interventions aimed at modifying the progression of PD is imperative and should be high on the research agenda. Moreover, participants' perceptions should be appraised to understand what works best for PwP and researchers should strive to adopt and/or modify strategies accordingly. The addition of PwP partners' input may provide valuable insights about the perceived effects of an exercise intervention for PwP from a different perspective (i.e., external) (Lamont, Morris,

Woollacott, & Brauer, 2012). Therefore, this qualitative study was designed to broaden understanding of the psychosocial benefits of group-based exercise programs for PwP by including participants who were active and non-active before joining the class and including a group of partners of PwP to share their experiences with a community-based exercise programme.

5.3 Methods

5.3.1 Ethical considerations

This study was approved by the SSES REAG, University of Kent (see reference code in **Chapter 2**). Ethical principles were adhered to regarding consent, confidentiality, and anonymity. Participants had the opportunity to ask questions before providing written informed consent and participating in the focus groups. They were reminded that they were not obliged to contribute to particular lines of discussion.

5.3.2 Participants

Two separate focus groups were held in March 2019 with 11 participants (PwP, n = 7; spouses of PwP, n = 4) purposely recruited from the exercise class. Considering the dynamic nature of the focus group interactions, participants with moderate-to-severe speech difficulties were not approached. Seven of the recruited participants (male, n = 7; age, 70 ± 9 yr) had a diagnosis of idiopathic Parkinson's as defined by the H&Y scale (H&Y stage, 2.0 ± 1.2), a disease duration of 4.6 ± 2.1 years, attended the exercise classes for 2.1 ± 0.7 years and their attendance rate was 74% ± 16% (with a range of 43-96%). Four spouses of PwP, who regularly accompanied their partners to the class and used to wait outside the exercise hall in a meeting room, took part (female, n = 4; age 68 ± 6 years). Participants were approached face-to-face two weeks before the focus group took place. This involved chatting to participants individually before the exercise class and providing them with a participant information sheet. Participants were then given a week to decide whether or not they would like to take part. Those who expressed interest and were available to arrive at the community centre earlier, were then contacted via telephone to confirm attendance. To rule out selection bias, partners and class participants were still invited to participate on the day if they turned up early.

5.3.3 Multi-modal exercise class

The Parkinson's specific multi-modal (MM) community-based group exercise class was a collaboration between the University of Kent and Medway Working Age Group. The project was set up in October 2016 with initial funding provided by two charities (the Medway Working Age Group and Parkinson's Equip) to support the delivery of a one-hour group exercise class once a week in a local community centre. The class was delivered by two qualified exercise professionals (Level 4

Specialist Exercise Instructor; Register of Exercise Professionals) and supported by undergraduate students from the university until March 2020 (the beginning of the COVID-19 pandemic). Approximately every four months (every 15 weeks on average) throughout the 3-year period, participants underwent cognitive and physical function assessments to monitor progress. Participants had a break from the class for approximately 4 weeks every summer.

A detailed description of the MM exercise class is provided in **Chapter 4**. In short, the exercise class began with a warm-up, which consisted of light aerobic and mobility exercise with an emphasis on maintaining good posture. The warm-up was followed by a 23-station circuit of aerobic and skilled exercises that combined resistance work, flexibility, balance, mobility, motor-cognitive training (i.e., dual-tasking; e.g., motor task with a cognitive challenge, such as counting backwards from 50 by 3) and cueing strategies (i.e., use of external visual cues to facilitate movement initiation), specifically designed to promote neuroplasticity and repair (Farley & Koshland, 2005; Fox et al., 2006). Intensity was self-prescribed, but participants were encouraged to work "as hard as you can" for 1-min. with a 30 seconds active-rest interval. Student volunteers worked closely with participants at each station providing instructions and verbal encouragement. The session ended with a cool-down that focused on balance, posture control and stretching exercise. Session rating of perceived exertion (sRPE) was recorded afterwards using the Borg 6-20 RPE scale (Borg, 1998; Foster et al., 2001; Penko et al., 2017). The mean sRPE for the group during the period October 2016 to March 2019 was 13.4 ± 1.5 (somewhat hard). The mean number of participants at each session during this period was 17.1 ± 2.5 and the mean attendance for the group was 76% ± 17%.

5.3.4 Study design

The present qualitative study used focus group meetings to explore participants with PD and partners' perceptions about a MM community-based exercise class. Focus groups are useful during all stages of a health research project, but may be particularly useful in this context to "elicit and analyse the range and depth of experiences" of the participants in the exercise class (Lehoux, Poland, & Daudelin, 2006). Focus groups can generate a rich understanding of individual needs and opinions on topics and, unlike one-to-one interviews, enable people to respond spontaneously and, thus, generate valuable ideas through participant interaction (Krueger, 2014). Given that the initiative is community based and there is a sense of ownership of the class, it was deemed important that participants and partners were able to provide feedback on the service, including what could be done to engage PwP from outside of the group. Therefore, this study sought to structure the discussion around participation, adherence and service delivery.

Two semi-structured focus group meetings were conducted on separate occasions at a local community centre. The focus groups were planned to coincide with the usual Tuesday night exercise class to maximise participation and to minimise time commitments. One focus group was scheduled before the exercise class and only included class participants (PwP), and the second focus group only

including partners was scheduled whilst the exercise class was running. Class participants arrived 120 minutes before their usual exercise class to take part in the focus group meeting, whereas partners took part in the focus group meeting whilst the 60-minute exercise class took place. Interviews with participants and partners lasted 87 minutes and 43 minutes, respectively. The team thought that the class participants would share information more easily when not in the presence of their partners, or someone without Parkinson's. Moreover, similar to what Lamont et al. (2012) considered, partners were interviewed separately in case some felt reluctant to express their feelings with honesty in front of PwP (Lamont et al., 2012).

A researcher at the University of Kent who had not previously supervised the exercise class, but was well-known to the participants, conducted both focus group discussions. It is conceivable that participants might have felt obliged to respond with socially desirable answers in the presence of the class instructors. Moreover, such rapport might encourage an individual to say more than he or she intended to. Each focus group began with a short round of introductions in which participants and partners briefly shared their experiences, before the moderator briefed participants about the focus group discussions. This briefing provided the moderator with the opportunity to emphasise issues of confidentiality and anonymity, manage expectations of the focus group, and give participants an opportunity to reflect on their decision to take part before the discussion began. Participants in both focus groups then discussed attitudes, opinions, and perceptions of exercise for PwP, reasons for and experiences of participating in the supervised group exercise class.

The focus group structure was the same for each meeting; however, partners did not comment on the nature of the exercise routines in the class itself as they had not observed participants exercising, which might explain why the focus groups varied in duration. Partners commented that, as much as they would like to know what goes on in the exercise class, their presence might induce anxiety and they had never been present in any of the exercise sessions. After the focus groups finished, the moderator debriefed participants by summarising content and reiterating ethical assurances regarding confidentiality. Finally, the moderator stayed in the room afterwards until the end of the exercise class to give individuals an opportunity to address any issues or concerns (Bloor, Frankland, Thomas, & Robson, 2001).

5.3.5 Data collection and focus group meeting guides

Topic guides for the focus groups were developed to prompt and encourage discussion about experiences of the exercise class. The questions were open-ended to ensure that participants responded using their own words. Context cues were provided before each discussion to help participants process the subsequent questions and probing questions were used when needed to encourage discussion.

The focus groups started broadly with questions about the exercise class experience (e.g., "When you think about the exercise class, what is the first thing that pops into your mind?"). The following set of questions were divided into general sections: i) motivation for participating in the exercise class (e.g., "What was your motivation to join the class?"); ii) benefits and challenges of taking part in the group class (e.g., "Have you noticed any positive effects on your health from the exercise?"); iii) factors associated with adherence to the exercise class (e.g., "Why do you think some of the local Parkinson's support group do [not] attend the exercise classes?"); iv) the exercise class structure/format (e.g., "Do you think the exercises are appropriate for people with Parkinson's?"); and v) feedback on the service (e.g., Are there any changes you would make to the exercise class to encourage greater participation?). The moderator also used prompts to elicit further responses and clarification, and participants were free to bring up any information they perceived as relevant.

5.3.6 Data analysis

Discussions were recorded using a dictaphone (Olympus VN-5500PC Digital Voice Recorder), subject to permission of each participant. An assistant (undergraduate dissertation student) acted as an observer, taking notes, and focused on verbal/non-verbal interactions. Audio recordings were transcribed verbatim. First names were replaced with pseudonyms (to make interviews coherent and easy to read) and all references to third parties and location were removed. Subsequently, the data was cross-checked with the audio recording. A copy of the transcripts was with two researchers and, using a process of repeated reading (Lamont et al., 2012), researchers noted initial thoughts and ideas in response to the data. Transcripts were imported into NVIVO 12 (QSR International Pty Ltd. Version 12, 2019) for coding. Researchers independently generated initial codes to give basic meaning to the transcripts and then discussed the different sets of coding to finally establish a list of codes. Subsequent discussions were held with the rest of the research team to challenge assumptions and interpretations before finalising the themes. A list was organised by grouping together similar codes and then coupling with raw data extracts (i.e., quotes). These groupings became the initial themes and miscellaneous codes were saved for later re-coding and analysis.

The focus group data were analysed using a reflexive thematic analysis (Braun & Clarke, 2019; Campbell et al., 2021). Thematic analysis is theoretically flexible and can be used within a variety of epistemological frameworks (Braun & Clarke, 2019). It is particularly useful in this context because of the applied nature of the research questions being asked. Analysis of focus group data should represent the fact that meaning is negotiated through dialogue and interaction in a group context. Thus, initial themes were generated based on analysis of discussions rather than individual statements (i.e., analysed in context) with a focus on identifying aspects of the discussions with practical and theoretical implications (Clarke & Braun, 2013).

Analysed transcripts were returned to participants to encourage reflection (Clarke & Braun, 2013).

5.4 Results

Research findings from both focus groups are organised around the five themes that were created and revised through reflection from the data. Themes (presented as individual sections) and subthemes (presented in bold within each theme section) were developed through an iterative process by comparing the transcripts between the two groups (PwP and partners).

Questions sought to explore participants' and partners' experiences with the group exercise class – as well as opinions on what they like and dislike about the class. The five higher-order themes that were created were: 1) Rallying to the challenge: '*We are fighting against it!* 2) Exercise as therapy: '*You will get a better quality of life*, 3) Perceptions of healthcare professionals' attitudes: '*It's different now, there is acceptance that exercise can offer people so much hope*, 4) Perceived beneficial and adverse effects of exercise: '*I feel tired and worn out, but it's a happy feeling*, and 5) Suggestions for improving the exercise class: '*Let's have two exercise classes a week!*. Although presented separately, the five different themes interact, overlap, and influence each other.

5.4.1 Rallying to the challenge: '*We are fighting against it!*'

Exercise class participants and partners discussed their personal reasons for joining/supporting the exercise class. Initial exercise participatory motives appeared to vary among participants. Some participants were keen to take part in as many of the community initiatives as possible, recognising that they provide a range of health and social benefits. The exercise class appears to be one of the more popular activities, and the fact that the class is grounded in evidence-based practice, appears to be a factor influencing participants and partners of its benefits. Some, however, drew on others for practical and affective support, which suggests that exercise was not necessarily the preferred intervention. However, there is a strong sense of community among those who attend the exercise class and, by joining, there would be a benefit to self and others.

Fighting back. The idea of 'fighting back' against Parkinson's is a well-known phenomenon in the Parkinson's literature and practice ('Fighting back: Tommy talks fitness, boxing and Parkinson's | Parkinson's UK', n.d.; Sjödahl Hammarlund, Westergren, Åström, Edberg, & Hagell, 2018); and is something that has also been reported in other health conditions, such as stroke and cancer (Kouwenhoven, Kirkevold, Engedal, Biong, & Kim, 2011; Williams & Jeanetta, 2016). However, whereas the 'fighting back' is often associated with binary success outcomes (i.e., victory or defeat), for the participants in this study, fighting back is concerned with preservation and holding onto health for as long as possible and delaying the progression of their condition. Importantly, whilst researchers might debate the use of military metaphors in chronic illness descriptions (Wiggins, 2012), and in keeping with our ontological position (i.e., meaning is co-constructed), we are in favour of using the very language PwP connect with. One participant said that by joining the class they [group members] were:

> Not letting it take over our lives . . . Fighting back . . . we are not passive . . . not letting it take over our lives . . . we are fighting against it. [PwP #5].

Altruistic motivation. Not all participants were intrinsically motivated to join the exercise class. As one partner stated, her husband's intention to exercise was very low, "Because he was quite negative when he first knew, which I suppose is quite understandable … it's a bit of . . . especially when he knew [it] is going to get worse as time went on . . . so he was very negative, it was 24/7 almost sitting on the sofa sleeping . . . and of course, it's not very good for us either." [Partner #2].

This view was echoed by other partners who commented that not everyone with Parkinson's shares the same motivation as people in this class and/or wants to exercise—or take part in the activities on offer to the local Parkinson's network support group.

Partner #2 commented that her husband was reluctant to attend the class as he did not want to see others who were at a later stage of Parkinson's (which has also been illustrated in a recently published qualitative study [Claesson et al., 2020]). However, she encouraged her husband to participate in the exercise class by suggesting that his involvement [in the project with the university] would help others:

> I'll be honest, [he said] he didn't really want to come here … But I said: 'You will not only be helping yourself, but you will also be helping others.' … And with that, he said: 'Alright, I'll go' … He doesn't want to know anything else … but he comes here because he thinks they're super here. [Partner #2]

Similar discussions emerged among PwP. As one participant stated, motivation to support research into Parkinson's was an important factor influencing participation:

> It's nice to be able to help other people as well, [be]cause from these studies, the feedback will be used to help other people, which is . . . you know . . . partly . . . the main [aim] of the exercise isn't it, really? [PwP #6]

Recent research has highlighted altruistic motivation to take part in community-based research for others' benefit (Carrera, Brown, Brody, & Morello-Frosch, 2018). In this study, both participants and partners discussed the notion of motivation to help researchers and other PwP. However, when considering possible altruistic motivations for research participation, it is difficult to know whether participants are genuinely motivated to help others, or whether their motivation is self-interested (or a combination of both). As some participants admitted, they were keen to try anything that is free, with one participant commenting that financial issues may influence the type of treatment sought to self-manage Parkinson's. Similarly, one partner [Partner #4] admitted that 'It [exercise] was just

suggested that it might do some good, and you'll get to a stage where you'll give anything a go, really.'

Spousal support. Partners' encouragement and support were seen as important factors contributing to participants' motivation, adherence, and compliance to the weekly exercise class. Motivation to attend the Tuesday evening exercise class often varied. However, participants described how structured group exercise encouraged adherence, despite fluctuating motivation and mood. One PwP commented how his partner provided encouragement when he did not feel like going to the class:

> That's interesting you say that because my wife always asks me about 5 o'clock on a Tuesday . . . says, are you going to Parkinson's class tonight? And if I say, I don't know, it's a bit cold and a bit miserable, I get a kick up the backside… [she says] 'get yourself out there, you miserable being!' [PwP #1]

However, as one partner explained, the lack of motivation of PwP to exercise outside of the class can be frustrating for those partners who also want to be physically active, and it would appear that they sometimes forgo their own physical activity goals to support their husbands':

> That's something I can't get mine [husband] to do . . . I would like . . . because I need to lose weight and I would like to go and do on walks, but . . . he cannot just see the point of just going for a walk, and I've . . . so, I've given up! [Partner #3]

5.4.2 Exercise as therapy: '*You will get a better quality of life*'

Participants described how exercise can provide ancillary benefits to standard medical management. Exercise was perceived to play a key role in their care, and when they exercised they felt better for it, describing how it can help overcome apathy and enhance quality of life.

> If you don't take this drive to do this exercise class you find the other non-motor symptoms of Parkinson's . . . anxiety, all those sorts of horrible things . . . apathy . . . all creeping in on you, especially coming back to the dark nights and wintertime. If you make that effort to come along to the group exercise, I believe, I do believe in my heart, it's much better for you and you will get a better quality of life for it. [PwP #3]

> . . . this class is just as good as the medication, if not better. I said it's part of my whole medication regime. [PwP 5]

Despite the acknowledgement that, as Parkinson's progresses, self-management will become increasingly difficult, participants in this focus group appear to have clear expectations about what

exercise can and cannot do for PwP. It is perhaps worth mentioning that participants from this group regularly attend both local and national networking events for PwP where the latest research findings are disseminated. There appeared to be general consensus that exercise can *at least* help limit the development and progression of the condition and that it can *at least* offer benefits for quality of life through socialisation and connecting with others (Claesson et al., 2020):

> I'm not progressing. . . at least I'm not . . . probably getting any better, but I'm not . . . I'm certainly not getting any worse… It's [exercise] maintaining your health. And I think that's an important thing. [PwP #2]

Not all participants were positive about the [protective] benefits of exercise, although this apparent resentment has not discouraged ongoing exercise participation:

> I have always been a member [of the gym] … I used to do karate, play rugby . . . Kept myself reasonably fit by going to the gym, but that didn't stop me from getting Parkinson's, though. [PwP #7]

5.4.3 Perceptions of healthcare professionals' attitudes: '*It's different now, there is acceptance that exercise can offer people so much hope'*

Participants and partners commented on how attitudes and opinions towards exercise among healthcare professionals varied. Some participants commented how they were initially discouraged to take part in high-intensity multi-modal exercise and recommended to stick to seated exercise, whilst others were referred to the class by their local Parkinson's nurse:

> There were very strong claims that … really strong exercise can actually put you back in your disease, but I … I don't believe that … what I do believe is that you can hold it … [PwP #3]

One participant went further, arguing that 'We should be doing exercise whether we've got Parkinson's or not! [PwP #6]. There were further discussions around experiences with healthcare professionals and stories focused on the lack of support for exercising with Parkinson's. For some participants, who were not previously physically active, motivation to start exercise was met with some resistance by healthcare professionals:

> I was told by a senior physiotherapist in _____ hospital that we . . . we won't ever run a Parkinson's exercise class because it wouldn't be supported. [PwP #3]

Along a similar line, one participant recalled how he had been discouraged to exercise, with healthcare professionals suggesting that exercising with 'fellow sufferers' (PwP #6; subtheme 'Fellowship') might not always positively impact individuals (through social comparison) and could be a potential barrier to exercise in this population:

> Well, you know, I don't think you want to go there … well because you don't want to see what's coming, do you? 'Oh really? You don't want to see what's coming. 'Oh right, what's coming?' When they say that, the first thing you want to know is: 'What's coming!' [PwP #2]

It is possible that such views discourage more people from joining the project. Ellis et al. (2013) reported that low outcome expectations is an important perceived barrier to engaging in exercise for PwP (Ellis et al., 2013). However, despite the mixed messages and advice regarding exercise as therapy, regular participation in the class appears to have positively influenced opinions and perceptions of exercise among some of the PwP:

> You see how successful this has been, but erm . . . erm, the official . . . NHS class is just seat-based and they walk around . . . [PwP #5]

> Well, apart from Parkinson's, surely the human body wasn't designed to sit still all day, was it? If you do, everything will just seize up—surely you've got to keep moving! [PwP #2]

The class participants appeared to be strong advocates of exercise for PwP (even though not everyone was positive about its protective benefits), there was acknowledgement that the attitudes of some among healthcare professionals are also beginning to change.

> I was being told by professionals … being put off exercising . . . not to exercise 'oh, don't do that you'll hurt yourself' Don't cycle, you'll do your back in!' This is [healthcare professional] talk. And she's totally different now . . . she's accepted that exercise can offer people so much, and so much hope. [PwP #3]

Partners' attitudes. Partners shared the view that exercise can be beneficial for PwP, but there was discussion that more could be done to recommend exercise as an adjunct therapy and encourage self-referral onto exercise programmes:

> Oh yeah, I think it's definitely a good thing … it definitely does them good … perhaps the people need to hear about it more because if I hadn't read the advert for

> this class in one of those little booklets that come through the letterbox, I wouldn't have known about it. [Partner #1]

Participation in such initiatives tends to be initiated through the local Parkinson's support network and, in consequence, active members appear to benefit from the relations. Communication appears to be a barrier to increased uptake among the local Parkinson's community and as one partner commented, doctors are often the first point of contact in the diagnosis process and so could do more to promote adjunctive treatments:

> Why don't they put them [the adverts] in Doctors' surgeries? You see everything else on the walls—what you can do or join! [Partner #2]

> The Doctors purely act as a prescribing mechanism. They don't really get involved . . . [Partner #4]

However, not everyone had experienced resistance or poor communication from healthcare professionals. As one partner explained, her husband was encouraged to participate in the exercise class following a referral from the local Parkinson's nurse:

> … she came to the house and she told me about, you know, what I could [for us] get as a carer, and she told me all the things that I, we could join, you know, like this [class]. [Partner #2]

5.4.4 Perceived beneficial and adverse effects of exercise: '*I feel tired and worn out, but it's a happy feeling*'

Participants discussed the positive and negative aspects of participating in the class, such as likes and dislikes about the exercise class format and content.

Enhanced mood and wellbeing. Participants in this study discussed how they perceived exercise to be important for emotional well-being and quality of life, highlighting that, as older adults, social isolation, and loneliness are a concern, which are known risk factors for poor psychological health (e.g., depression and anxiety) and wellbeing (Courtin & Knapp, 2017). Community-based groups provide opportunities for social interaction, something the participants in this study highlighted could be done before and after the class. Attending regular exercise classes is perceived to provide purpose (i.e., something to look forward to in the week) for these participants and an opportunity to make new friends, which may thus mitigate the psychological effects of social isolation. Participants also described how regular exercise has impacted their mood as well as their QoL:

> I just love to come here … I feel tired and worn out when I go back, but it's a happy feeling. I'm so pleased with what I'm doing. From my wife's point of view, there's been a change … I was very down … not depressed … but er . . . very quiet . . . and I suppose everything was going around . . . what was going to happen to me, but no . . . since I've met everybody else here and watched everybody and just the interaction, I think, between the group . . . apart from these . . . the activities, I think the interaction and meeting everybody every week . . . yeah, I think it's a brilliant idea. [PwP #2]

> … we've all got to do something … being given the dreaded disease…you've got to fill your time up with happiness … the exercise is one good step to filling up your happiness cup. You feel happy and excited, more so afterwards. [PwP #3]

Fellowship. Participants and partners frequently highlighted how the exercise class brought people together. Participants were not only able to share their individual experiences with other group members (also noted in other qualitative studies involving PwP [Charlton & Barrow, 2002]), but they were also able to learn about the disease through observation in the exercise classes and engagement with staff from the university. For this group, there appears to be a strong sense of peer support and group fellowship as participants and partners are united by a shared experience/shared condition (also highlighted by Claesson and colleagues [Claesson et al., 2020]):

> It's not just about the exercise, it's about meeting as a group, again it's a *very* important part of the situation we have found ourselves in . . . to become part of a group. [PwP #3]

> I'd miss all this … it's quite a strong bond [we have] actually… [PwP #7]

As one participant commented, group exercise connects people with similar challenges and shared experiences:

> It's nice to meet other people . . . fellow sufferers in other words . . . you know, different levels . . . some people are quite badly affected, some people are less badly affected. But it's nice to mix amongst them. Ask each other questions and get feedback from them. It's quite helpful, I think… Gives you a chance to chat to people, and pick up their experiences, which is . . . and the empathy . . . quite powerful, that. [PwP #6]

Partners are able to chat with one another whilst the exercise class is being delivered, and class participants are able to socialise with the instructors, student helpers, and other PwP. For some

partners, the exercise class can be ‘ … quite convenient nipping to the shops and having a break . . .’ [Partner #4]

Scheduled exercise can influence motivation to exercise by bringing people together to achieve shared goals:

> That’s the trouble … you don’t see me going to [the gym] . . . [I] don’t go anymore . . . [I get] bored. It’s me on my own. You know, for me, I need a group . . . a team, a team. [PwP #3]

Partners, too, discussed the impact that supervised exercise has on individual motivation to exercise:

> . . . I suppose he doesn't like to stand out . . . in front of, you know, like . . . because everybody else is doing an exercise he’s happy with it ... if they all did it in turns, they all did it in turns or something, he probably wouldn't want to stand out in the crowd, so to speak … [Partner #1]

> The various physiotherapists have given _____ exercises over the year and the pieces of paper are probably gathering dust in the magazine rack somewhere, but he will come and do this! [Partner #4]

As one partner commented, engagement with supervised group exercise may be due to the fact that it promotes greater autonomy support than prescriptive exercise interventions offered by healthcare professionals. Group exercise allows participants with different motivation levels to support one another but also work independently.

> Yes, it's not me telling him . . . because if he comes home with a piece of paper [from the physiotherapist] the only person that’s going to make him do it is me because he's not self-motivated. Some of the these . . . some of the people with Parkinson’s, are *very* self-motivated—*very* into beating Parkinson's finding the cure and, and what have you. My husband is one of the *least* motivated in that respect; he's just doing what he's got to do. [Partner #4]

Socialisation. Several of the participants commented that they frequently take part in all the activities on offer to the local Parkinson’s network support group and enjoy the benefits from forming strong social relationships with other class members. In this sense the class could be a place to create and maintain one’s social network. One participant described how they were keen to avoid being lonely, particularly in later life, and group activities such as the exercise class are an opportunity to interact with others.

> When do they say you've got Parkinson's? Usually, as you retire; I got mine in my 65th year . . . erm . . . I'm packing up work, I'm stopping work, I stopped work early . . . erm, I had the sort of job where I didn't work locally, so any people that I knew in Manchester and Sheffield . . . once you retire you all sort of drift away, once you pack up work you haven't got many friends, and this . . . a group . . . for exercise reasons, it's camaraderie as well. [Participant #3]

Moreover, in this study, both PwP and partners of PwP highlighted that exercise attendees enjoy the social interaction with the students during the exercise class. One participant highlighted the positive role that student assistants play in the exercise class:

> I think the students really enjoy it as well, which helps. [PwP #7]

Furthermore, another one of the partners also commented on:

> ... the number of volunteers is incredible, and the one-to-one ratio, yes it's very good, I think it's probably three-to-one tonight! [Partner #4]

However, a drawback is that during the summer months student support drops off. Some of the participants had formed close social bonds with the students and were sad to see students leave:

> I think the fact that the students are so good and friendly. It's a shame that when it comes to the end of the year, and they disappear. Then you start with another group, and they are all just as good as the last lot . . . and you work your way around. [PwP #4]

This level of support is unique, however, and such support is unlikely to be available elsewhere, which does pose a resource challenge to deliver a similar class experience elsewhere. For example, class participants commented that they would like a second class in the week; however, challenges include finding instructors, volunteer helpers to maintain a similar volunteer-to-participant ratio, and space to run the class. It is known that regular engagement in structured exercise programs is influenced by motivational factors and social support, both recognised correlates of exercise behaviour (Ravenek & Schneider, 2009). Participants also commented that they enjoy the exercise routines in the class.

Accomplishing goals. Participants were keen to highlight how the instructor supports motivation to exercise by varying the exercise routine, which maintains interest and enjoyment, as well as challenging participants to work vigorously. For example, one participant said:

> I'll make it competitive, [I'm] an old sportsman . . . really go for . . . if it doesn't hurt not going to do you any good. [PwP #6]

One partner described how positive encouragement from the instructor supports her husband's participation:

> The instructors have made him more positive … he comes to the class every week … it's quite wonderful … they say well done … he loves to hear that. [Partner #2]

Performance attainment has often been cited as an important source of efficacy information for sustained exercise participation and verbal encouragement from the instructors and helpers appears to increase internal motivation and self-efficacy (Dionigi, 2007). Ellis et al. (2013) suggest that cognitive-behavioural strategies such as goal-setting and feedback could be important targets for facilitating behavioural change in PwP (Ellis et al., 2013). Although our findings do not indicate which strategies are most effective, they do suggest that motivational support from the instructors and student helpers promote a perceived supportive environment to exercise, a finding evident in Rossi et al. (2018). Partners did not comment much on the class itself as they said they do not know what goes on. Whilst they said they would like to observe, they understand that the class is for participants to interact with other PwP and that exercising in front of spouses could increase state anxiety and motor performance disturbances (Lauterbach, Freeman, & Vogel, 2003; Witjas et al., 2002).

Functionality in active daily living. Partners were particularly vocal about the intensity and duration of fatigue and its effect on relationships and activities in daily life. As one partner described, her husband took some time to adapt to exercise:

> When he first came, he was absolutely shattered. You know, he could hardly walk to the car. But he seems . . . I don't know whether it's because his medication has increased, or whether he is getting used to it. I'm not saying it will stay like that exhausted spell like that, but he seems to be coping with it better now. I don't have to push him so much now, I just have to say, 'it's Tuesday now,' whereas but before had to coax him along, he seems to be more accepting of it now. [Partner #1]

One partner commented that her husband will do more in the session than at home, highlighting that the exercise class would leave him shattered and exhausted.

> Well, it takes my husband between two and three hours to get out of bed in the morning… sometimes he adds that in as an excuse, 'I can't move, exercise yesterday'. But it doesn't really impact him to that degree at all… I mean my

> husband will insist in the mornings that he can't stand up without using his arms, but I've just watched him in there! [Partner #4]

When discussing the short-term effects of exercise (i.e., in the hours/days afterwards), participants and partners did comment that fatigue and compromised functionality for daily living (e.g., getting dressed the following morning) are side-effects.

> I've got Parkinson's dystonia, which is muscle cramping and things, and I can almost guarantee that either . . . I will have a muscle cramp in the legs or the arms after the exercise class. But I still come and do it because I'm still fighting back Parkinson's (pause) and I really believe that. [PwP 5]

However, it was commented that this is not perceived to be a barrier; but it is worth recognising that this finding has implications for exercise prescription. Given that some participants in this study stated they would like to attend a second class in the week, more research is clearly needed to establish the optimal frequency for PwP. Recovery from and readiness to exercise might be worthwhile lines of investigation for researchers studying dose-effect responses to exercise in PwP. Whether this is something that impacts the rest of the class, or just a few, is unknown. Some members of the class do not participate in any other physical activity, and some were previously inactive. Therefore, it may be that these effects are more profound among those for whom exercise participation represents a marked lifestyle change.

On the topic of fatigue and reduced functionality, one partner did opine that the timing of the exercise class was not ideal; however, there was acknowledgement that the class was originally only offered to the working-age group (i.e., after working hours) but now includes retired people:

> There have also been concerns raised in the past about the timing of this class because a lot of people with Parkinson's are too tired at this time of the day, or their medication wouldn't last sufficiently for them to be able to attend this class. [Partner #4]

PwP were also asked about the timing of the class and one participant was quick to highlight that an acute negative consequence of the exercise class was impaired sleep:

> … I'm not sure, but I know one of the effects, certainly one of the effects that I have, and I certainly know [Participant #5] has, because I've got emails from him at 3 o'clock in the morning, is disturbed sleep pattern. And the one thing I do not get when I leave here is . . . I do not get a good night's sleep . . . and I don't normally but I thought sometimes I've left here, and I've thought like I'm going to sleep well

tonight, and I might do till about 3 o'clock, then I'm up and about downstairs making a cup of chocolate. [PwP #2]

5.4.5 Suggestions for improving the exercise class: '*Let's have two exercise classes a week!'*

These questions sought feedback on the exercise class content, aspects that could influence the service delivery of the exercise class, and any aspect that could be revised or developed going forward.

Provision. Participants discussed the success of the class (within the local Parkinson's group) and how they would like to see more classes delivered in the local area. Although some participants had discussed feeling tired after the class, participants discussed what could be done to reach more people in the group and accommodate increasing group size numbers. One practical solution put forward was wanted:

Let's have a second one a week. Let's have two exercise classes a week. [PwP #3]

Provide social opportunities after the class. Participants discussed how the group has successfully brought people together, but there is a sense that more could be done to strengthen these social bonds – in particular, linking social activities to the classes, which can provide a strong motivation for people to attend:

My, my mind goes to socialising . . . erm, I think we are missing a hell of a lot from not being able to socialise after the class. Erm, I think it will bring people closer together . . . it would knit it as a group a bit more. And er . . . a chance to have either a small beer, or a cup of tea or coffee, or something. [PwP #3]

5.5 Discussion

Research has provided evidence that exercise can improve both physical and cognitive performance in PwP. The development and maintenance of longer exercise interventions (>6 months) is imperative due to the chronic and progressive nature of PD, and consistent adherence to the exercise programmes and prolonged involvement is key to guarantee long-lasting positive results. Therefore, this qualitative study evaluates participants' experiences and perceptions about engaging with a community-based exercise class for PwP, its psychosocial benefits and the potential factors influencing participants adherence to the programme. Moreover, the unique setting of the class, being community-based with the implication of student helpers, has also been evaluated. The above discussion of themes that were created from focus groups including class participants and a group of

partners, revealed implications of the exercise class and provided valuable information about the project.

Participants who volunteer for exercise interventions or join community-based initiatives are often motivated to change. We found evidence of this with some participants in this study expressing that their initial motivation to join the class was based on a desire to change. It is highly likely that such motivations might influence their engagement with the class, whilst limiting the applicability of the findings to less motivated individuals. That said, we found evidence of a range of motivators. Not all participants were motivated to exercise initially with some class participants and partners discussing how they were encouraged to join the class for the potential benefits to self and others. As well as exploring reasons for joining the class, participants' opinions regarding exercise as therapy for Parkinson's suggests that the information and advice they receive might impact their engagement with interventions. For instance, we found evidence that healthcare professionals either encouraged or discouraged exercise participation, although there was consensus among the group of class participants that opinions about exercise for PwP were changing. There was also agreement that more could be done to promote exercise for PwP and explore a range of adjunctive non-pharmacological treatments. One particularly interesting, although not unsurprising, finding that emerged was the idea that researchers could do more with their findings. The class participants expressed that they are willing to devote time and effort to health research activities but would like to see more evidence that the findings are being used to benefit others, including the local Parkinson's community and healthcare professionals. Previous researchers have espoused the benefits of developing multidisciplinary team approaches to care for PwP and the views shared in this study echo those findings (Hirsch et al., 2011). Regular feedback and dissemination of research findings into the community is therefore key to supporting and sustaining interest in such activities.

A strength of the focus group approach used in this study, is that it involves users in the evaluation of the exercise class and elicits views on quality of life which are often under-described or isolated using quantitative measures (Dauwerse, Hendrikx, Schipper, Struiksma, & Abma, 2014; Den Oudsten, Lucas-Carrasco, Green, & Whoqol-Dis Group, 2011; O'Brien et al., 2008). Whilst the use of close-ended questions offers a number of advantages in pragmatic evaluation settings (Willig & Stainton-Rogers, 2017), participants can feel limited and such data often fails to fully capture their true attitudes, thoughts, beliefs and feelings, and many people would not assign values to them. This latter point was made by one participant at evaluation who did not complete a questionnaire on mood (as part of the periodic assessments) stating that they did not want to assign values to their mood states because they were concerned how such results might be interpreted.

An additional feature of the class is the high ratio of instructors to participants (1:2) with undergraduate students actively supporting the sessions. Whilst this level of support has benefitted participants and sustained the delivery of the class, it is unlikely that most community-based exercise

programs will be able to provide similar resources. However, support and supervision are clearly vital for increasing exercise self-efficacy (McAuley, Jerome, Marquez, Elavsky, & Blissmer, 2003).

The limitations of this study are centred around the homogeneity and characteristics of participants who took part in the exercise class, which limits external validity. All PwP who participated in this focus group study were male (all the spouses that took part in the second focus group were female), although this may not be all that surprising as men are reported to be at greater risk for Parkinson's than women (Taylor, Cook, & Counsell, 2007; Wooten, Currie, Bovbjerg, Lee, & Patrie, 2004). Thus, results from this study may only represent the opinions of dominant individuals in the exercise class and may, therefore, not represent the views of the rest of the class. Similarly, the views of the exercise class participants in this study may only represent the views of a subsample of participants in the class who share positive experiences of the class. It would be prudent to interview those PwP who have stopped attending the class, report low attendance, or those in the local Parkinson's network support group who do not exercise.

This study explored attitudes, opinions, and the subjective perceptions of exercise participation in PwP and partners of PwP recruited from a community-based group exercise class. We were primarily interested in topics of engagement and participation in exercise and service delivery. Some similarities can be found at the level of higher-order themes among participants in comparing the data from this study to other research that also explored key features of community-based exercise groups (Claesson et al., 2020; Rossi, Torres-Panchame, et al., 2018). Some differences, however, are apparent, such as reasons for joining the class (e.g., to benefit others) and a change in attitudes towards exercise as a therapeutic intervention (e.g., exercise can provide ancillary benefits beyond physical fitness). Many of the focus group participants commented positively on the impact of the exercise class, particularly, how exercise influences emotional well-being and quality of life. However, despite attendance data showing a high adherence rate, motivation to attend the class was susceptible to fluctuations in mood and functionality. Furthermore, in some instances, participants were apprehensive about joining the class at the very beginning. In terms of the delivery and accessibility of the exercise class, factors associated with adherence and prolonged involvement, included making the class accessible to PwP (all stages), creating fun and challenging exercises, providing encouragement, and nurturing relationships between class members, students, and instructors. Participants commented on the class itself, expressing their gratitude towards the instructor and student helpers and noted that variety and challenge were key factors influencing sustained motivation and ongoing participation.

5.6 Conclusions

Based on the findings of this study, supervised community-based group exercise appears to have utility as an effective adjunctive therapy for the management of Parkinson's through its perceived ability to positively impact physical fitness, psychological well-being, and quality of life. The class

provides members fellowship, meaningful social connections, and a perceived positive affective experience for both participants and partners, which appear to be important factors for sustaining exercise participation. These psychosocial factors have previously been shown to be important mediators of exercise and outcomes such as health-related quality of life and activities of daily living. The high level of supervision (1:2 ratio of instructor and student helpers to participants) undoubtedly has a positive impact, but it remains to be seen whether this model can be replicated in other community settings, which will be discussed in study 5 **(Chapter 7)**. The findings reported here add to the increasing evidence that community-based group exercise programmes can increase opportunities for PwP to be more active and have access to quality evidence-based exercise interventions.

Chapter 6. Study 4 – Aerobic Exercise vs Combined Cognitive and Aerobic Exercise in People with Parkinson's: Effects on Cognition, BDNF and pro-BDNF levels

6.1 Abstract

Introduction: Neuropsychological impairments, such as cognitive decline, mild cognitive impairment or dementia, are common in PwP and can impact their daily activities and quality of life. Pharmacological procedures have limited effects to treat those, and research suggests that non-pharmacological interventions, such as exercise or cognitive training, could have the potential to improve cognition. Considering the efficacy of exercise to also promote physical and cognitive improvements, the implementation of the combined modalities could be more beneficial than single-domain training. **Aim:** to investigate BDNF and pro-BDNF kinetics in PwP during different acute exercise interventions and their relationship with measures of cognitive function, with the overall aim to evaluate if there are added beneficial outcomes from combining physical and cognitive tasks compared to engaging with aerobic exercise only. **Methods**: 6 participants (age 61 ± 12 years; Hoehn and Yahr (H&Y) scale I to II) participated in 4 supervised conditions: a session of acute cycling (A), a second acute cycling session 24 h. after A (B), combined acute session of cycling and cognitive tasks (C) and a resting condition (D). In A, B and C participants completed 30 min of cycling at RPE 14 on a cycle ergometer. Outcome measures included blood and saliva biomarkers (BDNF and pro-BDNF) and measures of cognition (the Stroop test and the Free-Recall test). **Results**: visits B (second) and C (combined) were able to elicit larger improvements in the Stroop test with large (d=0.853) and small to medium (d=0.349) effects, respectively, and up to 30 and 40% improvements, respectively, in immediate long-term memory (LTM) compared to the resting control visit D. Serum and capillary BDNF levels were positively correlated with cognitive performance, whilst platelet-poor plasma BDNF correlations seemed to be headed in the opposite direction. **Conclusion**: due to the small sample size of this preliminary study, there was poor sensitivity to detect effects in cognition even though medium to large effect sizes were observed for some comparisons. Therefore, it is not yet fully known to what extent cycling combined with cognitively challenging tasks, compared to cycling alone, improves cognitive function and modulates biomarkers of neuroplasticity in PwP.

6.2 Introduction

Over the past two decades, there has been an increased interest in the protective effects of physical activity on neurological disorders, including PD and AD. In relation to PD, research has supported the beneficial effects that physical activity can have on improving functional capacity and cognitive function, and of slowing down the development of the condition (Alonso-Frech et al., 2011; Oguh et al., 2014). However, the biological mechanisms that lead to these beneficial effects are still poorly understood, as are the optimal nature of frequency, intensity, time, and type of exercise. Recently, it has been suggested that the effects of physical activity on cognitive improvements and neuroplasticity are linked to the enhancement of trophic factors signalling (Monteiro-Junior et al., 2015). Trophic factors, such as the BDNF, are important for brain neuroplasticity, survival, differentiation and neuronal growth, and they have been suggested to play important roles in exercise-induced cognitive enhancement (Campos et al., 2016; Ferris et al., 2007; Monteiro-Junior et al., 2015).

Different modalities of exercise have shown to provide significant benefits in cognitive and physical function in people with PD. These were observed after completion of walking and strength programmes (Corcos et al. 2013; Reuter et al. 2011); in addition, short and long-term improvements in balance, gait, ADLs and QoL were observed after individuals finished a boxing training programme (Combs et al., 2011). Also, indoor cycling and indoor tandem cycling proved to be effective in people with PD. These cycling modalities resulted in improved physical performance, functional mobility, balance, cognitive and upper extremity function, and reduced disease severity in people with mild to moderate PD (McGough et al., 2016; Uygur, Bellumori, & Knight, 2017). For instance, TUG (a measure of functional mobility) and single reaction time (SRT; a measure of cognitive function) significantly improved by 15% (i.e., 1.1-sec. faster) and 13% (i.e., 0.038-sec. faster), respectively, after completing 6 weeks of High-Speed-Low-Resistant recumbent cycling (Uygur et al., 2017). Moreover, both human and animal studies have observed that different forms of exercise, such as aerobic exercise (e.g., walking, running, etc), can also have an effect on the central nervous system (Erickson et al., 2011). That is, aerobic exercise increases BDNF synthesis both within the brain (Vaynman, Ying, & Gómez-Pinilla, 2004) and in the periphery (Erickson et al., 2011; Saucedo-Marquez et al., 2015; Dinoff et al., 2016; 2017).

During the last two decades, a large number of studies have investigated interventions aiming to increase BDNF and showed that BDNF levels gradually increase in the periphery during exercise that lasts at least 20 minutes, and return to baseline levels between 10 and 20 minutes after the cessation of exercise (Saucedo-Marquez et al., 2015; Schmidt-Kassow et al., 2012). Nevertheless, the timeline and kinetics of BDNF changes during and following exercise in PwP it is yet to be elucidated. Most of the research in exploring increases in BDNF synthesis has been performed on healthy young adults. Thus, there is a need to investigate potential links between the effects of exercise on BDNF and PD. Additionally, in 1999, Parain et al. showed that there is a reduced

expression of BDNF in the SN of PwP (Parain et al., 1999). Therefore, interventions aimed at modifying and reducing the decline of BDNF in PwP are needed. These could enhance our understanding of the biological underlying mechanisms by which exercise elicits a beneficial effect on PD and provides improvements in motor and cognitive functions. Moreover, the development of strategies with a clear description of frequency, intensity, duration, and type of exercise is imperative in order to develop interventions aimed at modifying and reducing the functional decline present in PD. Thus, monitoring biomarker levels and cognitive function changes would help to better understand the impact that an exercise intervention intervention might evoke.

Biomarkers related to performance, maintenance and plasticity of brain function are important to mark the progression of PD and, thus, to provide insight into the neurodegenerative processes that takes place. BDNF measured in blood has been proposed as a biomarker for cognitive benchmarks against different neurodegenerative diseases, such as PD and AD (Beeri & Sonnen, 2016; Costa et al., 2015). Although the most commonly used method for measuring BDNF is in blood collected through venepuncture or cannulation, these are invasive procedures that require skilled professionals, can cause distress, discomfort, and may involve the potential risk of infection. Moreover, these factors may limit the number of samples that can be taken throughout an intervention. In contrast, finger prick blood sampling, which also allows the collection of capillary blood, is less invasive than venepuncture, requires less clinical training, and easily allows repeated sample collection, which can improve the sampling timeline of a study and assist in evaluating changes in a specific biomarker. Thus, in the current study, finger prick BDNF measurements were used to investigate exercise-related changes whilst improving the temporal resolution of those measurements. Moreover, finger prick BDNF was compared to serum and platelet-poor plasma BDNF levels. Nevertheless, a definitive clinical validation and evaluations of clinical relevance are still lacking. Hence, a better understanding of therapies aimed at increasing BDNF levels, such as exercise, as well as the study of robust biomarkers on different sample types, are imperative to shed some light on the mechanistic responses, and help to further develop and improve potential treatment strategies for PD.

Currently, there is no consensus within the literature as to which is the best source to measure circulating BDNF: serum or plasma. Máderová and colleagues hypothesised that serum could be a good marker of long-term changes in BDNF production due to being comparable to the amount of BDNF stored in platelets (Fujimura et al., 2002; Máderová et al., 2019). However, although the majority of circulating BDNF is stored in platelets, its bioavailability may be restricted since platelets cannot cross the BBB. In contrast, plasma, which only stores small amounts of free BDNF, would contain BDNF that is able to cross the BBB (Pan et al., 1998; Poduslo & Curran, 1996). Therefore, plasma would represent a systemic readily available pool of BDNF (Serra-Millàs, 2016). However, similarly to serum, platelet-rich plasma measurements of BDNF can be easily affected by handling techniques due to the presence and activation of platelets, which presents highly variable results and hinders the accurate measurement of free active levels of BDNF in peripheral blood. To reduce the

influence of platelet-derived BDNF and accurately measure peripheral BDNF levels that reflect fluctuations in BDNF's secretion (and, potentially, the skeletal muscle), platelet-poor plasma would be a better source to evaluate acute or chronic exercise-induced changes in BDNF levels. Therefore, measuring BDNF in platelet-poor plasma could reduce sources of variability and be a better measure of bioactive available levels of BDNF.

First, this study aims to investigate the kinetics of BDNF and pro-BDNF in PD after a single exercise bout and to understand how BDNF and pro-BDNF kinetics behave after a subsequent exercise bout performed the following day. This would allow us to better understand the production of these neurotrophins in relation to exercise and start informing on the best exercise frequency to design an adequate exercise programme to enhance optimal BDNF production. To date, there remains a paucity of evidence on exercise-induced BDNF changes in PwP. Although BDNF kinetics following different intensities of exercise have been explored in young healthy adults (Schmidt-Kassow et al., 2012), to the best of our knowledge, no previous study has investigated BDNF kinetics in PwP, BDNF's cumulative effects on consecutive days of exercise and/or how BDNF and pro-BDNF respond to different exercise interventions in PwP (allowing the comparisons between cycling alone or cycling whilst performing cognitively challenging tasks). This would provide direction for future studies trying to elucidate how to schedule and distribute exercise sessions over a period of several days (e.g., over a week) and which intervention would elicit a higher BDNF response (e.g., cycling alone or combined with cognitive challenges).

Based on literature published on older adults (Máderová et al., 2019), we hypothesise that BDNF levels in people with PD will increase after the exercise bout. To date, there has been no detailed investigation of BDNF kinetics after a subsequent exercise bout on the following day in either healthy or PD populations. Nonetheless, studies from rodents have shown that repeated exercise can induce the increase of BDNF mRNA (Garza, Ha, Garcia, Chen, & Russo-Neustadt, 2004; Neeper, Gómez-Pinilla, Choi, & Cotman, 1996). Thus, we theorise that the cumulative increase of BDNF mRNA could translate to increased total levels of BDNF that will increase further following a subsequent exercise bout on the following day. Additionally, BDNF levels are not expected to change during the control resting condition. In regards to cognitive function and based on previously published research performed on young healthy adults (Ferris et al., 2007), it is expected that participants achieve better scores after the exercise bouts in comparison to the resting condition or baseline levels, and expect cognitive test scores to correlate with BDNF levels.

The second aim is to examine the relationship between biomarkers levels and cognitive measures, as well as how their relationship changes over time. While the study and treatment of motor symptoms in PwP has largely improved over the years, the management of cognitive impairments is still limited. Cognitive impairment can appear in the early course of PD and can range from subjective cognitive symptoms (e.g., losing a train of thought, forgetfulness, feeling overwhelmed making decisions or

planning, etc.) to mild cognitive impairment (MCI) (i.e., greater than the age- and education-expected cognitive decline that does not interfere with that individual's ability to perform ADLs) and, eventually, to dementia (Zhang, Aldridge, Narayanan, Anderson, & Uc, 2020). Parkinson's dementia, known as PDD, is characterised by a broad dysexecutive syndrome, severe impairments in visuospatial functioning, memory and attention, and neuropsychiatric symptoms such as hallucinations (Hanagasi, Tufekcioglu, & Emre, 2017). The prevalence of MCI in the elderly population is estimated to be 3–19%. In PD, although MCI is associated with older age at diagnosis and at disease onset, male gender, depression, more severe motor symptoms, and advanced disease stage, is found in about 20% of PD patients at the time of diagnosis (with a range of 19 to 38%) (Aarsland et al., 2010; Ritchie, 2004; Zhang et al., 2020). A longitudinal study identified that PwP that developed MCI would progress to PDD within 5 years (Pigott et al., 2015). PDD is progressive and presents severe cognitive deficits that significantly impair daily life. Pigott et al. (2015) results show that the transition from normal to impaired cognition in PwP is frequent and fast. Therefore, it is important to develop and evaluate interventions that can improve cognition. Research suggests that physical exercise may lessen motor symptoms and also improve non-motor symptoms such as cognition, mood and sleep (Reynolds et al., 2016). However, cognitive improvements are not only elicited through physical exercise interventions, such as interventions for functional mobility, aimed at maintaining or improving balance and locomotion (Gobbi et al., 2021). Cognitive training (combining intellectual and social aspects) also show the potential of maintaining cognition and psychological features in PwP, such as executive function, attention, working memory and anxiety (Gobbi et al., 2021). Recently, a combined approach has gained popularity. Mostly used in dementia prevention trials and guidelines, the combination of physical activity with cognitive training has gained interest. Research in young adults observed similar improvements in cognitive performance (assessed with a high-interference memory task) in both the combined intervention and the exercise training alone (Heisz et al., 2017). However, participants with greater fitness improvements (i.e., high responders to exercise) had better cognitive performance and greater BDNF increases with the combined intervention compared to exercise alone. This interesting observation could suggest that individuals' availability of neurotrophic factors might determine their ability to improve cognitive function. On the other hand, an extensive review evaluating studies in older adults with and without MCI, suggests that combining physical exercise and cognitive training interventions (completed either simultaneously or in a sequential manner) provides cognitive benefits that are comparable to those obtained with cognitive training alone (Gavelin et al., 2021). Considering the efficacy of physical activity to promote physical health in later life, researchers recommend the implementation of the combined modalities over single-domain training. However, there are inconsistencies not only in the FITT parameters used across research studies evaluating combined interventions, but also in the variety of cognitive training elements used (e.g., computerised, or non-computerised, targeting single or multiple cognitive domains, videogames, etc.). Moreover, although there is evidence showing that the combination of cognitive training with motor training seems to be most successful in improving cognitive function in PwP (Reuter, Mehnert, Sammer, Oechsner, & Engelhardt, 2012),

there is limited data in PD and this subject requires further evaluation. Therefore, in this study, we aim to continue to investigate the effects of both combined and isolated physical activity on cognitive function and neurotrophic factors in PwP.

Taken together, this study is designed to provide an in depth understanding of the rate of BDNF and pro-BDNF changes in PwP following an initial and subsequent bout of exercise (i.e., cycling session) in order to bring insight into the exercise-frequency that is needed to induce relevant changes that could have a beneficial neuroprotective impact and help slow PD's progression. Moreover, this study explores the benefits of engaging with cognitive tasks whilst exercising to evaluate if there are added beneficial outcomes (i.e., improved neurotrophic levels and cognitive function) from performing both tasks at the same time compared to engaging with exercise only.

6.3 Methods

6.3.1 Participants

Participants included people with PD (Hoehn & Yahr stage I, II or III) available and willing to commit to the time requirements of the study, able to see, hear, and use their hands well enough to successfully complete the assessments. Participants completed a pre-exercise screening and information form, requesting background medical information that was used to identify risks and limitations for exercise and symptoms indicating any underlying CVD. Participants were required to present low-risk status and be free from any conditions making participation in acute exercise inappropriate, according to the PAR-Q. An initial health screening was also conducted measuring BMI, BP and RHR to further check health status for CVD. Participants were excluded if they had any other neurological disease, apart from PD, cognitive decline (i.e., delirium or dementia), and any significant physical and/or sensory impairment. Participants also completed a screening test for cognitive impairment, the MMP, a sleep log at the beginning of each visit to rate sleep duration and sleep quality and the IPAQ to report their levels of physical activity during the last 7 days. Participants completed these questionnaires to control and avoid the interaction of confounding variables with the study results, such as significant changes in sleep duration, sleep quality or physical activity levels prior to each study visit.

To control for the effect of medication dosage on the study outcomes, participants were instructed to perform each assessment on an "on-medication" state (except for drug naïve participants) and take their medication between 45 min to 1h before starting the intervention, similar to Plotnik et al., 2011 described in their study. Participants were required to have had a stable medication dosage for the last 4 weeks before starting the study and maintain, if possible, the same medication and medication schedule throughout the study. Nevertheless, if a dosage or medication change was required it was not a reason to exclude this participant of the research. Any medication changes during the duration of the study would have been recorded, however, participant's medication remained constant. The

change in dosage or type of medication would have been transformed to Levodopa Equivalent Dose (Tomlinson et al., 2010) and added as a covariate in the analyses.

Following their usual Parkinson's medication regime as described above, participants were asked to refrain from consumption of food and fizzy drinks 1 hour prior to each visit and alcohol and caffeine 24 hours before each visit. Participants were also instructed to refrain from strenuous exercise for 24h before each visit and maintain a similar diet and activity habits throughout the study completion.

Participants taking part in our Observational Longitudinal Study (REF No. Prop 63_2018_19) were not allowed to take part.

Written informed consent was obtained from each participant before they took part in this project. Therefore, participants were only included if they were living independently and able to provide consent (i.e., no compromise in capacity to consent).

Participants were recruited via generic e-mail, word of mouth and attending meetings of Parkinson's UK and other PD related groups. In addition, social network sites (e.g., Facebook, Twitter, Parkinson's networks and websites, etc.) and support groups (Parkinson's Basecamp, Medway Working Age Group and others) were used to recruit participants. Tear-off leaflets, flyers and posters were used for recruitment and in order to share information about the project.

Table 6.1 Participants' demographic data at baseline. For continuous and ordinal vairables, mean values are listed with ± standard deviations. For categorical variables, frequency counts indicate the number of participants in each category followed by the proportion of the sample in parenthesis. BMI, body mass index. LEDD, levodopa equivalent daily dose. MMP, Mini Mental Parkinson's.

	N = 6
Gender	
Female	2 (33%)
Male	4 (67%)
Age (years)	61 ± 12
BMI (Kg/m^2)	27 ± 5
Years since PD diagnosis	5 ± 3
Hoehn and Yahr Stage	
Stage 1	4 (67%)
Stage 2	2 (33%)
Stage 3	0
Stage 4	0
PD Staging	
Early	5 (83%)
Moderate	1 (17%)
Advanced	0
LEDD	293 ± 370
MMP	30 ± 3

6.3.2 Study Design

Following a crossover design, each participant was randomised to a sequence of visits (each visit representing a different study condition [i.e., intervention]). In order to account for and eliminate any potential carry-over effect, a wash-out period of a minimum of one week was built into the design of the study (Evans, 2010). Conducting a crossover design allowed us to study individual participant responses to each of the conditions and examine participant-by-condition interactions.

6.3.2.1 Study Conditions

All study visits were completed at the same time of the day throughout the study, to avoid a potential effect of diurnal variations on biomarker levels and, also, to control for the effect of symptoms fluctuation on participants' performance.

The familiarisation visit was completed first and, subsequently, the order of each condition presented in **Figure 6.1** was randomised using a Latin square design approach (Kim & Stein, 2009).

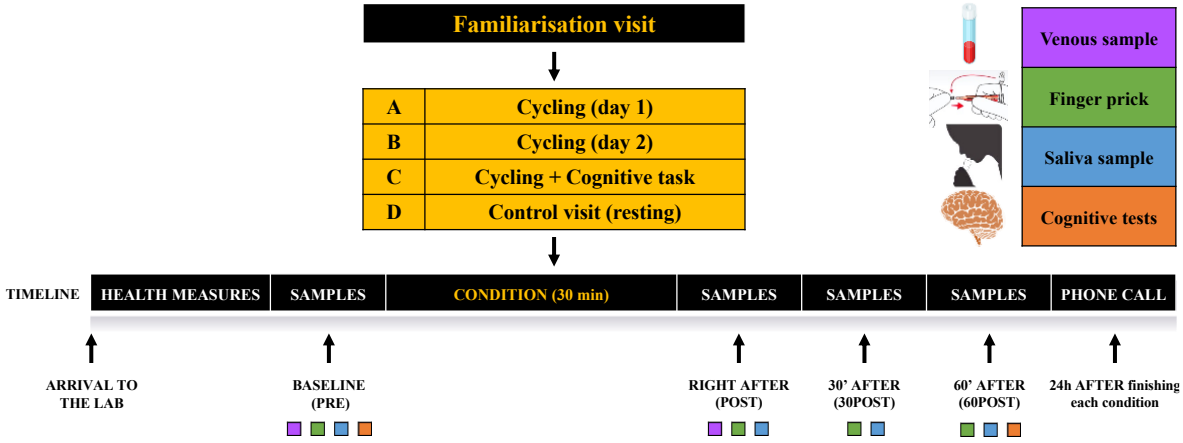

Figure 6.1 Timeline and measures schematic. This study involves a total of 5 visits to the lab. Conditions C and D are single visits, and condition A involves two visits in successive days (being condition B the subsequent visit). The study measures were performed in the following order: venous sample, finger prick and saliva samples, and cognitive tests. Participants were contacted by phone 24h after each visit in order to complete the last long-term memory test.

6.3.2.1.1 Familiarisation visit

During this visit, baseline health measures were performed (height, weight, waist circumference, resting BP and HR) and participants were familiarised to the cognitive tests and the cycling intervention in order to find the appropriate resistance equivalent to an RPE 14 for each individual (Borg, 1998). Participants completed a ramp test starting at a resistance that felt very light (i.e., RPE 9; participants' preferences initially ranged between 10 and 50 Watts) and resistance increased (e.g., 10 Watts) at the end of each minute corresponding to an increase in one stage in the RPE scale, in a

similar way to the RPE-clamped test protocol used by Mauger and Sculthorpe (Mauger & Sculthorpe, 2012), but without reaching maximal levels of exhaustion. Once the targeted intensity was found (i.e., RPE 14), participants cycled at the set resistance for 30 minutes. On this visit, participants were also familiarised to the cognitive tests that they would perform before and after each study condition.

6.3.2.1.2 Exercise (Visits A and B)

The protocol described below (see **section 6.3.3 Cycling Intervention**) was performed on two subsequent days. The timeline for taking the samples and performing the cognitive tests was kept the same for all conditions, as presented in **Figure 6.1**. This condition included one phone call 24h after the second day (i.e., visit B, in order to complete the long-term memory test). Visit A will be referred to as the 'single exercise visit' and Visit B will be referred to as the 'second exercise visit'.

6.3.2.1.3 Exercise combined with cognitive tasks (visit C)

This is a visit where participants completed the exercise intervention (see **section 6.3.3 Cycling Intervention**) whilst completing cognitively challenging tasks that consisted of a battery of 5 short cognitive challenges: the Implicit Association Task, Mental Rotation Task, Visual Search Task, Wisconsin Card Sorting and Go/No-Go Task; each of them lasting between 2 and 4 minutes approximately. The cognitively challenging tasks were performed whilst cycling for 30 minutes at RPE 14 (as explained in **section 6.3.3 Cycling Intervention**) and there was a standardised cognitive recovery of at least 1 min. between tasks. The timeline for taking the samples and performing the cognitive tests was kept the same for all conditions, as presented in **Figure 6.1**. Twenty-four hours after completing this visit, participants received a phone call to complete the long-term memory test. Visit C will be referred to as the 'combined visit'.

6.3.2.1.4 Control (visit D)

During the control visit participants followed the same schedule as the other visits and both the samples and cognitive tests were completed at the same time as the experimental visits. However, instead of the cycling task, participants sat in a chair and completed cognitively non-demanding tasks (e.g., chatting to the researcher, reading an easy book, colouring meditation/mandala, etc.). Twenty-four hours after completing this visit, participants received a phone call to complete the long-term memory test. Visit D will be referred to as the 'resting visit'.

6.3.3 Cycling Intervention

6.3.3.1 Exercise Intervention

The exercise intervention consisted of 30 min of cycling at RPE 14, which corresponds to a rating between "somewhat hard" and "hard" (Borg, 1998). This specific level of exertion for the exercise intervention was selected to ensure that participants were cycling at an intensity that was sufficient to promote neurotrophic factor increases. Based on previous research and current guidelines, an RPE of 14 approximately coincides to working at an intensity of 70% heart rate reserve (HRR) (Garber et al., 2011; Ross et al., 2019). Moreover, supported by Ross and colleagues, cycling at 70% HRR increases circulating BDNF levels (Ross et al., 2019). More specifically, the majority of exercise interventions used in PD research are of moderate-to-high exercise intensity with HRR levels ranging from 60 to 70%, which has been suggested to be equivalent to RPE 14 by PD guidelines (Bouça-Machado et al., 2020; Martignon et al., 2020). Therefore, this is an intensity that has been proven to be feasible and safe for PwP (Schenkman et al., 2017; van der Kolk et al., 2019). Moreover, the use of RPE ratings, which is a scale that has been validated for PwP, is recommended for all stages of PD (1–5 H&Y) by specific guidelines for tailored clinical testing and exercise prescription for PwP (Martignon et al., 2020; Penko et al., 2017). Therefore, participants completed 30 min of cycling at RPE 14 on a cycle ergometer (Corival, Lode B.V. Medical Technology, Groningen, Netherlands) as the exercise intervention.

Different methods are commonly used to estimate exercise intensity during cardiorespiratory exercise, such as HRR, percentage of the maximum HR (%HRmax), maximal oxygen consumption (VO_2max), RPE, amongst others. VO_2max, which requires stress testing for its measurement, is considered the gold standard measure of maximal cardiovascular capacity, however, the majority of published studies only include healthy young participants and data in older adults is limited (Huggett, Connelly, & Overend, 2005). Many older adults are unable to reach a maximal exercise effort to satisfactorily complete a VO_2max test (Gill, DiPietro, & Krumholz, 2000). Moreover, placing older persons under a maximal stress test could put them at risk of iatrogenic complications (e.g., due to the presence of asymptomatic coronary heart disease [CHD]). In both sexes, the risk of CVD (which includes CHD) increases with age (Hajar, 2017; Jousilahti, Vartiainen, Tuomilehto, & Puska, 1999), thus, in this study, participants completed a pre-exercise screening form providing background medical information that was used to identify risks and limitations for exercise linked to PD, and symptoms indicating any underlying CVD. However, it was predicted that most of the study participants would be aged over 60 years old (in fact, 50% of the study participants were older than 65) and it was, therefore, decided not to undergo maximal testing to reduce any potential risks of adverse cardiac events among non-symptomatic participants that were interested in taking part in this research.

The RPE-clamped test protocol described in the **section 6.3.2.1 Study Conditions** was used to find the resistance at which participants had to cycle at in order to reach an intensity of RPE 14. The Karvonen formula presented below was used to calculate the 70%HRR value for each participant on each visit, which was used as a reference to compare participants' HR to whilst cycling at RPE 14. The principal instruction given to participants was to cycle at an intensity of RPE 14 during the intervention, however, researchers used the 70%HRR information to ensure that participants' HR did not exceed this value (i.e., for safety reasons) or that it was not considerably and continuously lower than the 70%HRR target. Fixed percentages of HRR are commonly used for the prescription of exercise intensity in rehabilitation, disease prevention programs, and Parkinson's research (ACSM, 2014; Silveira et al., 2018; Uhrbrand et al., 2015). The %HRR is calculated according to the Karvonen formula (Karvonen, Kentala, & Mustala, 1957):

$$\%HRR = (\%\ exercise\ intensity) * (Maximal\ predicted\ HR - Resting\ HR) + Resting\ HR$$

For this study:

$$70\%\ HRR = 0.7 * (Maximal\ predicted\ HR - Resting\ HR) + Resting\ HR$$

To calculate the HRR with the Karvonen method, a value of maximal HR is required. Due to the safety reasons mentioned above, a maximal stress test was not performed, therefore, predictions of maximal HR were used instead. Maximal HR is commonly predicted with the equation 220 – age (Fox, Naughton, & Haskell, 1971; Karvonen et al., 1957). However, its validity has not been established in a study with older adults (i.e., > 60 years of age). Moreover, this equation underestimates maximal HR past the age of 40 years, markedly so with further increases in age (Tanaka, Monahan, & Seals, 2001). The Tanaka equation is a specialised regression equation that has proven to be superior to calculate age-predicted values of maximal HR for older participants (Tanaka et al., 2001). Therefore, the following equation was used to calculate the maximal predicted HR of each participant:

$$Maximal\ predicted\ HR = 208 - 0.7 * Age$$

6.3.3.2 Warm-up and Cool-down

Before each session, participants were fitted with a HR strap (Polar T31-Coded, Polar Electro, Warwick, UK) and both HR and RPE were monitored continuously and recorded every 5 minutes (HR monitored with a monitor [Polar FT1, Polar Electro, Warwick, UK]). At the beginning of the exercise intervention, participants completed a 5 min warm up cycling at RPE 11 and, in the last three minutes of the warm-up, the resistance was gradually increased until participants achieved their target of RPE 14. The targeted resistance was set during the familiarisation visit explained above

(see **section 6.3.2.1 Study Conditions**). At the end of the 30 minutes, participants performed a 2 min cool-down cycling with no resistance.

Throughout the intervention, participants would cycle at their preferred cadence and the workload would be adjusted by the cycle ergometer (Corival, Lode B.V. Medical Technology, Groningen, Netherlands). However, it was suggested to the participants to maintain at least a cadence of 60 rpm or above.

6.3.4 Samples

The samples used in this study were collected and analysed following the steps explained in **Chapter 2** (see **sections 2.6, 2.7** and **2.8**).

6.3.4.1 Blood sampling

Blood was collected via venepuncture on two occasions during each visit (before each condition [PRE] and immediately after [POST]) and via finger prick (before each condition [PRE], right after each condition following the blood sample [5'POST], 30 min after [30'POST] and 1h after [60´POST]). See **Figure 6.1**. On each occasion, 1 red and 1 purple vacutainer were used to obtain serum and platelet-poor plasma, respectively. Afterwards, samples were handled following the methodology explained **Chapter 2** (see **section 2.6**).

6.3.4.2 Saliva sampling

Saliva samples were also collected on each assessment on different occasions: before each condition (PRE), right after each condition following the blood sample (10'POST), 30 min after [35'POST] and 1h after [65´POST]). See **Figure 6.1**. Saliva sample collection and handling are described in **Chapter 2** (see **section 2.7**).

6.3.5 Cognitive and Mood Assessments

Cognitive function and mood were assessed before and after each condition; but always after blood and saliva samples were taken. We aimed to evaluate cognitive function independently by assessing attention, reaction time and response inhibition with the Stroop test, and long-term memory with the Free-Recall test, at different time points of the study. Both tests were computerised, programmed, and performed on MATLAB R2019b (The MathWorks, Inc.).

6.3.5.1 Stroop Test

Attention, reaction time and response inhibition were assessed with the Stroop test, where colour words (which were also coloured) were presented to participants who had to respond as quick as possible to these stimuli presented on the screen (Gualtieri & Johnson, 2006; Hsieh, Chen, Wang, & Lai, 2008). Over the years, several versions of the Stroop test have been developed. In this study, only three colours/colour words were used (red, green, and blue). On one hand, participants had to press the coloured key that corresponded to the colour of the word presented while fully ignoring the actual word meaning. There were congruent (i.e., the word corresponds to its colour, e.g., **red**) and incongruent stimuli (i.e., the word states a colour different than the colour of the word, e.g., **red**). However, when the word appeared in white colour, participants had to press the key that corresponded to the colour word meaning. The test was divided into four blocks, each separated by a 30-sec rest period. Thirty-six stimuli were presented in each block to the participants (i.e., 12 congruent, 12 incongruent and 12 neutral stimuli) in a random order, for a total of 144 stimuli per test. The stimuli were displayed in the middle of the computer screen and the interval response-stimulus onset was set at 2-sec. A fixation cross was shown in the screen for 1-sec between stimuli. The test was administrated at the start of each visit (before the start of each intervention) and after the intervention, and the total time to complete the four blocks was approximately four minutes. Outcome measures were accuracy (%) and reaction time (sec) (Bruyer & Brysbaert, 2011).

6.3.5.2 Free-Recall Test

The Free-Recall Test was used to assess long-term memory (LTM) and directed attention (Murdock, 1960, 1962; Tomporowski, Ellis, & Stephens, 1987). A total of four different sets of codes (i.e., stimuli) were created for each participant. Each code included 40 stimuli (i.e., images with their name written underneath) that were presented to the participants in two blocks of 20 stimuli each. Images were shown for 3 seconds, and between stimuli, there was a fixation pause of 3 seconds. In total, this test took approximately four minutes to complete. The stimuli were presented to participants at the start of each visit (before the intervention) and participants had to recall as many of the presented stimuli as possible on two different occasions: after the interventions (i.e., early free-recall) and 24h later via a phone call (i.e., delayed free-recall). Participants had a maximum of 7 min to provide a list of the recalled items and the number of correctly recalled words was used as the outcome measure.

6.3.5.3 Mood

Mood was assessed using the BRUMS (Terry, Lane and Fogarty, 2003) on different occasions. At the start of each visit, the standard instruction to ask was: How have you felt during the past week? After the intervention, the standard instruction to ask was: How do you feel right now?

6.3.6 Sample Size and Statistical Analysis

Sample size was estimated using data from a published study that investigated BDNF kinetics in an exercise intervention study design (Schmidt-Kassow et al., 2012). At an alpha level and power set at 0.05 and 0.90, respectively, the total sample size required to determine significant changes in BDNF before and after an exercise intervention was estimated to be 15 participants (based on Schmidt-Kassow *et al.* 2012) (G*Power software, version 3.1.9.6). Therefore, accounting for a 20% drop out, 18 participants were recruited. However, due to the interest generated in the study, a total of 20 individuals with PD were recruited and scheduled as participants. Unfortunately, the COVID-19 pandemic struck, and in March 2020, following UK Government guidance, the University of Kent released a statement saying that laboratory facilities and face-to-face contact with individuals outside of your household were not permitted. Hence, the study had to suddenly stop as well as any data collection that had already started (i.e., 18 participants in total). Seven months after the study was put on hold (October 2020), researchers were allowed to test again but in November 2020, the University of Kent (following the UK Government advice) released a communication prohibiting the participation of vulnerable public in research studies. The University dictated that adults above the age of 65 were deemed vulnerable and, therefore, they could only take part after December 2nd 2020. That meant that all the recruited participants, except for one, had to wait until restrictions were eased. When the University allowed the participation of older adults (>65 years old) in research studies, participants were contacted and those that were willing to take part throughout the month of December 2020 were tested. Initially, 10 participants were 're'-recruited and started the intervention. However, 4 participants dropped-out due to: not being able to complete past the second visit due to presenting COVID-19 symptomatology (1 participant), for safety reasons related to COVID-19 (2 participants) and CVD (1 participant suffered a stroke before attending the first visit after measures were eased). Adhering to all the restrictions that were put in place due to the COVID-19 pandemic, 6 participants were able to complete the study. Due to time restrictions, 1 participant completed all the visits except for the resting control visit, as stated in the results section.

Statistical analyses were conducted using software packages (SPSS 27 [IBM, Armonk, NY], GraphPad Prism Software version 8 for MacOS [GraphPad Software, San Diego, CA, USA] and R, version 4 [www.r-project.org]). Appropriate parametric assumptions were tested for each test and corrected by appropriate transformations if needed. If transformations were not successful, appropriate non-parametric tests were used. Spearman's rho was used to study the strength of the association between biomarker levels across sample types and cognitive outcomes. Moreover, to determine changes across different conditions and between different time points, repeated measures ANOVA and paired t-tests were performed. Significant interactions or main effects were analysed post-hoc using Bonferroni-corrected t-tests where appropriate. Otherwise stated, due to the small sample size of the study, the geometric mean was used to report the results presented in the following section.

6.4 Results

6.4.1 Session measurements

Participants' BP and RHR were measured at the beginning of each visit. Subsequently, maximal predicted HR and 70%HRR were calculated as described in **section 6.3.3.1** (see

Table 6.2).

Table 6.2 Participants' session measurements. Data are presented as mean ± standard deviation. SBP, systolic blood pressure. DBP, diastolic blood pressure RHR, resting heart rate. HR, heart rate. HRR, heart rate reserve.

Visit	SBP	DBP	RHR	Maximal HR	70%HRR
Familiarisation (n = 6)	136 ± 9	83 ± 10	74 ± 10	166 ± 9	138 ± 4
A (single) (n = 6)	137 ± 7	82 ± 16	78 ± 9	166 ± 9	139 ± 5
B (second) (n = 6)	139 ± 10	84 ± 17	74 ± 10	167 ± 9	138 ± 4
C (combined) (n = 6)	131 ± 12	83 ± 18	73 ± 11	167 ± 9	138 ± 3
D (resting) (n = 5)	133 ± 12	84 ± 10	74 ± 12	166 ± 9	139 ± 4

These parameters were continuously evaluated during the exercise intervention, at which participants cycled for 30 min at RPE 14, to ensure an appropriate exercise intensity as well as participants' safety during the exercise. On average, participants cycled at a load of 60 ± 21 Watts during 30 min whilst maintaining a cadence of 71 ± 12 revolutions per minute (RPM).

6.4.2 Cofounding variables

To control and avoid the interaction of confounding variables with the results and separate their influence from the intervention effects, participants had to fill in three questionnaires prior to each visit: the IPAQ short form (to estimate the levels of total physical activity in MET-hours/week during the 7 days prior to each visit), a sleep log (to rate sleep duration and quality the night before each visit), and BRUMS (to assess participants' mood during the 7 days prior to each visit). Moreover, using the computer programme MATLAB R2019b (The MathWorks, Inc.), a single measurement of participants' reaction time (SRT, Single Reaction Time) was completed at the beginning of every visit, before the battery of cognitive tests, to evaluate participants response execution (i.e., to detect significant increases or reductions in slowness of finger movements due to fluctuations in PD symptomatology or medication effects). Normality checks were carried out and one-way repeated

measures ANOVA (for normally distributed data) tests were performed to evaluate potential changes of the confounding variables during the study.

A one-way repeated measures ANOVA with visit (visit A/B, C and D) as the repeated measure, showed that IPAQ levels did not significantly change between visits ($F(2,8)=2.141$, $P=.180$). Visits A and B were performed on two subsequent days and only one IPAQ questionnaire was completed to evaluate the levels of physical activity during the 7 days prior to these consecutive visits. On average, participants weekly levels of physical activity were 45 ± 17 MET-hours.

Participants' sleep duration and quality the night before each visit remained constant throughout the four study visits ($F(3,12)=0.082$, $P=.968$, and $F(3,12)=2.019$, $P=.165$, respectively). Participants slept an average of 7.8 ± 1.0 hours rated as 3.7 ± 1.0 (1 being very poor to 5 being very good).

The completion of BRUMS questionnaire before each visit revealed no significant changes in anger ($F(2,12)=0.154$, $P=.859$), confusion ($F(2,12)=0.277$, $P=.763$), depression ($F(2012=0.497$, $P=.621$), fatigue ($F(2,12)=0.107$, $P=.899$), tension ($F(2,12)=0.131$, $P=.879$) or vigour ($F(2,12)=0.466$, $P=.638$) throughout the study. Visits A and B were performed on two consecutive days and only one BRUMS questionnaire was completed to assess participants' mood the week before these visits.

Finally, participants SRT did not significantly change across the study visits ($F(3,9)=0.688$, $P=.582$, with an average SRT of 0.062 ± 0.046 sec). Therefore, it could be assumed that participants' response execution before completing each of the study conditions remained fairly constant and did not directly affect the study outcome measures (e.g., reaction time measured in the Stroop test).

6.4.3 Cognitive and Mood Outcomes

6.4.3.1 Stroop Test

It is worth noting that one participant did not complete Visit D due to not being able to attend to the lab for personal reasons before COVID-19 restrictions were put in place. Therefore, two different data analyses were performed. First, data from 6 participants was used to evaluate differences between conditions A, B and C. The results of a 2 x 3 mixed ANOVA revealed that the main effect of time (i.e., PRE vs POST) or condition (visit A, B or C) on participant's reaction time was not significant for any of the stimulus type (i.e., congruent, incongruent or neutral, P values$\geq$.504, $\eta^2_p \leq 0.087$, i.e., ($F(2,15)=0.717$, $P=.504$, $\eta^2_p=0.087$). Secondly, the previously mentioned outcomes were compared to the results obtained from a 2 x 4 mixed ANOVA performed on the data of 5 participants who completed all 4 different study visits (these analyses included the data from the resting visit D).

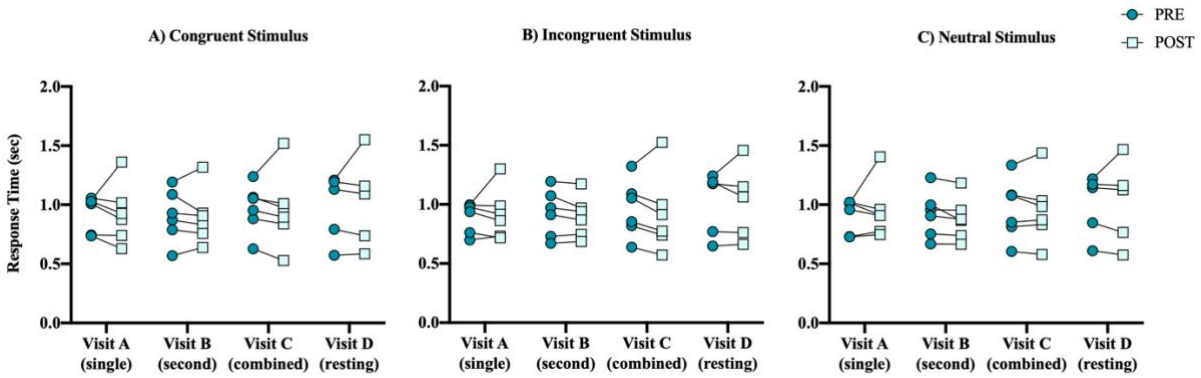

Figure 6.2 Individual response times for the Stroop test. Six participants completed visit A (single exercise), B (second exercise) and C (combined), and 5 participants completed visit D (resting).

A similar pattern of results was obtained, that is, the main effect of time, condition and their interaction, for each stimulus type, were not significant (P values≥.674, $\eta^2_p \leq 0.076$, i.e., ($F(3,19)=0.520$, P=.674, $\eta^2_p=0.076$, see **Figure 6.2** and **Table 6.3**). However, due to the small sample size of the data, it was also decided to evaluate PRE and POST measurements with both one-way repeated measures ANOVA and paired samples t-test (to individually assess each study condition). No significant changes were observed at PRE or POST across the different study visits for any of the stimulus type (P values≥.093). Moreover, analyses revealed that there were no significant differences between PRE and POST values across the different study conditions (P values≥.091). Nonetheless, it is worth noting that visits B and C provided the biggest improvements in Stroop test performance with medium to large and small to medium effects, respectively (see % change and effect sizes in **Table 6.3**) and, visit B, presented a trend towards the predicted direction that was observed for the Neutral Stimuli ($t(5)=2.089$, P=.091, d=0.853). During the resting visit (i.e., visit D), compared to PRE values, participants performed similarly, or worse at POST (see **Figure 6.2**).

Table 6.3 Stroop test performance presented in sec., percentage of change between PRE and POST assessments on each visit, and accuracy of the responses (percentage of correct responses). Six participants completed visit A (single exercise), B (second exercise) and C (combined), and 5 participants completed visit D (resting). GSD, Geometric Standard Deviation.

		Congruent		Incongruent		Neutral	
Visit	**Parameters**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
A (n = 6)	**Geometric Mean (GSD)**	0.924 (1.188)	0.898 (1.305)	0.886 (1.166)	0.901 (1.245)	0.901 (1.180)	0.930 (1.252)
	% Change	-3%		2%		3%	
	Cohen's *d* (95% CI)	0.057 (-0.746 – 0.856)		0.180 (-.979 – 0.636)		0.229 (-1.030 – 0.594)	
	% Accuracy (GSD)	97 (1.051)	98 (1.027)	97 (1.087)	98 (1.028)	98 (1.040)	99 (1.019)
B (n = 6)	**Geometric Mean (GSD)**	0.882 (1.298)	0.874 (1.276)	0.907 (1.249)	0.884 (1.212)	0.901 (1.240)	0.866 (1.222)
	% Change	-1%		-3%		-4%	
	Cohen's *d* (95% CI)	0.083 (-0.722 – 0.881)		0.598 (-0.303 – 1.452)		0.853 (-0.126 – 1.775)	
	% Accuracy (GSD)	99 (1.019)	99 (1.018)	97 (1.046)	97 (1.072)	99 (1.016)	99 (1.017)
C (n = 6)	**Geometric Mean (GSD)**	0.949 (1.262)	0.916 (1.405)	0.938 (1.292)	0.877 (1.394)	0.932 (1.320)	0.922 (1.348)
	% Change	-4%		-6%		-1%	
	Cohen's *d* (95% CI)	0.064 (-0.740 – 0.862)		0.349 (-0.494 – 1.161)		0.069 (-0.736 – 0.867)	
	% Accuracy (GSD)	97 (1.052)	97 (1.035)	96 (1.044)	99 (1.018)	100 (1.000)	99 (1.027)
D (n = 5)	**Geometric Mean (GSD)**	0.942 (1.388)	0.967 (1.471)	0.972 (1.347)	0.979 (1.376)	0.967 (1.343)	0.967 (1.452)
	% Change	3%		1%		0%	
	Cohen's *d* (95% CI)	0.266 (-1.146 – 0.643)		0.110 (-0.983 – 0.776)		0.155 (-1.028 – 0.737)	
	% Accuracy (GSD)	98 (1.027)	96 (1.035)	99 (1.012)	97 (1.032)	99 (1.019)	98 (1.028)

The accuracy data of the Stroop test were not normally distributed. Therefore, Wilcoxon signed ranks tests were used to evaluate differences between time points. Friedman tests were conducted to compare the accuracy of data across study visits. Overall, accuracy data did not significantly differ between study visits (P values≥.126) and between PRE and POST time points (P values≥.059).

The steps reported in this results section were repeated for all the outcome measures analysed in this chapter and the same pattern of results were observed between the two different mixed ANOVAs that were evaluated. Therefore, bearing in mind the small sample size of this study and to be consistent with the analyses used across the results section, one-way repeated measures ANOVA and

paired samples t-tests were used to investigate differences across study visits and between PRE and POST measurements, respectively.

6.4.3.2 Long-Term Memory

The geometric mean of the correctly recalled items immediately after each condition (i.e., POST) and 24 hours later are shown in **Figure 6.3** (n=5) and **Table 6.3** (n=6). During the visits A, B and C, participants performed slightly better than during the resting condition (visit D). However, the differences between study conditions, analysed with repeated measures ANOVA, were not significantly different (POST: $F(3,12)=0.665$, $P=.590$, $\eta^2_p=0.142$; 24h: $F(3,12)=0.732$, $P=.553$, $\eta^2_p=0.155$; see **Figure 6.3**).

Table 6.4 LTM test performance presented as the number of correctly recalled items after each intervention (POST) and 24 hours later (24h). Six participants completed visit A (single exercise), B (second exercise) and C (combined). Five participants completed visit D (resting). GSD, Geometric Standard Deviation.

Visit	Parameters	POST	24h
A (n = 6)	**Geometric Mean (GSD)**	12 (1.833)	11 (1.915)
B (n = 6)	**Geometric Mean (GSD)**	13 (1.907)	13 (1.821)
C (n = 6)	**Geometric Mean (GSD)**	14 (1.772)	12 (1.783)
D (n = 5)	**Geometric Mean (GSD)**	10 (2.747)	10 (2.543)

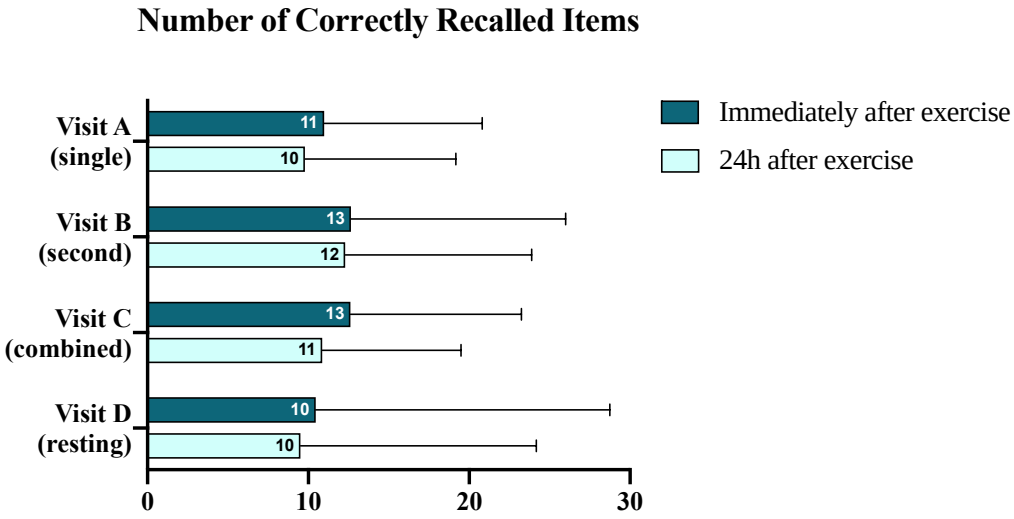

Figure 6.3 LTM performance presented as the number of correctly recalled items. Only participants who completed all 4 study visits are included in this figure (n = 5): visit A (single exercise), B (second exercise), C (combined), and D (resting). Data are presented as geometric mean and geometric standard deviation bars.

It is worth noting that one participant consistently exhibited greater Stroop colour-word interference (see **Figure 6.2**) and lower LTM scores than the other participants, which means that they experienced a higher delay in reaction time between automatic and controlled processing of information, inferior capacity to recall and worse directed attention compared to the other participants. Due to meeting all the inclusion criteria and the small sample size of the study, it was decided not to exclude this participant from the final analyses. However, Stroop and LTM test results were also evaluated without including this participant. Although a similar pattern of results was obtained for the Stroop test (i.e., interactions were non-significant), paired samples t-test showed significant improvements for the Congruent Stimuli after visit A (single exercise; (t(4)=3.238, P=.032, *d*=1.448) and visit C (combined; (t(4)=5.677, P=.005, *d*=2.539), and for the Incongruent Stimuli after visit C (combined; (t(4)=7.209, P=.002, *d*=3.224), with large effect sizes. Regarding the measures of LTM, there were no significant differences between conditions measured at POST or 24h. However, in line with the results that were initially obtained including all participants, cycling on two consecutive days was able to elicit the best LTM results, both, immediately after finishing visit B and 24 hours later. These results suggest that the cognitive processes and functions that are operating with each intervention might be different.

6.4.3.3 Mood

The data from the BRUMS questionnaire completed after each visit were not normally distributed. Therefore, Friedman tests were conducted to compare the POST scores of the six identifiable affective states on each study visit. No significant differences were found between study visits for any of the six affective states. Furthermore, Wilcoxon Signed Ranks Tests were used to evaluate differences between PRE (i.e., how have you felt during the past week?) and POST scores (i.e., how do you feel right now?). There was found to be a significant decrease in anger scores after completing visit A (*Z*=-2.226, P=.026). Although not reaching significance, all POST scores for anger in all visits were 0, thus, Cohen's *d* could not be calculated (Visit B, C and D [*Z*=-1.841, P=.066; *Z*=-1.826, P=.068; *Z*=-1.604, P=.109, respectively). Participants' depression and tension scores followed a similar trend. That is, all participants scored 0 at POST in visits A and B although significance was not achieved (depression: P=.068 and .066, respectively; and, tension: P=.066 and .059, respectively). Interestingly, participant's perceptions of fatigue were lower at POST for all study visits (presenting moderate to large effect sizes), but only visit D presented significantly lower levels of fatigue compared to PRE values (*Z*=-2.032, P=.042, *d*= 1.574). Confusion and vigour scores did not significantly change after any of the study visits.

6.4.4 Samples Analyses

6.4.4.1 Saliva BDNF & pro-BDNF

As explained in Chapter 3 (**section 3.4.1 Results of the BDNF Spike/Recovery Assays**), during the ELISA development procedure, saliva samples did not provide acceptable recovery results. Therefore, it was recommended that all future saliva assays should add a spike recovery test for each sample being analysed and only samples with recovery values between 80% and 120% would be included. This recommendation meant that the analysis of saliva samples would require double the amount of sample volume and ELISA wells needed to complete the analyses (i.e., in total, more than 5 ELISA plates would be needed to solely analyse saliva BDNF, without accounting for pro-BDNF measurements). Unfortunately, the funding for this thesis was limited, which restricted the number of ELISA kits that could be purchased. Although saliva collection was completed, handled, and appropriately stored throughout the study, due to the difficulties experienced in saliva analysis and the limited available ELISA kits, it was decided to prioritise BDNF and pro-BDNF blood analyses and saliva BDNF and pro-BDNF were not assessed. The latter samples could be analysed in future research.

6.4.4.2 Blood BDNF & pro-BDNF

In order to evaluate the effect of different interventions on BDNF and pro-BDNF levels, blood samples were collected and analysed. Accordingly, serum and PP-P samples were collected through venepuncture before and after each condition (i.e., PRE and POST). In order to improve the temporal resolution of the sampling timeline for the neurotrophic factors, FPP samples, which are finger-tip capillary blood samples, were collected from a finger prick on each visit on 4 occasions: before each condition (PRE), right after each condition following the blood sample (5'POST), 30 min after (30'POST) and 1h after (60´POST), as shown in **Figure 6.1**.
BDNF levels were measured in PP-P, serum and FPP samples. Pro-BDNF levels were measured in PP-P to allow the measurement of the pro-BDNF/BDNF ratio.

A 2 x 4 mixed ANOVA revelated a non-significant interaction between sample collection time and study condition ($F(9,57)=.997$, $P=.453$), although a large effect size was found ($\eta^2_p=0.136$).

Initially, a repeated measures ANOVA showed a significant main effect of time for PRE levels of FPP across the different study visits ($F(3,12)=3.936$, $P=.036$, $\eta^2_p=0.496$). However, Bonferroni adjusted post hoc pairwise comparisons did not reveal any significant differences in PRE levels of FPP across study visits. Although FPP BDNF levels behaved differently in each study visit, as shown in **Figure 6.4**, no significant changes were observed between time points or study visits.

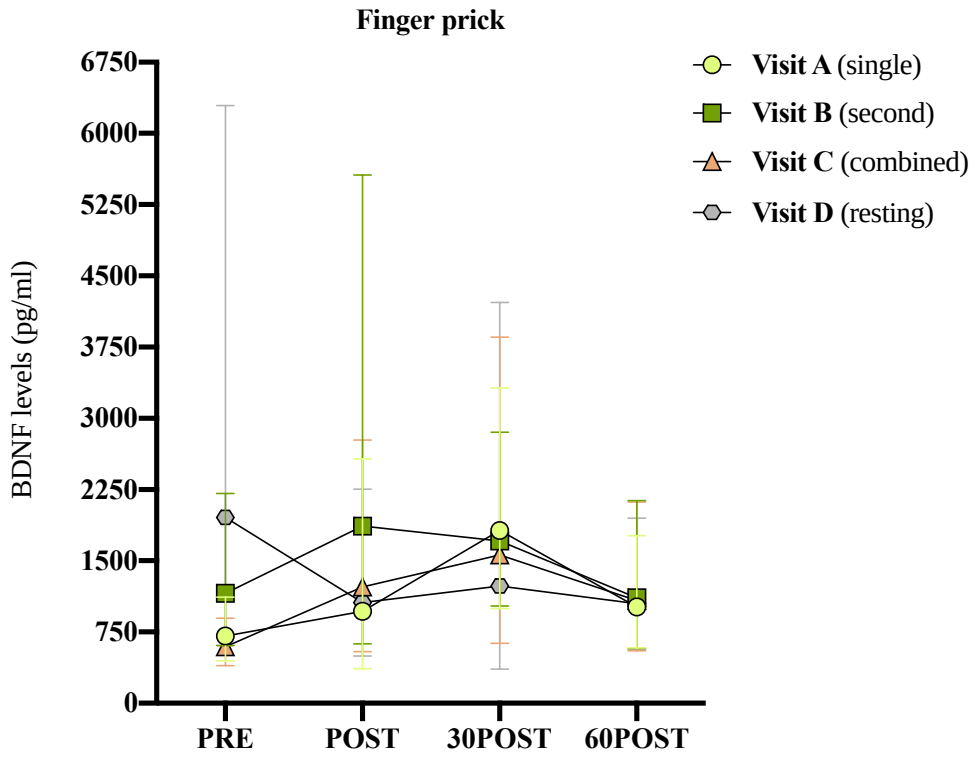

Figure 6.4 Finger prick BDNF values measured in pg/ml over 4 different time points. Only participants who completed all 4 study visits are included in this figure (n = 5): visit A (single exercise), B (second exercise), C (combined), and D (resting). Data are presented as the geometric mean and geometric standard deviation bars of each time point.

FPP BDNF values presented some individual variability as shown in **Figure 6.5**.

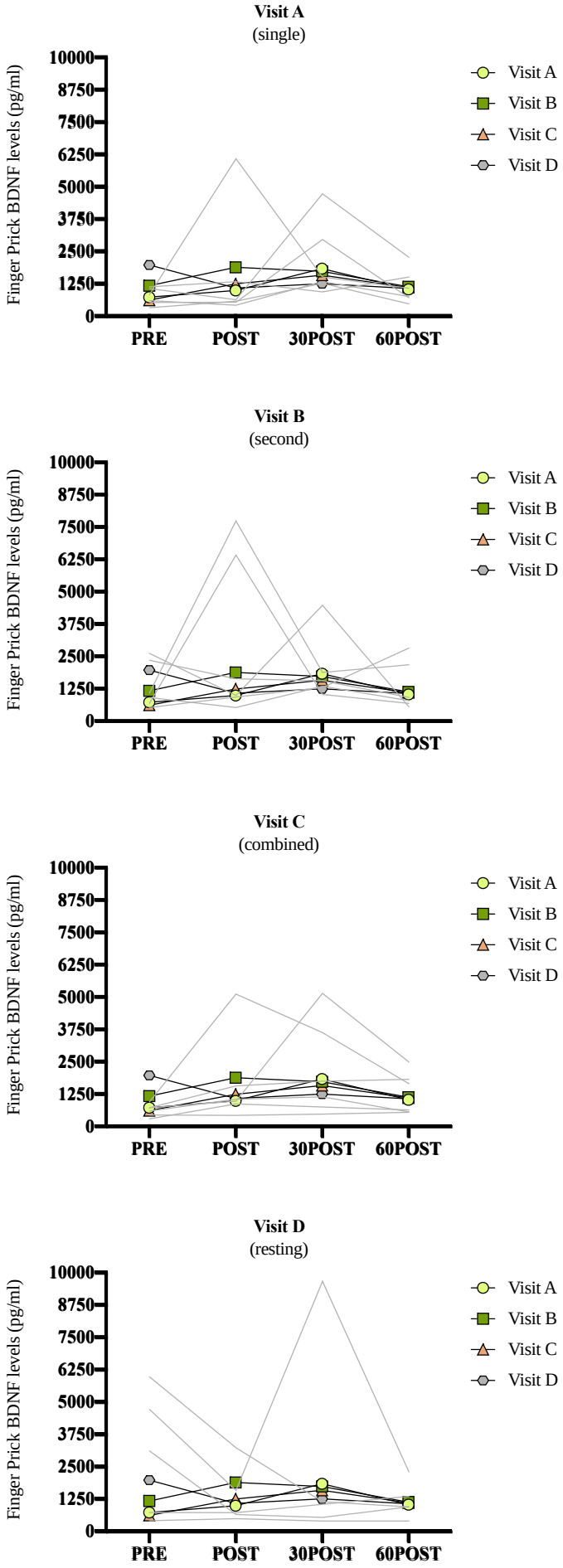

Figure 6.5 Individual responses of finger prick BDNF over time (represented in grey lines) separated by study visit: A (single exercise), B (second exercise), C (combined), and D (resting).

PP-P levels of BDNF did not significantly change at POST compared to their PRE levels (individual values are presented in **Figure 6.6**).

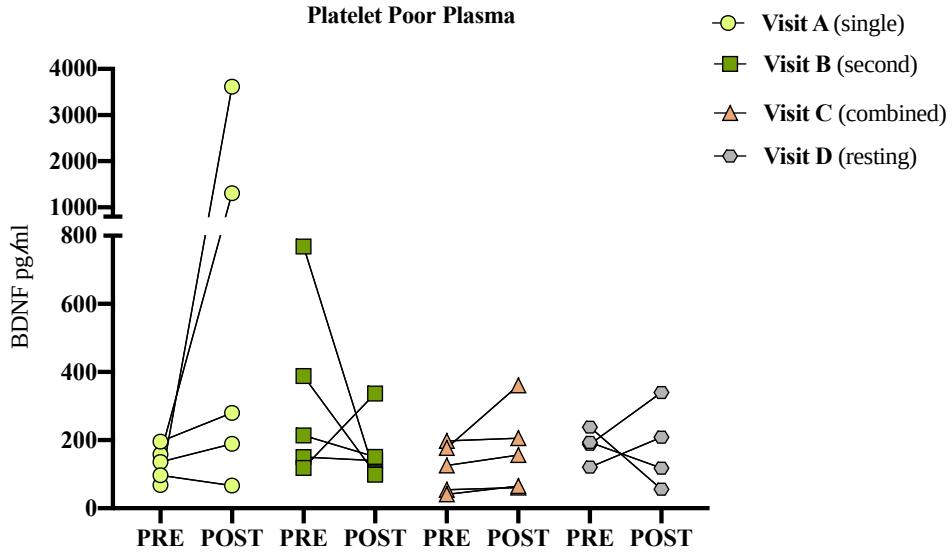

Figure 6.6 Individual responses of platelet poor plasma BDNF at PRE and POST separated by study visit: A (single exercise), B (second exercise), C (combined), and D (resting).

In visit B, POST Pro-BDNF levels measured in PP-P significantly increased compared to PRE values (t(4)=-7.595, P=.002, *d*=3.397), whilst none of the changes observed at POST in the other visits were significantly different from their PRE values (see **Figure 6.7**).

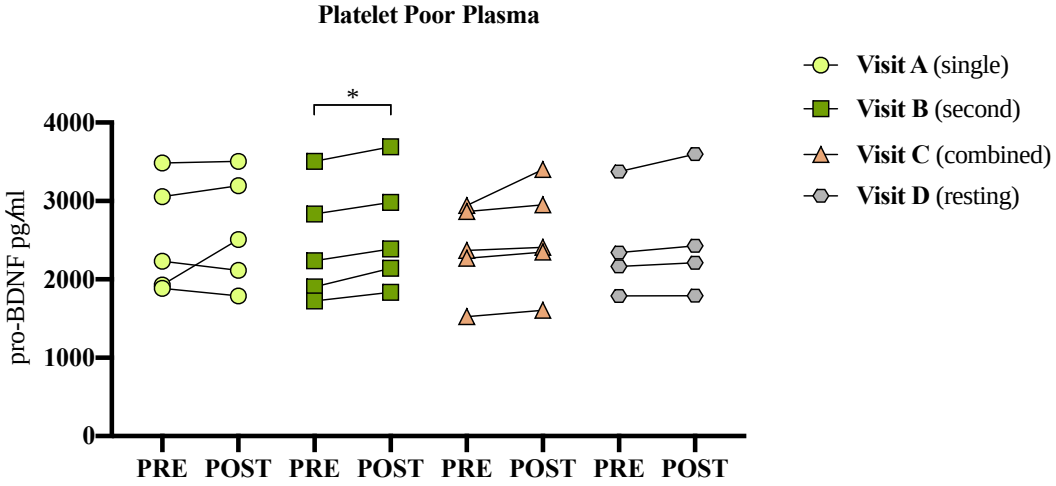

Figure 6.7 Individual responses of platelet poor plasma pro-BDNF at PRE and POST separated by study visit: A (single exercise), B (second exercise), C (combined), and D (resting). *Significant difference between PRE and POST values ($p<0.05$).

The pro-BDNF/BDNF ratio was evaluated in PP-P. Although the pro-BDNF/BDNF ratio behaved differently across study visits (see **Figure 6.8**), there were not statistically significant changes across timepoints and study visits.

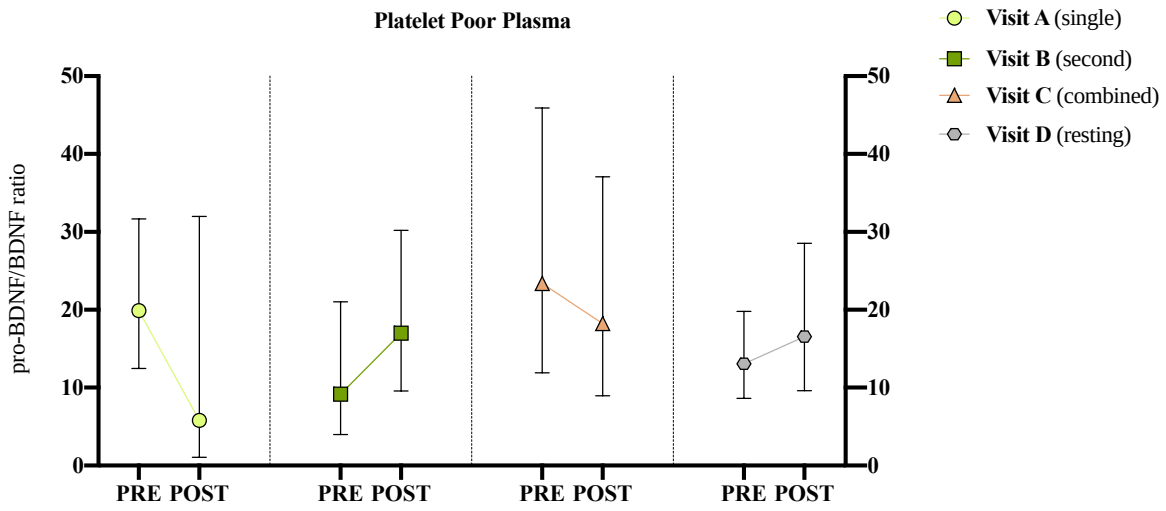

Figure 6.8 Geometric mean with geometric standard deviation bars of the brain-derived neurotrophic factor (BDNF)/pro-BDNF ratio at PRE and POST for each study visit: A (single exercise), B (second exercise), C (combined), and D (resting).

Paired samples t-tests analyses revealed that, except from visit B (*d*=0.930) and D (*d*=0.308), serum levels of BDNF significantly increased after completing visits A and C (t(4)=-3.627, P=.022, *d*=1.622 and t(4)=-3.633, P=.022, *d*=1.625, respectively). See **Figure 6.9** for a representation of individual values.

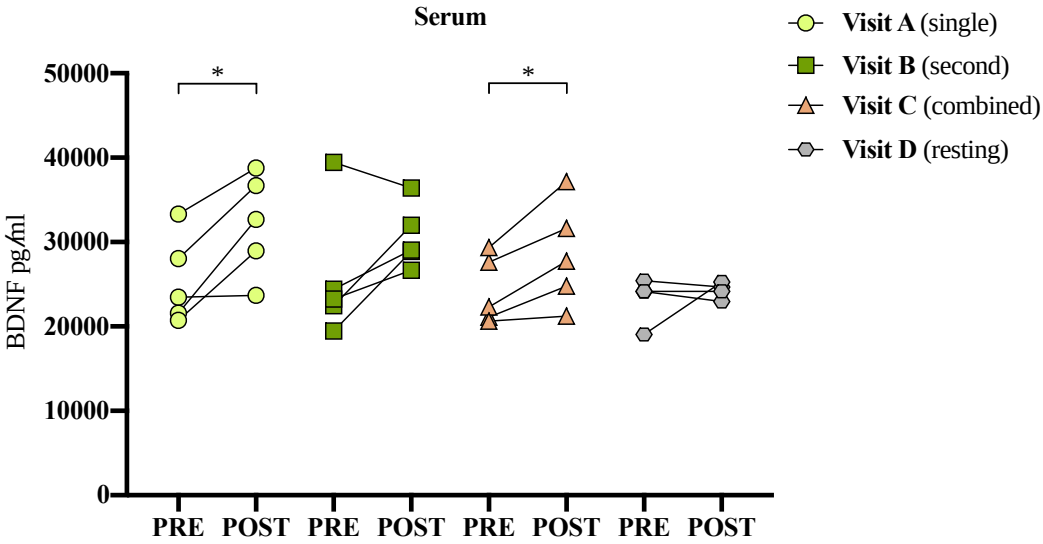

Figure 6.9 Individual responses of serum BDNF at PRE and POST separated by study visit: A (single exercise), B (second exercise), C (combined), and D (resting). *Significant difference between PRE and POST values ($p<0.05$).

Due to presenting less variability than the other study conditions, Visit C (combined condition) sample levels were chosen to evaluate correlations across sample types. As expected, a significant positive correlation between PRE and POST levels was observed for FPP BDNF (r_s=.900, P=.037), PP-P pro-BDNF (r_s>.999, P<.001) and serum BDNF (r_s>.999, P<.001). However, there was no significant correlation between PP-P BDNF levels at PRE and at POST (r_s=.800, P=.104). This

pattern of results was generally consistent across the other study visits; however, it is worth mentioning that in visit B (the second exercise condition), PRE levels of PP-P BDNF were negatively correlated with POST levels of PP-P (r_s=-.900, P=.037), which was not observed in any other visit.

6.4.4.3 Biomarker levels and cognitive function

PRE values of finger prick BDNF were positively correlated with LTM performance immediately after completing the cycling condition and 24 hours later in visits A and B, respectively (r_s=.812, P=.049 and r_s=.812, P=.0499, correspondingly).

In visit A, POST biomarker levels showed a positive correlation between PP-P BDNF and Stroop reaction time at POST (congruent stimuli: r_s=.900, P=.037, neutral stimuli: : r_s=.900, P=.037), a negative correlation between serum BDNF and Stroop reaction time at POST (congruent stimuli: r_s=-.900, P=.037, neutral stimuli: r_s=-.900, P=.037), a positive correlation between finger prick BDNF and LTM performance immediately after completing the cycling condition (r_s=.841, P=.036), and a negative correlation between finger prick BDNF and Stroop reaction time at POST (incongruent stimuli: r_s=-.943, P=.005, neutral stimuli: r_s=-.829, P=.042). Interestingly, finger prick BDNF at 30POST and 60POST did not correlate with any of the cognitive function measures.

In visit C, PRE biomarker levels showed a positive correlation between PP-P BDNF and Stroop reaction time at PRE (congruent stimuli: r_s=.900, P=.037, incongruent stimuli: r_s=.900, P=.037, neutral stimuli: r_s=.900, P=.037) and at POST (incongruent stimuli: r_s=.900, P=.037, neutral stimuli: r_s=.900, P=.037).

In visits B and C, none of the POST levels of biomarker across different sample types were significantly correlated with any of the cognitive function measures at PRE or POST.

In visit D, none of the PRE levels of biomarker across different sample types were significantly correlated with any of the cognitive function measures at PRE or POST. At POST, PP-P BDNF levels were negatively correlated with LTM performance immediately after the resting visit and at 24 hours (r_s=1.000, P<.001, both), and POST finger prick BDNF levels were negatively correlated with Stroop reaction time measures at PRE (congruent stimuli: r_s=-.900, P=.037, incongruent stimuli: r_s=-.900, P=.037, neutral stimuli: r_s=-.900, P=.037) and at POST (congruent stimuli: r_s=-.900, P=.037, neutral stimuli: r_s=-.900, P=.037).

6.5 Discussion

The aims of this study were to assess whether different acute exercise interventions (cycling alone, cycling on consecutive days or cycling combined with cognitively challenging tasks) compared to a resting condition could enhance participant's cognitive function (with an assessment of long-term

memory [LTM test] and attention [Stroop test]) and evaluate whether potential improvements in cognition could be explained by changes in peripheral BDNF levels. Moreover, possible associations between LTM and Stroop results, and BDNF levels across different sample types (serum, finger prick and PP-P) were investigated. Additionally, pro-BDNF levels in PP-P were also examined. The main findings of this study reveal that the second bout of cycling (visit B) and cycling combined with cognitive tasks (visit C) were able to elicit larger improvements in the Stroop test with large (d=0.853) and small to medium (d=0.349) effects, respectively, compared to the other visits. Regarding biomarker levels, serum and capillary BDNF levels were positively correlated with cognitive performance, whilst platelet-poor plasma BDNF correlations seemed to be headed in the opposite direction.

Cycling as a safe high intensity intervention for PwP

Walking is the most popular exercise regime that PwP engage in, however, many PwP can present significant walking difficulties (due to impaired dynamic postural control, increased rigidity, low strength, and freezing of gait) that can lead to activity avoidance, social isolation, and reduced independence and QoL (Afshari et al., 2017; Allen, Sherrington, et al., 2010; Lindh-Rengifo, Jonasson, Ullén, Mattsson-Carlgren, & Nilsson, 2021). Moreover, in most instances, walking is considered low-intensity, which, as an intervention, would miss the heightened benefits that PwP can get from performing exercise at higher-intensities. Nonetheless, even when walking is substantially compromised, PwP can preserve their cycling ability, probably due to the continuous external cuing generated by the pedals' motion (Snijders & Bloem, 2010; Snijders, Toni, Ružička, & Bloem, 2011). Thus, cycling may be a practical approach for PwP and may allow the completion of exercise interventions at higher intensities. In the current study, participants cycled at an intensity that they perceived between "somewhat hard" and "hard" (RPE 14), which corresponded, approximately, to cycling at 70%HRR; an intensity that had previously proved to be sufficient to elicit increases in circulating BDNF levels (Garber et al., 2011; Ross et al., 2019). On average, participants' HR was 126 bpm, which was equivalent to cycling at 63%HRR and classed as high intensity (also named as hard or vigorous intensity) by current guidelines (Garber et al., 2011; Martignon et al., 2020). It might be argued that participants did not reach the minimum required intensity for the intervention, however, PwP's commonly experience altered hemodynamic responses due to autonomic dysfunction, which may attenuate HR responses and difficulty in achieving designated training zones (DiFrancisco-Donoghue, Elokda, Lamberg, Bono, & Werner, 2009). Thus, using RPE ratings may be a better and safer approach that, importantly, can be easily reproducible at home settings without requiring the presence of a specialised instructor or researcher (Alberts & Rosenfeldt, 2020).

Cognitive changes after different acute bouts of aerobic exercise

In healthy adults, aerobic exercise, has shown to induce psychological changes, such as improvements in mood, motivation, and cognitive performance (Chang, Labban, Gapin, & Etnier, 2012; Colcombe & Kramer, 2003; Dilorenzo et al., 1999). However, research suggests that the implementation of combined modalities (e.g., exercise and cognitive training) could be more beneficial than single-domain training (Shatil, 2013). Since research in PwP evaluating this is scarce, the current study compared the effects of cycling combined with cognitively challenging tasks to cycling alone, cycling on subsequent days, or resting (control condition) in PwP. There was a slight nonsignificant tendency towards improvement in the measures of cognition after completing all study visits that was not observed in the resting condition. Furthermore, in agreement with the results obtained with older adults, the combined condition (aerobic exercise plus cognitive tasks) was able to elicit bigger improvements in LTM, attention, reaction time and response inhibition, immediately after finishing the intervention. Interestingly, cycling on two consecutive days was able to elicit the best LTM results after 24 hours.

Intervention-induced neurotrophic factor responses

Subsequently, to evaluate whether the cognitive benefits observed were due to changes in mediators of neuroplasticity (e.g., neurotrophic factors), BDNF and pro-BDNF levels were evaluated in different sample types. Indeed, serum BDNF levels were significantly higher after all study visits apart from visit B (subsequent days of cycling) and D (resting condition). Nonetheless, it is worth noting that after visit B, all participants' serum BDNF levels increased apart from one participant, whilst in the resting condition D, as expected, this general tendency towards a BDNF improvement was not observed. Moreover, in line with previous studies, serum and capillary BDNF levels were positively correlated with cognitive performance after completing visit A (cycling alone condition) (Khalil, Alomari, Khabour, Al-Hieshan, & Bajwa, 2016). A significant association between BDNF levels and measures of cognitive function was not observed in the study conditions B (subsequent days of cycling) and C (combined), which could suggest that the cognitive improvements observed in those visits might be mediated by different mechanisms. In visit D, capillary BDNF levels were related with better performance in the Stroop test, suggesting that levels of BDNF could be related with cognitive performance at rest. However, future studies should continue evaluating biomarkers and focus on the potential mechanisms that might drive cognitive improvements to allow a better prescription of exercise type and dose to enhance cognition in PwP.

In contrast to the outcomes discussed above, the results obtained from the analyses performed on PP-P seemed to be heading in the opposite direction. Generally, participants' biomarker levels before the interventions correlated with their levels at POST, apart from PP-P, where BDNF levels after visits A and C did not seem to correlate with their baseline levels. Moreover, after visits A and C,

participants with higher levels of PP-P BDNF seemed to obtain worse results in the Stroop test. Interestingly, in visit B, participants with higher levels of PP-P BDNF before starting the second exercise bout, presented the lowest BDNF levels results after completing the intervention. These pattern of results could be related due to the hormetic-like behaviour of BDNF in response to exercise, a concept that has recently become more popular (Gradari et al., 2016). A hormetic response is a biphasic dose-response where a factor (e.g., exercise) induces a beneficial effect or an up-regulation (stimulatory effect) at low doses and inhibitory effects at high doses (Mattson, 2008). In accordance with the results obtained in this study, previous research has shown that BDNF levels can decrease to, or drop below baseline levels after an exercise intervention (Dinoff et al., 2016; Ross et al., 2019; Schmidt-Kassow et al., 2012), however, the timeline and kinetics of BDNF changes during and following exercise in PwP remained unexplored. To evaluate these and improve the temporal resolution of the BDNF response, several capillary samples were collected and results also insinuated an hormetic-like biphasic dose-response behaviour of BDNF (see **Figure 6.4**). Interestingly, capillary BDNF levels after visit A were correlated with better cognitive performance (Stroop test and LTM immediately after the exercise), but capillary samples taken 30 and 60 min after completing the intervention did not correlate with any of the cognitive function measures. These findings should, however, be viewed with some caution due to the small sample size of the study.

The differences observed in BDNF levels measured in different sample types at PRE and POST, suggest that BDNF responds differently to the interventions (cycling alone or combined with cognitive challenges) depending on whether it is measured in PP-P, serum, or capillary plasma (which it is believed to resemble PR-P levels). Thus, regarding the opposite direction of the results obtained in PP-P, several mechanisms might explain these outcomes. First, it is important to bear in mind that blood compartments, such as serum or PR-P, contain platelets, which store the majority of circulating BDNF and release it upon activation (e.g., during the coagulation process) (Fujimura et al., 2002). Therefore, serum or PR-P BDNF levels may not always be freely available and, although our data suggests that their BDNF levels are less variable and positively correlated with cognitive performance, they could have a different physiological role from PP-P levels, which are less influenced by platelet activation and may be a potential marker for central BDNF levels (Klein et al., 2011; Pillai et al., 2010). Notwithstanding, evidence in humans is still limited, and it cannot be completely inferred that changes in peripheral PP-P BDNF reflect changes in the brain. Second, BDNF's half-life in plasma is very short and the observed negative correlation between its levels after exercise and cognitive performance may be due to the rapid uptake of BDNF by platelets or the liver, cellular reuptake or binding to its neuronal receptor TrkB (Dieni et al., 2012; Fujimura et al., 2002; Fumagalli et al., 2006; Hernandez-Baltazar et al., 2019; Pardridge et al., 1994). The molecular mechanisms that regulate the origin, uptake and distribution of PP-P BDNF remain to be fully elucidated, however, recent developments in the field of biomarkers have identified brain-derived extracellular vesicles, specifically exosomes of neuronal origin released to blood circulation, that act as BDNF carriers and can cross the BBB (Rani et al., 2019). The measurement of neuronal exosomes

may better reflect central changes (compared to serum or plasma) and could shed some light on whether, where and how the enhancement of BDNF through exercise takes place. Moreover, it could be a potential source of biomarkers for PD (Ohmichi et al., 2018), since peripheral measures of plasma and serum BDNF have regularly presented controversies.

BDNF has two active forms, the mature form (which is referred to as BDNF throughout this thesis) and its precursor form, pro-BDNF. Most of the research evaluating neurotrophins has focused on BDNF, whilst its precursor has received less attention. In the current study, pro-BDNF was measured in PP-P, which also allowed the evaluation of the pro-BDNF/BDNF ratio. The outcomes obtained show that pro-BDNF levels were significantly higher after cycling for 30 min on two consecutive days (visit B), whilst they did not significantly change after completing the other study visits. The pro-BDNF/BDNF ratio behaved differently across study visits, and nonsignificant increases were observed in visit B and D, whilst presenting decreases after visit A and C. In accordance with the results obtained in study 1 (see Chapter 3), higher levels of pro-BDNF were observed in PwP compared to non-PD participants, suggesting that the cleavage of pro-BDNF to BDNF could be affected or inhibited in PD (Yi et al., 2021). Although Yi et al.'s (2021) results suggest that using the ratio might be more informative than using BDNF and pro-BDNF measures alone, this could not be statistically proved in the present study and further work is required to confirm this in PwP at different stages. Nonetheless, the current findings add important information about the underlying mechanisms that may elicit exercise-induced responses in PwP and postulate questions to be answered in future studies, such as: are the observed higher levels of pro-BDNF the result of an increased activity-dependant synthesis similar to the mechanism that mediates the conversion of pro-BDNF to BDNF in response to factors such as physical activity, neuronal activity or enriched environment (Cao et al., 2014; Je et al., 2012; Schiera, Maria, Liegro, & Liegro, 2020)? What are the mechanisms that mediate increases in pro-BDNF levels that are only observed on the second day of subsequent bouts of cycling for 30 min? Are the high pro-BDNF levels the result of a cumulative increase of BDNF mRNA (which translates the pro-BDNF protein)? Proteases, such as plasmin, tissue plasminogen activator (tPA), furin or matrix metalloproteinases (e.g., MMP-3, MMP-7 or MMP-9) can cleave off the N-terminal pro-domain to obtain mature BDNF (intercellularly or extracellularly) (Nagappan et al., 2009; Pang, Nagappan, Guo, & Lu, 2016; Pang et al., 2004). A potential cause of the observed elevated pro-BDNF levels could be the result of the malfunctioning or inhibition of proteases, which could lead to reduced BDNF maturation and secretion due to the lack of pro-BDNF cleavage. Accordingly, evidence suggests that polymorphisms in the MMP-9 gene may be involved in the pathogenesis of PD and that elevated levels of endogenous tissue inhibitors of metalloproteinases (TIMP-1) had been found in CSF samples of PwP as well as in the SN (as observed in post-mortem brain tissue analyses) (He et al., 2013). What remains unclear is whether these factors are the cause of the elevated pro-BDNF and lower BDNF levels that were observed in PP-P after visit B, or whether they would represent later increases in BDNF levels instead.

The relationship between study condition and biomarker response might not be straightforward

Overall, it is difficult to conclude whether the observed changes in biomarker levels were due to exercise (cycling alone or combined with cognitive tasks) or changes in other correlates of the evaluated neurotrophins. Previous research has already reported that exercise-induced increases in BDNF levels rarely correlate with clinical outcome measures and are highly influenced by pre-analysis storage, handling and analysis conditions (Hirsch, van Wegen, Newman, & Heyn, 2018; Tsuchimine et al., 2014). However, trying to account for these factors, participants were always assessed at the same time of the day, were instructed not to change their lifestyle during the study, and none of them reported changes in their medication regime. Also, standardised and rigorously conducted sample collection, handling and analysis methodologies were followed to ensure the reliability of BDNF and pro-BDNF measurements. Importantly, the results obtained from the visits A, B and C tended to differ from the resting visit D, hence, suggesting a true effect of the interventions. Certainly, when analysing participants' mood in comparison to how they felt during the week prior to each visit, participants felt less angry and confusion 1 hour after completing all the study visits apart from the resting condition. Also, depression and tension mood states tended to be lower after visits B and C, which could be a result of exercise-induced hormonal changes (i.e., fluctuations in beta-endorphins and monoamine levels) (Pedersen, Klarlund & Saltin, 2015), altogether providing further benefits of the study interventions compared to the resting condition.

It is important to design interventions that can be transferred from controlled laboratory settings to real-world settings, such as home-based exercise programmes, which may be of interest for PwP. Several studies have provided evidence of the benefits that high-intensity aerobic exercise (e.g., cycling on a stationary bicycle) may have for PwP (Duchesne et al., 2015). However, although it has been proven to be safe and feasible for PwP (Uhrbrand et al., 2015), a rigorous evaluation of the practicability and efficacy of implementing these lab-based design interventions in PwP's own homes is generally not implemented. Van der Kolk and their team decided to investigate this and designed a double-blind 6 months study where PD participants aged between 30 and 75 years with a sedentary lifestyle were randomised into a stretching active control condition or aerobic exercise that consisted in cycling on a stationary home trainer (equipped with a virtual reality software) 3 times a week for 30 min (van der Kolk et al., 2015). Participants exercised, on average, at 59%HRR and were able to improve their physical fitness after 6 months (van der Kolk et al., 2018). Overall, this study provided preliminary evidence that PwP can safely and reliably complete intense aerobic exercise at home. These results have later been complemented with a recently completed larger home-based aerobic exercise RCT (named Park-in Shape study, clinical trial identifier: NTR4743) (van der Kolk et al., 2019). Researchers not only identified that aerobic exercise attenuated participants' motor symptoms and improved cognitive control, but also recently showed that aerobic exercise was able to reduce global brain atrophy and stimulate functional and structural changes related with protective and restorative neuroplasticity in both motor and cognitive brain networks in

PwP (Johansson et al., 2021). These results provide important information about the mechanisms underlying the disease-modifying effects of exercise through functional and structural adaptive plasticity in corticostriatal sensorimotor and cognitive control networks.

Taking all this evidence together, this provides a strong rationale for future studies to evaluate whether successful and beneficial lab-based studies can be implemented at PwP's homes. Indeed, a recent systematic review has reported that high-intensity home-based exercise may be as effective as centre/lab-based exercise interventions for PwP (Hare, Hill, & Clegg, 2020). Thus, the experimental work presented here evaluates different short (i.e., acute) interventions in a controlled laboratory setting that have the potential to be implemented in a home setting. This transfer to PwP's homes could facilitate prolonged adherence and allow the transition from acute interventions with short-lived effects into prolonged exercise programmes with long-term benefits.

Limitations

Nonetheless, we are aware of the limitations of the current study. Although researchers recruited more than the desired number of participants required for this study based on sample size calculations (n = 20), the restrictions brought in by the COVID-19 limited the number of participants that were able to complete the study (n = 6). Hence, the study design had poor sensitivity to detect effects in the evaluated outcomes even though medium to large effect sizes were observed for some comparisons (as seen with the cognitive measurements). Although interesting preliminary results were observed, inter-participants variability within measurements significantly impacted the overall results. That is, one participant exhibited opposite responses to the rest of the participants throughout the study conditions (i.e., worse cognitive performance after the intervention). This used to be described as "non-responder to exercise" in the literature. However, this categorisation highly depends on the variables being measured in a study and it is, therefore, more accurate to state that one participant did not respond to the study interventions (Pickering & Kiely, 2019). With a larger sample size, we would be able to elucidate whether these were normal responses due to individual variations in exercise and cognitive adaptations and or outliers. Unfortunately, blood could not be obtained from this participant and, therefore, the relationship between their cognitive levels and neurotrophic factors could not be explored. Nonetheless, this study serves as an initial step to future studies with larger sample sizes. Second, due to the limited economic resources available for this thesis, we were not able to evaluate participants' BDNF polymorphism to investigate its possible relationship with participants cognitive outcomes. Finally, only small volumes of blood were obtained from finger prick samples. Thus, it was not possible to assess whether capillary BDNF was more similar to PR-P or PP-P BDNF. Since a second centrifugation step was not performed to obtain capillary plasma and finger prick BDNF correlated with serum BDNF, it is hypothesised that finger prick BDNF would resemble PR-P BDNF levels.

6.6 Conclusions

Although the current study is limited by a small sample of participants, the findings suggest that cycling and its combination with cognitively challenging tasks might enhance participant's cognition providing improvements that are meaningful and translatable to real-life tasks (i.e., playing indispensable roles in advanced cognitive processes, such as decision-making in the face of complex and limited information, regulating emotions, etc.) (Miyake et al., 2000). However, it is yet unknown to what extent combining different modalities compared to cycling alone improves cognitive function and modulates biomarkers of neuroplasticity in PwP. Thus, the pilot data presented in this chapter warrants further studies with larger sample sizes to allow for more discerning analyses. Nonetheless, the outcomes of this study highlight the importance of evaluating BDNF levels in different sample types to comprehensively study the behaviour of this neurotrophic factor in PwP in response to exercise. Moreover, adding pro-BDNF measurements is recommended.

Chapter 7. Study 5 – Suitability, Usefulness, and Perceptions of Online Delivery of Multi-modal Exercise for People with Parkinson's: A Focus Group Study

7.1 Abstract

Introduction: The restriction measures introduced by authorities to control the COVID-19 pandemic reinforced stay-at-home lockdowns, limited in-person activities and social meetings, which created barriers for people with Parkinson's (PwP) to start engaging or maintain regular physical activities (such as attending group exercise classes). Physical inactivity has been associated with a worsening of PwP's symptomatology, therefore, videoconferencing and telematic communication options to deliver exercise programmes are valuable alternatives to traditional face-to-face methods. However, their suitability, usefulness and PwP perceptions about the change of platform to digital modes need to be explored. **Aim:** to examine participants' perceptions about a change of exercise setting and mode of delivery (from face-to-face towards online delivery), capture participants' experiences and thoughts about both modes of delivery, perceived benefits, challenges, and barriers of exercising at home, and investigate factors associated with adherence. Furthermore, we aimed to develop guidelines for an appropriate online delivery of exercise programmes for health-care professionals and researchers working with PwP. **Methods:** Nine participants (male, n = 7; female, n = 2; age, 64 ± 8 yr; H&Y scale I to III) who were regular attendees of the same community-based group class and had been engaging with the online version of the class for more than 6 months were recruited using purposive sampling. Participant's perceptions and experiences were assessed via online focus groups and a short survey with open-ended questions. **Results:** Using a reflexive thematic analysis framework, five main themes were created: 'Reasons to attend the online exercise class', 'Practicalities of exercising in a home environment', 'The human element retains social contact and familiarity', 'The calibre of the exercise instructor' and 'Reasons to drop out'. **Conclusions:** although there was a general preference towards face-to-face group exercise activities, participants highly valued the online-based version of the exercise class, which proved to be feasible, useful, and safe for PwP. The themes identified from this study bear insights presented as guidelines for researchers or health-care professionals that should be considered when designing online exercise programmes or interventions for PwP.

7.2 Introduction

On the 23rd of March 2020, the UK Government announced the first national stay-at-home order to control the spread of the of COVID-19 and slow down infection rates. By April 2020 well over 100 countries worldwide mandated similar lockdowns to restrict public movement and social gatherings. Moreover, restrictions hampered care by abruptly limiting patient access to clinics, physiotherapy, and neurology wards to prevent patients from being infected (Fasano et al., 2020), as well as 'protecting the NHS' (in means of patient capacity, expenditure time, effort of hospital staff, etc.) (Home Office, 2020). In light of this situation, researchers and exercise professionals predicted an overall decrease in physical activity levels in the population due to an abrupt shift to remote working (i.e., working from home), gym closures, suspension of group activities (e.g., community-based group exercise programmes, such as the long-term intervention described in study 2 [Chapter 4]), restricted access to parks and cancelled outdoor events (Brand, Timme, & Nosrat, 2020). Thus, the COVID-19 pandemic created a range of unforeseen challenges for PwP when starting or maintaining their regular physical activities (such as community-based group exercise classes). For people with chronic conditions, the consequences of having limited access to health care services can be devastating. Furthermore, for PwP, the forced absence of physical therapy could pose additional risks, such as worsening of both motor (affecting aspects of ADLs) and non-motor symptoms (with mental health implications affecting psychosocial wellbeing, increased sense of loneliness, depression, sleep problems, etc.) (Domingos, Família, Fernandes, Dean, & Godinho, 2022). As a countermeasure, videoconferencing and telematic communication options have been received with high interest by both exercise professionals and PwP as a valuable alternative to traditional face-to-face methods commonly used for the delivery of exercise programmes (being group-based or with one-to-one supervision). However, the need remained for research that explored how PwP perceive this change of platform (from face-to-face to digital modes of exercise delivery), its suitability and usefulness.

The effects of exercise for PwP have been extensively discussed within the literature and have long been postulated to be useful for the management of Parkinson's symptoms. However, most of the research in this area evaluates animal and human exercise interventions (both clinical-based and community based) that typically only last between 4 and 24 weeks (Da Silva et al., 2016; Schenkman et al., 2018; Shu et al., 2014; Uc et al., 2014; van der Kolk et al., 2019), and there is limited follow-up data evaluating whether benefits can be prolonged beyond the intervention and how long would they last for (Rosenfeldt, Koop, Fernandez, & Alberts, 2021; Ryan, Ryan, Peacock, & Ridgel, 2020). Nonetheless, there is some evidence from research across different populations, including PwP, which suggests that, upon cessation of exercise programmes, improvements (especially related to motor execution) tend to dissipate and levels of physical inactivity tend to increase (Garber et al., 2011; Ridgel, Vitek, & Alberts, 2009; Rosenfeldt et al., 2021). This is important for PwP, particularly when physical inactivity levels have been associated not only with worse walking performance, more disability in daily life, and greater disease severity, but also with an acceleration of the degenerative

process of dopaminergic neurons (Fox et al., 2006; Nimwegen et al., 2011). Together, these observations emphasise the meaning of the adage that says, "*if you don't use it, you lose it*", and highlights the need to develop strategies that allow for the long-term maintenance of exercise programmes. The integration of exercise into PwP's lives is, thus, imperative and consistent participation should be incorporated into PwP's routines (similarly to antiparkinsonian medication) to achieve and maintain benefits (Alberts & Rosenfeldt, 2020).

On top of the burden that the COVID-19 pandemic has placed on health care services, current trends show a demographic upturn in the number of elderly people (as well as people with long term conditions, such as PD) living at home in the UK, however, fewer are receiving appropriate home support (Barrett & Kirk, 2000; Mortimer & Green, 2016). Since health care services are failing to cope with both the demographic change and the high demand experienced due to the global pandemic, alternative models of care (e.g., telemedicine) and home-based interventions could be implemented and have the potential to improve patients' medical care, health and functional performance (Fasano et al., 2020). Regarding home-based exercise, published research shows that older adults undergoing physical activity in home settings can achieve equivalent health outcomes to those that attended a face-to-face group (Baez et al., 2017). Therefore, this provides a rationale to develop exercise interventions that can be delivered online and allow participants to take part from the comfort and safety of their own homes.

The highlighted need to develop alternatives to face-to-face delivered programmes to overcome unpredictable situations, such as the COVID-19 pandemic, has led to a quick implementation of telematic home-based exercise alternatives. For instance, supervised circuit-based multi-modal exercise (as discussed in the present study), yoga or cycling on a stationary bicycle, have been recommended by several associations and organisations (American Physical Therapy Association, the Chartered Society of Physiotherapy, the World Confederation for Physical Therapy, the International Network of Physiotherapy Regulatory Authorities, Parkinson's UK) ('Guide for rapid implementation of remote physiotherapy delivery | The Chartered Society of Physiotherapy', n.d.; 'Staying active at home when you have Parkinson's | Parkinson's UK', n.d.; 'Telehealth in Practice | APTA', n.d.; Lee, 2020). However, it is important that such exercise regimes are professionally guided and adequately dosed in terms of duration, frequency, and intensity of exercise.

Thus, this study aimed to follow-up regular attendees of the community-based multi-modal group exercise class for PwP who recently adapted to the online delivery of these sessions. Using focus group methods, we sought to investigate their perceptions about the change of exercise setting and mode of delivery (from face-to-face towards online delivery) and understand why some participants may not adhere to this change of platform. Specifically, we aimed to capture the [psychosocial] factors for and against online delivery versus face-to-face delivery, perceived benefits and challenges of exercising at home, and understand what support and resources may be needed to improve online

delivery and minimise the impact of suspending face-to-face classes. Using these findings, we aim provide health-care professionals and researchers with guidelines to facilitate the development of appropriate online delivery of exercise programmes for PwP.

7.3 Methods

7.3.1 Ethical considerations

This study was approved by the SSES REAG, University of Kent (see reference code in **Chapter 2**). Ethical principles were adhered to regarding consent, confidentiality, and anonymity. Participants had the opportunity to ask questions before providing written informed consent and participating in the focus groups. They were reminded that they were not obliged to contribute to particular lines of discussion.

7.3.2 Participants

Nine participants (male, n = 7; female, n = 2) who attended both the Parkinson's Exercise Class at St Mary's Island Community Centre (Medway, UK) for at least 6 months and the online classes (which started at the end of March 2020) were purposely recruited to take part in two focus groups that were held in November 2020. Participants (age, 64 ± 8 yr) had a diagnosis of idiopathic Parkinson's as defined by the H&Y scale (H&Y stage, 2.0 ± 1.0), a disease duration of 5.0 ± 2.4 yr, attended the face-to-face exercise group classes for 3.0 ± 0.9 years with an attendance rate of 73% ± 9% (range, 54-84%), and attended the online exercise classes for 8 months (at the time when the focus groups took place) with an attendance rate of 65% ± 24% (range, 29-94%). There was an average of 13 participants per online class (range: 8 – 18).

Participants were sent the same invitation letter providing them with a participant information sheet and were given a week to decide whether they would like to take part. Those that were interested provided a signed consent form.

Additionally, participants who attended the Parkinson's Exercise Class at St Mary's Island for at least 6 months but did not engage with the online classes were also recruited to complete a short survey with open-ended questions (male, n = 1, age, 40, H&Y stage, I, disease duration, 1 yr, attended the exercise group class for 7 months with an attendance rate of 100%).

7.3.3 Multi-modal online exercise class

The Parkinson's specific multi-modal (MM) online class was based on the weekly MM community-based group exercise class that participants used to attend face-to-face. This used to be delivered in

a local community centre by two qualified exercise professionals (Level 4 Specialist Exercise Instructor; Register of Exercise Professionals) and supported by undergraduate students from the university until March 2020 (the beginning of the COVID-19 pandemic). With the development of pandemic restrictions, one researcher (a regular class instructor) adapted the face-to-face class into an online form. Despite this change in modality, the nature of the face-to-face MM class and its components (strength, aerobic, balance, flexibility, symmetry, goal-related, and posture control exercises) were maintained (a detailed description of the MM exercise class is provided in Chapter 4) and readjusted accordingly to allow for its completion in participants' own homes. For example, instead of performing a fast shuttle walk with large arm swing movements across the hall, participants were instructed to safely walk around their own designated training areas (e.g., lounge or garden) or to perform a single step forwards and back maintaining the arm swing, whilst trying to improve their step length. The nature of the face-to-face MM class (i.e., a 23-station circuit as a multi-component exercise session) was maintained, however, instead of each participant completing a different exercise at a time, all participants performed the same exercise following the movements, verbal instructions and encouragement of the online exercise instructor. The timings of the class remained consistent (i.e., 1 hour divided in a full body warm up, the MM circuit and cool-down including mobility and balance exercises and stretches; see **section 4.4.4** and **Table 4.2** in **Chapter 4**).

A risk assessment evaluation covering the delivery and participation to an online-delivered exercise class for PwP was performed prior to its start. The appropriately created risk assessment form was reviewed and subsequently accepted by the Health and Safety Co-ordinator and the Head of School of Sport and Exercise Sciences at the University of Kent. Moreover, prior to each online weekly session, the instructor sent a reminder email to participants including a health and safety check/reminder of health and home safety, asking participants to: choose a space without tripping hazards, remember to drink water throughout the class (the instructor adds 'water breaks' during the session) and ensure there is someone else in the house during the class. The instructor was aware of participants individual capabilities and regularly screened whether participants experienced any changes in function, pain or injuries, which allowed the instructor to tailor exercises to individual needs. Participants used their own devices (tablets, computers, laptops) to access the online class.

Prior to the start of the live online MM group class, all participants received a clear description of the structure of the MM class and a video of a complete MM exercise session. To date, the instructor has recorded several videos of the exercise class that are used as an introductory resource for new participants joining the online session and/or as additional content for class participants wishing to exercise in their own time.

7.3.4 Study design

Following the same study design described in study 3 **(Chapter 5)**, two online semi-structured focus group meetings were conducted on separate occasions in two small groups in order to more easily

allow the participation of all participants in the discussions. Both focus group meetings lasted approximately 70 minutes and were facilitated by a researcher that, although being familiar to the participants, was not directly involved with the delivery of the face-to-face and online exercise classes. The researcher/instructor in charge of delivering the online MM class was not present during the focus group meetings in case some participants felt inhibited to express their true feelings (as described in study 3, **Chapter 5**). The focus group structure was the same for both meetings and, through a series of open-ended questions, participants discussed attitudes (i.e., participation), interaction, class delivery (e.g., instructions, type of exercises, new setting [online],…) benefits and limitations of participating in a supervised online exercise class for PwP.

Moreover, participants' attendance and adverse events (defined as any adverse medical or psycho-social event in a participant during the online class) were recorded to provide a feasibility analysis of the online delivery.

7.3.5 Data collection and focus group meeting guides

As described in study 3 **(Chapter 5)**, topic guides were created and followed to encourage participants discussions about the online exercise class. Probing questions were prepared within the interview guide to extend the narrative and gain clarity if necessary. The focus groups started broadly with questions about participation to the online class (e.g., "What made you start attending the online class and why?"). The following set of questions were divided into general sections that focused on three aspects of the exercise programme: i) perceived effects of the online class (e.g., "Have you noticed any positive/negative effects from the online [exercise] class?"); ii) barriers and motivators (e.g., "What do you think some of the barriers are to people attending a class like this?"); iii) the online intervention setting and delivery (e.g., "Do you have the appropriate space to perform the class safely?"). An additional section evaluating potential factors associated with the non-attendance of some participants was also included (e.g., "Why do you think some people dropped out of the [online] class?").

7.3.6 Data analysis

Discussions were recorded by the meeting moderator with the videoconferencing platform used to run the focus group meetings (Zoom [San Jose, CA: Zoom Video Communications Inc]) (Boland et al., 2021). Audio recordings were transcribed verbatim and data analysis was performed as described in **section 5.3.6** in **Chapter 5** using a reflexive thematic analysis.

7.4 Results

The online MM group exercise class was found to be feasible for a group of PwP that used to regularly attend a community-based face-to-face MM exercise class delivered by the same exercise

instructors. Focus group participants' attendance ranged between 29% and 94%. Different participants occasionally experienced short-term incidences of internet outage, however, they were able to log back into the class. Only on one occasion, the exercise instructor had network connection issues, but as one participant recalled '. . . we all kept on going and stayed with it. None of us backed out.' [Participant #9]. Apart from those, no adverse events (such as falls) or safety issues were experienced during the online sessions.

Research findings from both focus groups are organised around the five themes that were created and revised through reflection from the data. Themes (presented as individual sections) and subthemes (presented in bold within each theme section) were developed through an iterative process by comparing the transcripts between the focus groups.

Questions sought to explore participants' experiences with the online group exercise class, its digital delivery and change of setting – as well as perceptions about the psychosocial benefits (and drawbacks) of an online group exercise class for PwP. The five higher-order themes that were created were: 1) Reasons to attend the online exercise class: '*I see no difference between the face-to-face classes and online classes'* 2) Practicalities of exercising in a home environment: '*It is what you put into it'* 3) The human element retains social contact and familiarity*: 'There are points of humour and interaction'* 4) The calibre of the exercise instructor: '*You have got them motivating you'* and 5) Reasons to drop out: '*There's quite a few that haven't turned up'*. Although presented separately, the five different themes interact, overlap, and influence each other.

7.4.1 Reasons to attend the online exercise class: '*I see no difference between the face-to-face classes and online classes*'

The community restrictions brought about by COVID-19 caused the immediate cessation of the community-based face-to-face MM group exercise class that participants used to attend on a weekly basis. This class, grounded in evidence-based practice, was a popular activity for the participants of this focus group, who quickly transitioned to a change of delivery mode. Nonetheless, participants gave different reasons for why they continued attending the group exercise class even after reverting to an online platform. The success of the face-to-face community-based class seemed to be a pivotal reason that encouraged participants to engage with this new mode of delivery in the first instance.

Motivational factors. Almost all participants viewed exercise as a means to slow PD progression and maintain function, however, participants provided different reasons for why they chose to engage with the online exercise class. Whilst some were intrinsically self-motivated to exercise, others cited extrinsic motives, such as being part of a group (i.e., seeing others engaging with the exercise motivated them to complete the class), maintaining interaction with their colleagues, and the role that their partner plays by providing encouragement and support during the class (the role of the exercise

instructor was also an important source of motivation, which has been discussed under the theme: The calibre of the exercise instructor: '*You have got them motivating you'*).

> 'To carry on and seeing people that I've met over the last couple years. And you'll see them and interact with them a little bit. Also, to carry on doing exercise. It's the motivation to do it.' [Participant #3]

> 'Yeah, I think the group itself is motivation as well. Sometimes you're thinking "oh crumbs", especially me, I don't finish work till about half past five . . . And I think, "oh crumbs, am I going to go home do that?" But I'm glad I do. Once I get there and get thinking everybody else is doing it, I need to be doing it as well . . . we've got to keep it moving.' [Participant #6]

> 'The reason I attended the live course right from the start is it allows to exercise in a class with like-minded people. And I've really enjoyed it.' [Participant #5]

> 'Motivation is my biggest problem . . . If nobody says anything, I'll slide under the mat and disappear.' [Participant #5]

Participants also reported that their partners encouraged them to be more active by regularly reminding them to exercise, providing physical assistance to overcome functional limitations (e.g., freezing of gait), and/or by using attention or cueing strategies to assist their partner during the class. Some participants described the role of their partner as being 'almost like a personal trainer' trying to mimic what the instructor is doing and getting the class participant to follow their movements:

> 'I have a lot of difficulty with moving my legs. If my husband wasn't here with me, I don't know if I could cope without him.' [Participant #9]

> 'I suppose I'm fairly lucky, because my wife will take me out and find me. She'll say, "come on!" . . . And I will say "yeah in a minute, in a minute". But she'll go, "let's go now".' [Participant #1]

For other participants, their source of motivation was strongly linked to their interest in Parkinson's research, awareness of research outcomes supporting the notion that exercise is beneficial for PwP and the possibility of contributing to research.

> 'Lots of research is showing that exercise slows down the progression of Parkinson's. So, for me, that is a motivation enough.' [Participant #2]

Contrary to the above-mentioned external sources of motivation, participants also discussed the importance of being self-motivated and aware of the impact that, both, PD's and exercise have on motivation:

> 'You've got to have the motivation. And I mean, I see no difference between the [face-to-face] classes and [online] classes, whether I go or not, I just go or went to all. I hardly missed any of the [face-to-face] classes, or this [online]. I've just stuck to it . . . People often tell me that exercise is good for Parkinson's. And I've got to take their word for it.' [Participant #1]

> 'Motivation is difficult as the progression of Parkinson's increases . . . if I don't do it, I think it increases demotivation.' [Participant #2]

New technologies can be perceived as threatening by most older adults, which can lead to lack of engagement with them (Leonardi, Mennecozzi, Not, Pianesi, & Zancanaro, 2008). Nonetheless, being familiar with the face-to-face session, class participants and the instructors, were perceived by one participant as pivotal factors to attend the online class.

> 'Because I enjoyed the face-to-face courses so much, it is natural progression to go with the online course.' [Participant #2]

To fill the void. Beyond the motivating factors described above, there was a shared sense of lack of available exercise resources for PwP. This vacuum was already perceived prior to the start of the COVID-19 pandemic and was accentuated with its emergence. From the point of diagnosis, participants feel that there is an inadequate provision of exercise programmes offered by healthcare providers (e.g., National Health Service [NHS]). Therefore, additional available programmes, such as the original community-based face-to-face class and its online version, filled the void of exercise prescription by health authorities.

> '. . . provision from the NHS is appallingly poor. You know, there are no [exercise] classes in Kent or South England or the UK. So, if this class we've got, which is fantastic, could be somehow spread and publicised within the NHS, and done via video throughout the UK that would be beneficial to everyone.' [Participant #4]

This links to the perception that was shared amongst participants, who recurrently described the online version of the MM group class as being '*better than doing nothing*' and '*the second-best option*' (after the face-to-face session).

> '…doing it [online] and staying with it is better than doing nothing. And it's enjoyable as well.' [Participant #8]

The online alternative, however, was perceived as being a 'temporal solution' and a 'stop gap'. For some participants, their reason to attend was to temporarily substitute the group exercise class until the face-to-face delivery returned.

> 'I just see, the [online] type meetings for exercise classes as a stop gap. I don't see it in the mainstream as an interesting . . . enough for anybody to know that that's all you're going to get. I think I'll be the first one down to the local gymnasium starting the whole thing over again and looking for an exercise class that's suitable for people with Parkinson's disease.' [Participant #5]

> '. . . I'd say it is a substitute [to] face-to-face, but it's not all of it. There is the gold standard, and this comes far short with it. But it is still something that we should do to keep us together and to get motivated to exercise.' [Participant #2]

Although there was overwhelming endorsement and preference for the face-to-face exercise group experience, participants valued the alternative that a live-online exercise class does offer.

> 'What you just said there that it is better than nothing, I disagree with that. It is much, much better than nothing. But it's not quite as good as a face to face.' [Participant #5]

7.4.2 Practicalities of exercising in a home environment: '*It is what you put into it*'

Use of videoconferencing platforms. The platform used to deliver the online class was discussed and deemed as one of the most important factors to ensure an appropriate online-stability of the class. For example, Zoom was preferred over other platforms that had previously been used at the very beginning of the change of delivery mode (i.e., Jitsi Meet [https://jitsi.org]). Importantly, participants highlighted the need for a stable internet connection, the importance of IT literacy and support of their partner/family members to set-up the connection. Connection failure and instability, internet cost and/or no access to digital devices, such as personal computers or tablets, were perceived as barriers to attendance at the online exercise class.

> '. . . it's relying on whoever wants to be a part of the meeting to have up to date software and up to date laptops, PCs.' [Participant #4]

> 'Not everybody is a computer person.' [Participant #5]

One participant pointed out that, as a group, they were more IT-user friendly than other PD support groups that they regularly were involved with. On the other side, two participants mentioned that they required the help of their family members/partners to connect to the online class.

> ‘I call my son, can you come down and help me please?’ [Participant #5]

> ‘I wouldn't be able to do it without [husband] because I don't know what to do [with] the computer side.’ [Participant #9]

Other participants also mentioned how they noticed that other online class participants had the support of family members to set everything up for the class.

Household environment. Two important factors influenced the ability of participants to exercise at home. First, the physical space to properly exercise is an important point to consider. Whilst most of the participants reported having an appropriate and suitable space to exercise at home, some considered that it was a limitation and stated that the lack of an open space compromised the execution of some of the original exercises (some of which had already been modified by the instructor to allow their completion from home).

> ‘. . . I think it's limited what you can do, because I used to throw the ball on the wall and . . . up in the air. Now, we've all got low ceilings.’ [Participant #7]

> ‘The issue as well is the space. So, for example, in the exercise class, there is the ball catch. Now in the hall, obviously, you can chuck toss the ball very high and catch it again. And in your living room, you can only toss it about two feet before the light hits. It's not quite the same thing.’ [Participant #4]

Some participants were confined to the room where they had their personal computer, which established a set physical space to exercise that sometimes was not big enough:

> ‘My room isn't that big so I can’t get the full body length.’ [Participant #8]

However, others said that they could make some space by moving their furniture, and some participants also mentioned that the adjustments made by the instructor to allow the completion of the exercises from home had not changed the nature of the class and could be completed at home.

> ‘You make room for it easily.’ [Participant #5]

> ‘I wouldn’t say [the exercises] have changed a lot really.’ [Participant #6]

Second, the social dynamics of the home are important influencing factors to participants’ attendance. Family members were reported to be both a facilitator (as described under the subtheme **Motivational factors**) and a barrier to engaging in online home-based exercise.

> ‘I haven't done all the numbers of the exercise classes I should have done because I'm at home, the family will still come around, thank goodness, but the family or friends will pop around or something will happen . . . I find that if I've arranged to go out [to the face-to-face exercise class] it's another matter. You know, I've gone. But if you're in your house environment, I find I won't do the classes as regular as I should. I just opt out of it and decide to do something else.’ [Participant #5]

As much as exercising from home is deemed convenient for some, home is the ‘meeting point’ for other participants, which stops them from exercising.

Suitability of the MM class for online delivery. When participants were asked whether the exercise class format was appropriate and suitable for online delivery, all participants agreed. Moreover, all participants were receptive to the idea of recommending the class to other people with PD:

> ‘I'm sure it is. I'd recommend it to anybody else who doesn't do it now. Again, just purely to get them into exercising, even though they can't do face to face. But if rather than that they are just sitting down doing nothing, the exercises are doing good.’ [Participant #1]

> ‘Yeah. I'm very pleased that the exercise class is available. It's good. And of its type, I think, one of the best, and we're lucky, we've got the best [instructors] as well . . . The quality of the staff is excellent.’ [Participant #5]

Participants also noted the potential that the online format has to welcome participants who may not be able to attend the face-to-face class:

> ‘And there must be quite a few people who've got PD who can't actually get to a class.’ [Participant #3]

As much as participants approved the suitability and usefulness of the online class, some participants were quick to report that they lacked the motivation and self-discipline to exercise at higher intensity. Although the instructor regularly uses direct and individualised prompts to motivate participants and ensure an appropriate technique throughout the class, one participant felt that by being on a “tiny

screen" the instructor could not see them and they were "not driven so hard". A second participant added that the online format allowed them to "take a step back".

> 'The online one is easier. Because I say, you get away with it . . . not everybody does it naturally, but you just can't be seen . . . You don't know whether I'm doing that or not.' [Participant #5]

> 'In [the face-to-face group class], they are with you all the time, "do this, do that, keep moving, keep marching". But here [online], when you're in your own place, you don't get full. You can take a step back…' [Participant #8]

On the contrary, other participants found the class challenging enough:

> 'Definitely. Yeah. And in all honesty, it's over an hour (…) so it's quite it's quite a time.' [P7]

Furthermore, participants emphasised that personal motivation is important and there was consensus towards the fact that:

> 'It is what you put into it as well, I think.' [Participant #6]

Safety. Overall, participants expressed that it was feasible and safe to exercise from home. Although the instructor regularly emphasised the need of having a safe environment to exercise and advised participants to exercise whilst another person was at home and/or aware of their exercise session, as the discussion progressed, some safety concerns arose in the conversations questioning what could be done if someone suffered a fall or lost consciousness.

> 'Yeah, I feel safe. I suppose. If anything happened, I've got the wife there as well. She's always coming up.' [Participant #1]

> 'I'm fine with exercise on my own . . . I'm fine with it. But I can't say about everyone because everyone is in…, you know what is like with Parkinson's, we're in different stages. Some of the ones that are a lot further down the line would find it harder.' [Participant #8]

> '[The instructor] does stress it all the time "make sure you can hold onto something, the wall or whatever".' [Participant #9]

‘But there is a certain element there which says there's a risk. We don’t have our blood pulse taken anyway on the . . . [online] meetings, we don't do blood pressure and things like that . . . I assume they will look at you.’ [Participant #5]

7.4.3 The human element retains social contact and familiarity: ‘*There are points of humour and interaction*’

The majority of participants stated that social contact and interaction were a very important part of the live group exercise experience. There were mixed opinions regarding whether the online class was able to foster enough social contact and interaction. Some participants strongly defended that the online class was not able to provide the same social connectedness and interaction as the face-to-face class. However, some participants were aware of the limitations of online platforms to provide enough “human contact” and suggestions were made to try to provide more social opportunities. It is important to consider that the possibility of having a more social/interactive experience might be linked to being more familiar and confident using online platforms.

‘. . . the social interaction is not really there. And I can't see how that is going to be easily rectified . . . It's just an issue with the medium itself. Yeah, it's a limitation on the medium of being on the internet.’ [Participant #4]

‘By taking away this interaction, it takes away a big part of our lives.’ [Participant #5]

‘I wonder if instead of the class finishing straight off the exercises, they could go on for 15 to 20 min just for us to socialise.’ [Participant #2]

‘Everyone is trying to chat together. It's like it comes back down to one being able to control the computer as well. We are being computer literate.’ [Participant #8]

Nonetheless, there was also popular consensus that the online class could still be interactive. Especially, compared to non-live home-based exercise resources, such as the pre-recorded video of the exercise session that was distributed to all participants prior to the start of the live-online class. This resource was highly valued amongst participants and allowed them to follow a structured exercise class on demand, however, given the choice, they would prefer to exercise live with the instructor.

‘And that's the point of being in live, you know, rather than a recorded there are points of humour and interaction.’ [Participant #7]

‘I think is a good interactive, because if it weren't live . . . I've been doing classes that are recorded and you do the exercise recorded on your own. [But live] You get that motivation from others doing it as well.’ [Participant #2]

‘You do actually get to see people in this live [online class]. You can actually talk a bit. [The instructor] does encourage us in her emails: get there a bit early, you can talk to each other. They're usually more of a meeting group as well.’ [Participant #8]

‘Can I just mention as well that, in addition to the online course, there is the video, where [the instructor] does the exercise class. I've got difficulties and I haven't got a laptop or PC anymore, because that was my company's stuff that's gone. So I haven't actually got any IT that can log on to the live class, but I can log on to the [video] class. So, I've been doing that on my own. That's, that's invaluable. It is a full pack.’ [Participant #4]

7.4.4 The calibre of the exercise instructor: ‘*You have got them motivating you*’

The calibre of the exercise instructor was instrumental in terms of the quality of exercise delivery, attention to detail, feedback provided, and professionalism (mention has been made in the second quote under the subtheme **Suitability of the MM class for online delivery** [see **Practicalities of exercising in a home environment: ‘*It is what you put into it’***], however, additional aspects were discussed regarding the calibre of the instructor).

Live interaction. Although participants were appreciative of the [pre-recorded] video, there was a general sense that doing the online class live was far better than exercising alone.

‘. . . when it went live, it became a totally better experience for everybody. The instructor feels something coming back from these classes, rather than, you know, rather than going into a void.’ [Participant #7]

‘Because you have got [the instructor] motivating you and… talking to you at the time. If you feel as though you're doing it wrong, you could always sort of ask whereas if it was pre-recorded, you just carry on.’ [Participant #6]

‘[The instructor] has used [a recording of the class], but now that’s properly live [online] it's far, far better, . . ., because there is the one to one even via the internet.’ [Participant #9]

Simply telling PwP to exercise is not effective. Instead, adequate and personalised instructions are important to facilitate competence, provide greater engagement and benefits (Ellis & Rochester, 2018). With the change of delivery mode, some participants felt that those participants at more advanced stages would struggle to follow the commands as easily as they could in the face-to-face class. Participants also discussed how feedback could not be provided as instantaneously in the online class, which was perceived as being directly affected by the lack of support of student helpers:

> 'Obviously . . . [it] is better with the students if you're there with them. Because some of us are a lot further advanced, you know, and cannot take an instruction . . . So, students basically manoeuvre their arms around when you're stretching, they manoeuvre their arms for them but that way [online] we miss out . . ., but you cannot do anything else, can you?' [Participant #8]

Instructor's set up. Participants perceived changes in the set-up of the instructor. For example, participants noticed an improvement in the feedback provided when a bigger screen was used.

> '[Real time interaction] it's a lot better because this week [the instructor] said [they] had a larger screen to see people better. And [they] told to move the arms or stretch, to move the legs . . . But when [the instructor] was with a bigger screen, [the instructor] can actually interact with us a lot more and tell us to do things.' [Participant #8]

7.4.5 Reasons to drop out: '*There's quite a few that haven't turned up*'

Some participants that regularly attended the face-to-face exercise class never engaged with the online format. In order to investigate the reasons behind their lack of engagement with the change of delivery mode, non-attendees were invited to complete a short survey and open-ended questions to understand why they did not opt into the online class, express their concerns, identify potential limitations or barriers that they might have encountered (one participant took part). Furthermore, regular attendees discussed potential reasons for other people's non-attendance.

Potential obstacles to attend the online class. Regular attendees of the online class were asked to discuss potential reasons that could explain why some participants dropped out and did not engage with the online delivery of the exercise class. Participants gave some suggestions, which were mainly related to technical and logistical aspects of being able to connect to the online class, or lack of appropriate digital equipment:

> 'Some might be 80 or 70 and might not understand what they're doing' [Participant #7]

> ‘I lost my computer. And then I've got withdrawn’ [Participant #4]

Reasons to not-attend from a non-attendee. Additionally, non-attendees were asked to complete a survey reporting their reasons for not attending the online exercise class. One participant reported that, as much as they do not feel comfortable turning on their camera during a videocall, their home was not suitable to perform the MM online class (which linked to the subtheme **Household environment** discussed above):

> ‘I don’t have the room to exercise in front of my laptop. I also feel embarrassed about exercising in front of my family. My dog would also probably attack me!’ [Participant #10]

This participant preferred to follow the recording of the session on their own time or complete a training session on their stationary bike. Nonetheless, above all, they preferred the face-to-face group format of the class:

> ‘I just prefer and am more motivated about exercising as a group in a physical location.’ [Participant #10]

7.4.6 Summary of advantages and disadvantages

Participants’ perceived advantages and disadvantages of online exercise delivery for a group class of PwP are summarised in **Table 7.1**.

Table 7.1. Overview of advantages and disadvantages of online exercise delivery for a group of PwP.

Advantages of Online Exercise Delivery	Disadvantages of Online Exercise Delivery
Not affected by adverse weather (i.e., no travel required)	**Distractions / interruptions / lack of piece at home** (e.g., family or friends popping around)
No travelling required (reduces physical burden, transport difficulties [such as needing a lift], traveling costs and geographic restrictions)	**Requires appropriate digital skills** (not all participants had sufficient digital skills or technical support to log in)
Convenience (i.e., saves time [time flexibility] and resources [venue hire costs])	**Requires appropriate digital equipment** (the loss of equipment, such as a computer, may imply withdrawal from the online class)
Offers an interactive option to exercise live with an instructor and group (instead of exercising completely alone)	Having appropriate digital equipment and a stable internet connection have a **cost** and **connectivity might not be available** in certain locations (e.g., rural environments)
Inclusiveness (offers participants at different disease stages the possibility to join the exercise at different intensities and it is not limited to PwP from the same support group or area)	**Less quality and amount of instantaneous feedback** from the instructor (participants perceive that feedback is richer and more immediate in face-to-face class, which may be attributed to the higher number of instructors and student helpers present in the face-to-face group class)
Safe (offers participants that have limited mobility the opportunity to safely join from their own homes. Whilst environmental enrichment may be positive for PD, it can trigger motor problems such as freezing of gait)	**Lacks physical and social interaction** with the rest of the group and **less opportunities for interaction** with other class members **during the class** (due to participants being muted during the exercise)
Allows continuation over the summer and winter months (periods when the community-hall and university were closed and forced the delivery of the face-to-face class to stop)	**Perceived less effort expended** during exercise online (appropriate motivating cues are required to ensure participants engagement)
Flexibility and recurrence (having a recorded video of the session provides the opportunity to review the content of the class and complete it at a time that suits the participant)	**Limited space at home** (due to having less room for exercise at home some exercises cannot be replicated from the face-to-face class)
Allows exercising at high intensity (with appropriate motivating cues to ensure a safe and consistent engagement from participants)	**Less motivating** (no one else physically exercising alongside, plus some participants experienced lack of self-discipline)
Allows the possibility of introducing a blended exercise delivery (which can combine home-based and in-person sessions alternated throughout a week or at the same time [allowing participants to choose a home-based practise on in-person attendance]. Moreover, this blended approach could fill the void experienced during summer/winter holidays when the community-based class used to stop)	**Not monitored closely during exercise** (pulse, RPE, exercise technique, difficulty/intensity adjustments, etc.)

7.5 Discussion

The primary aim of this study was to gain insights into how PwP perceived a change of exercise setting and mode of delivery (from community-based face-to-face exercise towards online-based delivery) recognising potential benefits, challenges, and barriers of exercising at home. This was investigated with a reflexive thematic analysis of two focus group discussions, which revealed that the online-based group exercise class for PwP was appropriate for online delivery, feasible and safe. These outcomes agree with previous research providing evidence that home-based exercise programmes (e.g., dance programmes for PD, remotely-monitored physiotherapy, combined aerobic and strength exercises, etc.) are accessible and usable for PwP (Flynn, Preston, Dennis, Canning, & Allen, 2021; Hill et al., 2021; Lai et al., 2020; Morris et al., 2021). Moreover, the current study provides evidence that a live MM exercise class delivered in an online group setting for PwP with direct remote supervision is feasible and safe when guided by exercise specialists that are known and trusted. However, results highlight that, given the choice, most participants preferred the face-to-face group format of the class.

Participants highly valued the alternative that a live-online exercise class did offer to keep exercising whilst community halls, gyms and face-to-face activities remained closed. The progressive nature of PD can lead, over time, to disability and disengagement with active lifestyles and social activities, which can further exacerbate PD symptomatology (Lubomski, Davis, & Sue, 2021; Perepezko et al., 2019). Therefore, it is important for PwP to stay engaged with physical, cognitively stimulating and social activities for maintaining or improving QoL, motor and non-motor symptoms (Perepezko et al., 2019; Rodrigues de Paula, Teixeira-Salmela, Coelho de Morais Faria, Rocha de Brito, & Cardoso, 2006). The prolonged cessation of regular activities for PwP, such as group exercise classes, may revert any of the experienced functional improvements, increase levels of inactivity, and lead to social isolation (i.e., lack of integration with the social environment, which PwP are already at risk of suffering) (Garber et al., 2011; Ridgel et al., 2009; Rosenfeldt et al., 2021; Soleimani, Negarandeh, Bastani, & Greysen, 2014). Importantly, a disruption with the social environment in PwP has been associated with a diminished QoL, social dissatisfaction, and worsening of PD severity (Subramanian, Farahnik, & Mischley, 2020). All these factors may synergise with, and be intensified by, the consequences of the COVID-19 pandemic, where in-person group exercise classes were stopped. Fortunately, telematic programmes and the use of digital tools have shown to be an effective mode of providing home-based exercise alternatives for PwP mainly delivered on one-to-one online sessions (Allen et al., 2017; Lai et al., 2020; Langer et al., 2021; Morris et al., 2021; Song et al., 2018; Tunur et al., 2020). However, to the best of our knowledge, limited research had investigated the practicalities, usefulness, suitability of engaging with a group exercise class for PwP delivered online, or participants perceptions about transitioning from a community-based exercise programme to an online exercise class.

A strong reason for participants to start attending and adhere to the online exercise programme was to keep exercising (i.e., stated as '*better than doing nothing*') when all in-person exercise sessions and gyms were closed following COVID-19 restrictions. That participants were urged to stay active reinforced the detrimental effects that reduced exercise levels or inactivity can have for PwP. Moreover, participants' reflections highlighted a perceived void of accessible Parkinson's specific exercise programmes run by health authorities that was being covered by independent programmes (such as the community-based/online-base exercise class discussed in study 2 [Chapter 4] and in this chapter). Although the regular engagement with exercise regimes has well-defined motor and non-motor benefits for PwP, there is a perceived lack of exercise prescription and delivery by physicians that has previously been reported by PwP and now, several years later, has been unresolved (Ravenek & Schneider, 2009). Thus, the development, establishment and evaluation of independent long-term exercise programmes are highly needed.

In addition to maintaining their exercise levels, another important reason to attend the online class was being familiar with the exercise programme and class participants. According to the theory of familiarity, it is 'the relationship between an individual and something that the individual has had considerable experience with. The experience is sufficient to advance to the development of an internal model of that something' involving fast and automatic recognition processes to recognise a previous experience without the need to retrieve specific details from the encoding episode (Kaplan & Kaplan, 1982; Mollison & Curran, 2012). Therefore, this sense of familiarity, guided by enjoyable past experiences (i.e., previous attendance to the community-based version of the class), turns the online class into an activity that is recognisable and gives sense to the investment of participants' personal resources (e.g., time, focus, willpower, etc.) to perform the activity (Leonardi et al., 2008). This could, consequently, mean that participants who previously attended the face-to-face class might be more likely to adhere to the online programme compared to new attendees. However, further research evaluating new participants' attendance and experiences with online exercise programmes are required to confirm this hypothesis.

Participants viewed the online exercise class as an enjoyable and positively challenging experience that could aid and modify PD progression and help control their symptoms. Moreover, participants considered that being knowledgeable about the benefits that exercise has for PwP was an important source of motivation to continue engagement with exercise programmes, especially when there was a change to online delivery. Nonetheless, not all participants treated 'exercise as medicine', and occasionally they prioritised friends and family gatherings over the weekly exercise class. To enhance motivation and improve attendance rates, it is suggested that providing regular information about the effects of exercise for PwP supported by scientific evidence may help motivating participants, family members and friends to maintain PwP's engagement with the exercise programme and more easily include it in their day-to-day routines, in a similar way to pharmacological medication regimes. It is important to stress that researchers are not saying that PwP

should abandon social gatherings to attend exercise classes, but instead PwP, family and friends, may treat exercise training as an adjuvant treatment that PwP must methodically take.

Regarding motivation to exercise, participants also identified that their participation with the online programme, in terms of adherence (i.e., attendance to the online class) and compliance (i.e., meeting the exercise prescription required during each session), was strongly dependant on their personal motivation, which was distinguished in two types: those requiring extrinsic motivators and those that were intrinsically self-motivated. The first group of participants were extrinsically motivated by being part of a group (i.e., seeing others exercising), positive feedback and constant encouragement during the class provided by the exercise instructor, partners, and/or other class participants. These participants admitted that by exercising in-person, they felt more motivated due to the presence of other fellow class participants and the exercise instructors. Moreover, they admitted that their lack of self-confidence and low outcome expectations from the online exercise class (i.e., participants did not expect to benefit from the online exercise class as much as the face-to-face class) made them fear that their motivation levels would eventually drop, compromising their competence for the class (i.e., ability to exercise online) and leading to their disengagement with the online programme. In line with the theoretical propositions of the social cognitive theory (SCT), individuals with higher self-efficacy (i.e., those who believe that they have the ability to participate, adhere and complete the exercise class to a specified standard) and better outcome expectations (i.e., those who expect favourable results from the exercise programme) are more likely to implement self-regulatory strategies (e.g., goal setting, self-persuasion, planning, and problem solving) that are essential to adopting and maintaining an active engagement with the programme (Bandura, 2004). In other words, we observed that participants who did not believe in their personal ability to engage with the online exercise programme lacked self-efficacy and self-motivation, which are important factors for exercise programme initiation, adherence and long-term maintenance (Bandura, 1997). In fact, self-efficacy is a catalyst for maintaining motivation to exercise. Therefore, as recommended in **Table 7.2**, identifying participants that require external-motivating factors may be key to ensure adherence in exercise programmes.

Exercising gradually, providing achievable goals, feedback (e.g., emphasising achievements when barriers to exercise are overcome, such as attending to the exercise class when the participant did not feel like they could), and offer different exercise choices (providing problem-solving strategies to overcome barriers to perform a specific exercise), are measures that can be implemented to help researchers and exercise instructors address aspects related with reduced exercise self-efficacy when running exercise interventions and programmes for PwP (Stevens, Stanton, & Rebar, 2019). Additionally, including additional resources, such as a recorded video of the class, enables participants to decide where and when to exercise (more convenient), which can be used as a strategy to increase exercise self-efficacy. Nonetheless, it is important that researchers and the exercise instructors are aware of PD non-motor symptoms, such as apathy and depression, which can

contribute to lower motivation to actively engage with the exercise (Perepezko et al., 2019). Therefore, appropriate supervision is key to provide direct feedback, prompts and motivation to ensure the completion of exercise programmes at rates beyond participants' voluntary limits, which is known to be needed to induce global improvements in function (Ridgel et al., 2009). On the other hand, supported by previous research, participants with higher self-efficacy were able to appreciate more the social connectiveness that the online class was able to provide, foster their confidence and increase their motivation to engage and adhere with the exercise programme (Borrero, Miller, & Hoffman, 2020). Hence, participants that are more intrinsically motivated to exercise may need less support in the self-efficacy domain during the class. Nonetheless, in accordance with previous research exploring PwP experiences of participating in a group exercise, both types of class participants concurred that the group was the 'glue' and preferred exercising in a group setting (being face-to-face or online) rather than alone, which has the potential to improve social connections, fellowship and motivation (being all important factors to ensure PwP participation to exercise) (Claesson et al., 2020; Ravenek & Schneider, 2009). Moreover, the social bonds formed with fellow exercisers and instructors were key to promoting sustained motivation and adherence to the programme.

With focus on social connections and interaction, participants largely preferred the face-to-face group format of the class. However, amongst most participants, there was agreement that the online class was also interactive. This is particularly important for PwP, since most PD treatments (e.g., medication, DBS) have no effects on social and leisure roles and exercise seems to be the only revised activity type that can help improve social interaction (Perepezko et al., 2019).

This study presents some limitations that should be taken into account. During the global pandemic where in-person activities were cancelled, international guidelines recommended engaging with moderate-intensity exercise at home for 5 to 7 days per week to compensate for the decrease in normal daily physical activity levels (Jiménez-Pavón, Carbonell-Baeza, & Lavie, 2020). Although the live-online MM class aimed to maintain PwP's engagement with the MM programme, it was only available 1 day a week and, thus, did not meet the frequency and volume of exercise required by physical activity guidelines. Nonetheless, participants were persistently encouraged to stay active and try to achieve the activity levels recommended and were provided with video recordings of the MM class to assist them in achieving the required exercise target (some participants reported completing the video recording of the class on a daily basis). With the rapidly adopted measures by authorities, the abrupt cancellation of face-to-face contact limited our ability to measure outcomes of physical and cognitive function to allow the comparison of the effects of online versus face-to-face exercise on PwP function and cognition. Interestingly, there is research that suggests that telematic exercise for PwP may not be inferior to traditional in-person approaches (van der Kolk et al., 2019). However, this observation should be further investigated in future studies. Finally, further research should evaluate whether participants perceptions about the online exercise programme change over

time and try to elucidate whether, with the extension of the global pandemic and lengthened period of restrictions, participants would be more inclined to engage with online exercise programmes.

7.6 Conclusions

It is important to contemplate PwP's experiences, perceived benefits, and challenges to understand what needs to be considered when planning exercise programmes and strategies for the future. The present study examined participants' perceptions of an online-based exercise programme, and supports the feasibility, usefulness and safety of an online exercise class that uses SCT components in PwP. This study reveals that online-based exercise programmes present some disadvantages (presented in **Table 7.1**) mainly linked to reduced sense of social interaction compared to in-person programmes and there was a general preference towards face-to-face group exercise activities. Nonetheless, participants spoke highly of the online exercise class and confirmed that it was well organised, challenging enough, diverse, and interactive. Therefore, this study revealed several advantages linked to online delivery of exercise programmes for PwP and highlights the following qualities for PwP: inclusiveness, disappearance of geographical restrictions and costs, convenience, ability to complete challenging and intense exercise from home, amongst others (see **Table 7.1**). For exercise instructors, online exercise delivery may be a more cost-effective option, in terms of time and resources. Furthermore, the results of this study bear insights integrated into recommendations for researchers or health-care professionals that intend to design online exercise programmes or interventions for PwP (see **Table 7.2**).

Table 7.2 Guidelines and recommendations for an appropriate setting-up and delivery of online exercise programmes for health-care professionals and researchers working with PwP.

Observations	Recommendations
1. Class participants strongly recommended the online class to other PwP It is suitable and feasible for PwP.	Organise 1-to-1 meetings (or detailed email correspondence) with new participants prior to their enrolment with the online class to collect information (diagnosis date, medication, main symptoms, prevailing or important past injuries, movement limitations, etc.), introduce them to the nature of the class and the correct technique for the exercises. In addition to the exchange of information with the instructor, provide participants with a video of the class and allow them to ask questions/concerns they may have before joining the online group.
2. Related to the above observation, **participants provided recommendations for instructors to safely incorporate new participants**	**1st**. Instructor to explain the movements (in an individualised meeting). **2nd**. Start slow and build intensity up gradually (i.e., complexity, repetitions, range of motion). **3rd**. Eventually, get up to the speed and amplitude of the instructor.

	4th. If needed, create smaller groups of people with similar abilities/confidence with the exercises. Invite new participants to observe a live-online class prior to joining.
3. Importance of knowing individuals' capacities, PD stage and physical form	As described in Recommendation number 1, taking time to meet new participant and acquire appropriate information prior to their engagement is key to allow the instructor to evaluate participants' suitability for the online class format. Instructors may consider creating smaller groups based on intensity preferences or difficulty of movements/functional (and cognitive) abilities/PD stage. Further, instructors could consider creating a separate group for participants who need assistance and invite their partners/family members/support person to attend and assist throughout the class.
4. Importance of the platform Zoom is a reliable platform preferred by class participants (compared to Jitsi Meet [a free open-source video conferencing platform that did not require downloading an app but was deemed as more unstable than Zoom]). Downside of Zoom: it has an associated cost for holding meetings longer than 40 min.	Simplify participants access to the online sessions and provide clear instructions on how to download specific programmes (should this step be needed). For example, providing clear instructions on how to download an app and links to direct participants straight to the meeting room of the platform of preference made the access to the online class easier (from participants perspective). **Important**: be aware of platform security risks and adhere to platform provided guidelines to make a meeting more secure and avoid being hijacked (e.g., 'Zoombombing').
5. Record one (or several) online class Participants revisited the videoed version of the class and regularly utilised this resource (on some occasions on a daily basis) to exercise outside of the online class hours.	Provide recorded content to allow participants to exercise on their own time or in addition to the live-online exercise class.
6. Instructor set up The use of a big screen (at least >13 inches) allows the provision of better observation of participants technique and more detailed feedback.	Participants perceive whether the instructor has a better setup (with the use of bigger screens it allows the instructor to observe participants in more detail, and this was perceived by them as more detailed feedback was provided).
7. Instructor's interpersonal skills and PD expertise Participants value the professionalism of the instructor, their ability to provide a caring environment, being supportive, as well as challenging them in a positive way.	It is instrumental that the instructor has PD expertise, attention to detail, professionalism, outstanding communication skills to ensure an instrumental quality of exercise delivery (i.e., concise direct instructions are key, describing how certain exercises can focus on movements required for ADL, provide challenging content but always be ready to modify exercises to suit individual participants needs), ability to

	provide high-quality feedback and provide a caring motivating environment. (These extend to potential class helpers providing invaluable exercise monitoring and supervision).
8. The room and floor type where participants exercise are important Participants perceive that it is more difficult to exercise when family or friends are around the house. Participants perceive that it is more difficult to move on carpet.	Exercising in a quiet room not used for social gatherings is recommended to allow participants to solely focus on the exercise and avoid distractions. If possible, exercising on hard flooring (e.g., wooden floor) instead of carpet (thin carpet is preferable). Instruct participants to avoid uneven surfaces (e.g., rugs and thick carpets may affect balance and/or how forces are applied into the ground). They may also present tripping hazards and difficult ambulation in participants with a shuffling gait.
9. Safety Participants mentioned that they were aware that they were doing the class at their own risk, however, it is the instructor's duty to provide appropriate health and safety instructions and ensure that participants exercise in a safe area free of tripping hazards.	Complete a health and safety evaluation, which may be accompanied by asking participants to complete a home risk assessment. Ask participants to report any changes in medication or symptomatology prior to the start of the class. Always ensure that there is someone around and/or ask participants to provide an emergency contact (some participants may live and exercise on their own). Remove risks of falling by instructing participants on how to maintain the exercising area hazard-free, allow them to have water breaks, provide reminders to use the wall or chair as support when needed and provide adjustments to the exercises to be completed from a seated position should be needed.
10. The household environment is important Social factors associated to the household environment may affect participants' participation and attendance.	Discuss with participants the importance of exercising on a regular basis for PwP, treating 'exercise as medicine' and following a regular exercise schedule. Be opened to discuss the benefits of exercise to family members if they show interest, which may help them respecting participants space and time to exercise.
11. Motivation may be an issue Some participants need external sources of motivation whilst others are more intrinsically motivated	Identify which participants require more external-motivating factors and are less intrinsically driven so as to provide motivating prompts (e.g., identify participants' individual goals and get them to work towards their goals. For example, increasing the number of sit-to-stands correctly performed for one minute compared to their previous week). These

	participants may also benefit from receiving directed prompts. Provide active and positive encouragement throughout the class.
12. Instructor helpers Participants valued when the instructor had an extra 'pair of eyes' to help them supervising the class participants. It gave participants a sense of being more closely supervised and evaluated.	Having more than 15 participants (16 including the instructor) in your screen decreases the size of participants videos in gallery view (in gallery view up to 25 participants can be displayed per screen by default [using Zoom]). If having more than 15 class participants is difficult for the ability of the instructor to supervise participants and provide appropriate feedback, having a helper in the class is strongly recommended. (After the completion of this focus group study, the presently discussed online class had more than 15 attendees, thus, two undergraduate students from the University of Kent attended the online exercise sessions to provide additional individualised feedback and prompts to participants during the class whilst the instructor focused on delivering the exercises and providing general feedback and supervision).
13. Weekly engagement Participants need clear instructions on when to exercise.	Regular emails are useful to remind and motivate participants to attend the online class. Include the link to the preferred online platform in each reminder email to facilitate access to the online class.
14. Enable feedback and information traffic Additional individualised feedback provided by email is appreciated and valued by participants.	Email trafficking between the instructor and participants can help in providing extra feedback in a private individualised way that might not be able to be provided during the class.
15. People are not sure whether they would have joined an online class for PD had the pandemic not happened Being familiar with the class instructor and the other class participants is an important factor contributing to participants enrolment with the online exercise class. Some participants report that they would not feel confident to join an online class should they not have known any of the participants or the instructor.	Set a friendly and welcoming environment. Allow time for new participants to introduce themselves to the group and let the group participants welcome new members. Make the online room available for participants to log in before to the class and maintain the room available to allow opportunities to socialise after the exercise class.

Chapter 8. General Discussion

8.1 Summary of findings

This PhD thesis sought to investigate the disease-modifying potential of exercise for PwP. Both acute and long-term interventions were developed to explore different facets of putative mechanisms involving the beneficial effects that exercise may promote in PwP. Moreover, using a qualitative methodology, participants perceptions, opinions and motivations influencing participation and adherence in both a community-based and an online group exercise class were evaluated. Additionally, participants' partners also provided insights about the perceived effects that the exercise intervention may have for PwP from a carer's perspective. There is a tremendous amount of evidence that exercise can help in managing PD symptoms, improve both physical and cognitive function, and increase the levels of neurotrophic factors. However, there have been contrasting findings between studies due to differences in the evaluated exercise interventions (mode, timing, amount, and intensity of the exercise) and analytical methods used to measure biomarkers, which makes it difficult to generate conclusions and definitive exercise guidelines for PwP. Moreover, most studies take place in highly controlled laboratory settings (e.g., university research centres and clinical research units), which limits the translation of findings outside of these settings, such as in the community. These interventions may not always be easily available for PwP and their establishment is usually limited to the length of research studies. Therefore, the overall aim of the series of studies reported in this thesis was to develop exercise interventions that have a positive impact on the progressive nature of PD, can be implemented in real-world settings (at home or in the community) and can be maintained in the long-term, whilst also attempting to understand the underlying mechanisms that elicit responses to exercise training in PwP.

Reagent diluent optimisation is key to ensure optimal ELISA performance

The first study of this thesis (presented in chapter 3) was key to detail and suggest methodological steps for an accurate measurement of neurotrophins (BDNF and pro-BDNF) across different sample types (serum, plasma [PP-P and PR-P] and saliva). Widely varying levels of BDNF have been reported in the literature depending on the body fluid or method used for its detection, which are not standardised, nor usually reported in sufficient detail. Therefore, study 1 (Chapter 3) provides a thorough analysis with the widely used DuoSet ELISA Development System (cat #DY248 and cat #DY3175). Results revealed that each sample type had specific matrices that required the optimisation of their own reagent diluent to ensure assay performance and measurement accuracy. More precisely, we found that the reagent diluent used to measure BDNF in platelet-rich plasma, platelet-poor plasma and serum required combinations of 40%, 30% and 40% goat serum with PBS, respectively. Also, for pro-BDNF measurements in PR-P and PP-P, combinations of 10% goat serum with PBS were the most appropriate. The optimised reagent diluent was then used to create the serial dilutions for the standards and dilute the samples. It is important to mention that saliva BDNF and serum pro-BDNF measurements presented some difficulties. Regarding saliva BDNF measurements, previous research had already experienced difficulties in measuring saliva BDNF, however,

researchers had followed standard protocols of commercial kits, without adapting or optimising the protocol for this sample type (Vrijen et al., 2017). Their results were rarely above the minimum detection level of the ELISA kit and were, therefore, considered unreliable. Trying to overcome those limitations, we optimised the ELISA assay for the detection of BDNF in saliva and obtained both detectable and undetectable levels of BDNF. However, we found that there were factors in the saliva matrix causing a falsely elevated or depressed BDNF value in most of the measured samples. This is an important observation since, due to the high percentage of samples presenting with unacceptable recovery results, future studies intending to measure BDNF in saliva should always run a spike recovery test for each sample being analysed and only samples with recovery values between 80% and 120% should be included in their final analyses. Otherwise, the accuracy of the results could be doubted. Furthermore, previous studies that did not perform or report these steps must be interpreted with caution. Regarding serum pro-BDNF, the analyses revealed that the assay that was used was not sensitive enough to pro-BDNF measurements; most fell below the lowest detection level of the assay. It remains unclear as to why serum pro-BDNF levels are mostly undetectable, contrary to what was observed for plasma pro-BDNF.

Once an appropriate methodology for the sample analyses was established, the samples collected for the studies 1, 2 and 4, presented in Chapter 3, 4 and 6, respectively, were analysed following the above-mentioned methodological steps to measure BDNF and pro-BDNF. The link between these neurotrohpins and neurological conditions has received significant attention over the past two decades and research studies have shown that BDNF levels and function may be reduced and altered in PwP due to the vulnerability of dopaminergic neurons present in PD's neuropathology (Chauhan et al., 2001; Howells et al., 2000; Mogi et al., 1999; Parain et al., 1999; Rahmani et al., 2019; Scalzo et al., 2010). Moreover, it has been suggested that peripheral BDNF levels can be used as a proxy for central levels of BDNF (Pan et al., 1998). Nonetheless, BDNF data on PD participants are rather controversial and there is no general consensus as to whether PwP present lower peripheral BDNF levels compared to healthy controls and whether peripheral BDNF measurements are an accurate reflexion of brain BDNF levels (Allen et al., 2013; Numakawa, Odaka, & Adachi, 2018; Ventriglia et al., 2013). Nonetheless, to measure fluctuations in peripheral BDNF levels, measurements performed in PP-P would be a better representation of the systemic readily available levels of BDNF and pro-BDNF (although pro-BDNF has received considerably less attention and little is known about its peripheral levels and origin) (Serra-Millàs, 2016).

In our observational study, we evaluated plasma (PP-P and PR-P) and serum levels of BDNF in a PwP group that classed themselves as not being physically active and healthy participants that were not given instructions regarding their levels of physical activity. Differences in BDNF levels between PwP and healthy adults were not observed for any of the sample types that were assessed, however, we were able to highlight differences between groups in terms of pro-BDNF levels measured in PP-P (i.e., PwP presented significantly higher pro-BDNF levels) and BDNF genotype. That is, PwP

presented higher frequency of the Val allele ($BDNF_{VAL}$) and, related with their functional outcomes, it could be hypothesised that PD individuals with the $BDNF_{VAL}$ polymorphism could be better responders to exercise and more susceptible to the deleterious effects of physical inactivity than Met carriers (de las Heras et al., 2020). Nevertheless, further studies are required to confirm these hypotheses. The lack of significant differences in BDNF levels between PwP and healthy adults may be explained by compensatory mechanisms that upregulate peripheral BDNF in PwP to counter neuronal insults (Ng et al., 2021; Scalzo et al., 2010). Scalzo et al. (2010) found that BDNF levels were lower only in the newly diagnosed patients with PD, whilst increases in BDNF levels were observed in patients at advanced stages of the disease (Scalzo et al., 2010). However, due to the heterogeneity of PD disease duration, a recent meta-regression analysis could not justify that disease duration was the reason for BDNF differences between PwP and healthy controls (Rahmani et al., 2019). BDNF measurements were complemented with measures of pro-BDNF in PP-P, which showed significant differences between groups. Thus, similarly to other research in PD, PwP presented higher levels of pro-BDNF, which were not always accompanied by changes in BDNF levels (Suire et al., 2017; Yi et al., 2021). Although BDNF signalling has been suggested by several studies as one of the most prominent molecules to evaluate the effects of interventions for PwP or holding diagnostic value for PD, our research highlights the complexity of its measurements. Moreover, due to the multi-factorial nature of PD, it is unlikely that one single biomarker or measure can characterise this complex phenomenon. Therefore, adding pro-BDNF measures may provide additional information about the pathophysiological changes present in PD. Nonetheless, more research is needed to elucidate the relevance of using the pro-BDNF/BDNF ratio as a biomarker.

Benefits of the MM exercise intervention for PwP

Physical activity has been extensively studied and regarded as an effective adjuvant treatment for PwP. However, important outcomes such as physical function, cognition and neuroprotective biomarker levels are not usually studied concurrently, which may risk missing important information (as highlighted above). To address the multi-faceted impairments presented in PD and modify (i.e., halt or delay) their development, a weekly MM exercise class specifically designed for PwP in a community-based group setting was evaluated in study 2 (Chapter 4). The MM intervention successfully targeted both physical and mental faculties with the overall aim to slow down the rate of decline present in PD. A strength of the study is that the effects of age, disease duration, disease stage (H&Y scale) and medication (LEDD) were taken into account when interpreting the results, which showed improvements in walking capacity (5% improvement after 8 months), functional mobility (11% improvement after 4 months), lower extremity strength (15% increase after 4 months) and bilateral grip strength (9% increase after 28 months) and a maintenance in function across 1, 2 or 3 years (importantly, the results of the mixed-effects models across all time points showed that none of the measured outcomes decreased more than their MCD). Furthermore, group comparisons showed that MM exercisers significantly improved their mobility, lower extremity strength,

cognition and BDNF levels opposed to PwP that did not engage with MM exercise. Therefore, the potential for MM exercise to increase physical function, cognition and peripheral BDNF levels in PD patients, justifies that this mode of training might provide optimal treatment for both motor and non-motor symptoms in PwP and slow down deterioration, compared to single-mode exercise training (e.g., cardiovascular exercise alone).

Objective measurements were complemented with a qualitative evaluation of themes related to participants' experiences and perceptions about engaging with a community-based exercise class for PwP, its psychosocial benefits and the potential factors influencing participants adherence to the programme (presented in study 3, Chapter 5). However, it is worth bearing in mind that, as a stand-alone intervention, the weekly MM programme did not meet the overall levels of physical activity recommended by exercise guidelines (ACSM, 2016, 2017; Davies, Atherton, McBride, & Calderwood, 2019). Although, contrary to our outcomes, previous studies in older adults have found that completing an exercise programme once a week is not sufficient to improve function (Stiggelbout, Popkema, Hopman-Rock, De Greef, & Van Mechelen, 2004), studies evaluating interventions that are completed twice or three times a week may compromise participants attendance and retention in the long-term. For example, Flynn et al. (2021) designed a 10 week intervention where participants took part in three 60 min sessions a week of individualised exercises prescribed by a physiotherapist (Flynn et al., 2021). Although the intervention was able to provide improvements in balance and walking speed and was deemed feasible and acceptable for PwP, it is important to highlight that 25 people declined to be involved in the study as they could not commit to attending the centre more than once a week. As such, researchers subsequently recognised that their findings may have overestimated the burden of the intervention (Flynn et al., 2021).

There are disease-specific barriers that may impede the participation of PwP with exercise regimes with a frequency higher than once a week, such as non-motor symptoms (e.g., fatigue and apathy), low self-efficacy, lack of time of participants or the burden that it can place on carers (Ellis et al., 2013; Prado, Hadley, & Rose, 2020; Schootemeijer et al., 2020). Regarding the latter, finding balance between caring for a person with PD and having time to attend one's own needs is important to preserve carer's wellbeing, who usually are members of their family and, commonly, the spouse of the PwP (Goy, Carter, & Ganzini, 2008; Prado et al., 2020). None of the carers (spouses) that took part in the focus group meetings presented in study 3 (Chapter 5) defined the MM programme as burdensome. Thus, providing sustainable community solutions (such as the MM community-based exercise class proposed in this thesis) that are both beneficial for PwP and not overburdensome for carers is necessary. Overall, the study findings suggest that the MM community-based exercise class was feasible, safe and beneficial for PwP at H&Y scale I to IV. Moreover, bearing in mind the chronic progressive nature of PD, this study emphasises the importance of developing and completing long-term MM exercise in order to see functional, cognitive and neuroplastic improvements in PwP. Nonetheless, further studies should evaluate whether increasing the frequency of the MM programme

might further improve the observed benefits or impact participants retention, attendance, and carers burden.

Effects of different acute bouts of aerobic exercise on cognition and neurotrophic factors

Based upon the findings of study 2 (Chapter 4), which presents the beneficial effects of long-term engagement with a MM programme, study 4 (presented in Chapter 6) aimed to specifically investigate whether there were added benefits from combining physical and cognitive tasks compared to engaging with aerobic exercise only. Moreover, this study provides a preliminary acute evaluation of participants' neurotrophin levels (BDNF and pro-BDNF), kinetics and cognitive function under 4 different conditions: a cycling session (visit A), a second cycling session 24 h. after A (visit B), combined session of cycling and cognitive tasks (visit C) and a resting condition (visit D). Research supports that cognition plays a pivotal role in the regulation and control of mobility (Amboni, Barone, & Hausdorff, 2013). A multi-centre RCT that evaluated the effects of long-term exercise on cognitive and physical function in participants older than 70 years, found that physical and cognitive performance were correlated and exercise could be used as strategy to not only improve physical function but also improve cognition in older adults (Williamson et al., 2009). These observations are particularly important for PwP, where declines in cognitive function (e.g., executive impairment) are an independent risk factor for the development of physical frailty with PD progression (Lin et al., 2019). Therefore, knowing that cognitive skills can positively impact functional and physical abilities, developing interventions that can modulate PwP's cognition is imperative. It is suggested that increases in information speed, improved attention and decreases in reaction time are cognitive skills that can positively impact functional and physical abilities (e.g., mental flexibility and attention are important for balance skills, and reduced processing speed was found to be associated with difficulty in performing turns in PwP) and can be modified with appropriate training (Figueiredo Sousa & Macedo, 2019; Pal et al., 2016).

Therefore, study 4, presented in Chapter 6, evaluated participants' cognition with the Stroop Test, which has successfully been administered in PwP as a measure of cognitive flexibility, attention, processing speed and response inhibition (Djamshidian, O'Sullivan, Lees, & Averbeck, 2011; Hsieh et al., 2008), and the Free-Recall Test (for LTM), since the effects of the current interventions on PwP's memory have not been investigated (Teixeira-Arroyo et al., 2014). Our results showed that both visits B (second cycling bout) and C (combined) were able to elicit larger improvements in the Stroop test with large (d=0.853) and small to medium (d=0.349) effects and up to 30 and 40% improvements in immediate LTM, respectively, compared to the resting control visit. Moreover, cognitive performance was positively correlated with serum and capillary BDNF levels. Together, these outcomes shed some light on the beneficial effects that performing aerobic exercise on subsequent days or in combination with cognitive tasks may have for PwP's cognitive function, as well as the underlying mechanisms that may be causing the changes. Further research is needed to

determine the extent to which aerobic exercise (alone or repeated on subsequent days) or combined with cognitive tasks can improve cognition and levels of neurotrophic factors in PwP. Additionally, future studies should evaluate the implications that exercise-induced improvements in cognition could have on disease progression.

Participants' experiences and perceived psychosocial benefits of in-person and online group-based exercise programs for PwP

Most interventions providing evidence of the beneficial effects that exercise has on PD's symptomatology do not consider the participant's experience of these interventions and whether they may perceive any effects beyond the objective outcomes (e.g., effects of the intervention beyond the assessments, enjoyment, dislike, improvements in social functioning, etc.). Participant's thoughts and perceptions should be considered and taken into account for the successful development of long-term exercise interventions. Thus, alongside the long-term community-based group exercise intervention that was evaluated in study 2 (Chapter 4), rigorous checklists and guidelines were thoroughly followed to develop qualitative studies using a reflexive thematic analysis (Braun & Clarke, 2006, 2019, 2021; Campbell et al., 2021; Clarke & Braun, 2013). This allowed us to provide insights into the effectiveness of real-world exercise interventions (both community-based and online) and capture important information about the participants' experiences and perspectives. The first qualitative study presented in this thesis (Chapter 5) explored the attitudes, opinions, and subjective perceptions of exercise participation from class participants (PwP) and their partners. Results revealed that both participants and partners felt that the group class provided PwP with an opportunity to proactively self-manage their health, as well as fellowship, meaningful social connections, and a perceived positive affective experience, which were important factors for sustaining exercise participation and improving physical fitness, psychological well-being, and QoL. Due to the COVID-19 pandemic, the MM community-based class had to change delivery mode, so at the end of March 2020, started being delivered online. Then, a process evaluation of the change in mode of delivery of the Parkinson's specific MM exercise class was conducted: from face-to-face towards online delivery. This change of setting proved to be feasible and safe for PwP, who identified that being familiar with both the exercise programme and the exercise instructor, as important reasons to engage with the online format of the class. Moreover, results highlighted that, although this delivery mode was highly suitable, practical, and interactive, most participants preferred the face-to-face group format of the class. This second qualitative study also provided a natural 'spin-off' opportunity from the sudden switch to online delivery and the knowledge gleaned provided an opportunity to develop guidelines to other researchers or health-care professionals intending to follow a similar format of exercise delivery for PwP.

Key points

1) All the proposed studies are original in the literature in PwP.
2) Evaluating the levels of both BDNF and pro-BDNF and genotype provide valuable insights into potential mechanisms that regulate exercise-induced benefits on PwP's physical and cognitive functions. However, careful, and thorough methodological steps must be followed to ensure an accurate measurement of these neurotrophic factors, which include appropriate reagent optimisation and spike-recovery checks when using ELISA assays.
3) The MM intervention approach, combining motor training and cognitive challenges, designed for the longitudinal community-based study, as well as the comprehensive assessment of neurotrophins, motor and cognitive function, are innovative.
4) The length of the MM intervention (more than 3 years; 154 weeks).
5) The practical utility of the MM programme in both in-person and online formats (specific, supervised, safe, reproducible, low-cost, disease-modifying programme with clinical applicability).
6) Study 4 (Chapter 6) serves as initial step for future studies evaluating whether combined exercise elicits more synergistic benefits than performing aerobic exercise alone or on two consecutive days in PwP.

8.2 Limitations

The thesis presents original and innovative work in multiple ways and these are presented above. However, there are also some implicit limitations that should be taken into consideration.

The clinimetrics (term used to indicate the practice of using indexes, rating scales, etc. to describe or measure symptoms, physical signs and other clinical outcomes) of Parkinson's are significantly challenging. The high inter- and intra- person variability and different responses to treatment make it a particularly difficult task. In our research, we have included measurements of the H&Y scale as well as other measures specified in the methods section. We are aware that, currently, the MDS-UPDRS is one of the most evaluated, valid and reliable scales, commonly used as the gold standard in the field (Lim & Tan, 2018; Ramaker, Marinus, Stiggelbout, & van Hilten, 2002). Not including participants' MDS-UPDRS scores might be interpreted as a limitation, however, it is suggested that other more detailed scales should be used to assess specific parameters (e.g., non-motor symptoms or specific motor features such as gait and balance, amongst others), which might be less contaminated by medication effects (Ahlskog, 2018; van der Kolk et al., 2019). For instance, the 6MWT, the TUG and tests of upper limb motor function are recommended and widely used in PD research. The rationale behind the tests chosen in this thesis is supported by the fact that an objective scoring, with specific, valid and reliable measures is less time consuming, clinically relevant and may be more sensitive to change compared with the UPDRS (Lim & Tan, 2018). Moreover, these measures also better reflected change in performance as they closely imitate the exercise intervention

(i.e., sit-to-stands, shuttle walking, turnings, etc.) and challenges presented in everyday life (e.g., walking, getting out of a chair/bed/ toilet, etc.).

We also established participants' physical activity levels on the basis of the IPAQ Short Form questionnaire. However, the use of self-report questionnaires to measure physical activity levels is challenging and, in some instances, may not be accurate for actual levels of physical activity. Utilising measured techniques, like accelerometery would be recommended.

As already mentioned, the restrictions put in place to control the spread of the COVID-19 pandemic severely impacted the sample size of the study presented in study 4 (Chapter 6). Although the preliminary results obtained presented medium to large effect sizes for some of the evaluated outcomes, there was poor sensitivity to significantly detect effects due to the sample size of the study (n = 6).

The studies presented in this thesis did not evaluate whether the observed benefits were maintained beyond the period of engagement with the exercise programme. Due to the unexpected cessation of the MM group class due to the COVID-19 pandemic and restrictions limiting research testing, researchers were unable to evaluate participants function after the intervention had stopped. Thus, the long-term follow-up effect of the exercise interventions after their completion should be evaluated in future studies, which is not commonly assessed in exercise trials (Tomlinson et al., 2014). This information would help to determine how long the effects of an exercise intervention last in an individual with PD after the cessation of the programme.

Finally, researchers intended to recruit an homogeneous sample of participants following strategies that had been developed to improve recruitment, participant selection and maximise inclusivity (Picillo, Kou, Barone, & Fasano, 2015). However, PD is a highly heterogenous disease and participants' involvement with the studies was voluntary. Therefore, the responsibility to be involved in the studies depended on participant's willingness. Most of those who took part in the studies 2 and 4, presented in Chapter 4 and 6, were self-selected members of active PD support groups. Therefore, our exercise strategies might have caught the attention of participants that were especially motivated, proactive and knowledgeable about PD and the impact that exercise can have and may not be representative of all PD communities. On top of the recruitment strategies that were already followed in the current thesis (e.g., flyer postings, research meetings, etc.), future studies should implement approaches to improve challenges associated with participants recruitment, such as working closely with movement disorder neurologists, which would allow for fast-tracking clinic patients and greater coverage of in-person clinic recruitment (Hall et al., 2018).

8.3 Practical Applications and Future Directions

Measuring BDNF in PP-P has the potential of reflecting systemic readily available levels of BDNF that do not come from platelets, which would have a restricted bioavailability and be measured in serum or PR-P samples. Thus, it is suggested that the acute decrease observed in PP-P BDNF after the exercise interventions could be due to the rapid uptake of BDNF by platelets or the liver, cellular reuptake or binding to its neuronal receptor TrkB (Dieni et al., 2012; Fujimura et al., 2002; Fumagalli et al., 2006; Hernandez-Baltazar et al., 2019; Pardridge et al., 1994). Further studies improving the temporal resolution of the sampling timeline will be able to elucidate this and discuss the practical utility of measuring BDNF in PP-P.

Increases in peripheral basal levels of BDNF were observed after engaging with MM exercise for approximately 8 months. However, it was outside of the scope of this thesis to investigate its origin. Our aim was to increase peripheral levels of BDNF and investigate its implications for physical and cognitive functions. Nonetheless, this observation provides possible goals for future work. For instance, recent developments in the field of biomarkers have identified brain-derived extracellular vesicles, specifically exosomes of neuronal origin, that can be detected in peripheral blood, as BDNF carriers. These have been proposed as a useful biomarker for PD since they provide an *in vivo* window to the CNS allowing researchers to better monitor long-term changes in important mediating factors (Ohmichi et al., 2018; Rani et al., 2019). Moreover, we were able to observe differences in pro-BDNF levels between PwP and healthy adults, which may indicate potential mechanisms that could be playing a role in PD pathology. Related with the previous point, there is research suggesting that a decline in walking speed in older adults is associated with elevated pro-BDNF in plasma extracellular vesicles (Suire et al., 2017). Therefore, the study of exosomes and other molecular mechanisms that may be affecting the bioavailability of neurotrophic factors (such as MMP-9, cathepsin B, lactate, irisin, histone methylation or acetylation, peroxisome proliferator-activated receptor-γ coactivator 1α [PGC-1α], amongst others) is important to develop efficient strategies and interventions to generate the largest possible physical and cognitive benefits to modulate PD pathology (Chen, Wu, Mesri, & Chen, 2016; He et al., 2013; Moon et al., 2016; Nay et al., 2021; Norheim et al., 2011; Toker et al., 2021; Wen et al., 2016; Wrann et al., 2013).

Finally, MM exercise is recommended as an exercise strategy to improve and maintain physical function and cognition in PwP, and it is feasible to run in the long-term in home, community-based and online settings. Further studies should evaluate whether the functional and cognitive benefits observed in the longitudinal and acute exercise interventions included in this thesis develop from the aerobic component or due to synergistic effects of the combination of strength, aerobic and cognitive elements. Moreover, future research should also investigate whether increasing the frequency of the MM class would be feasible and provide further benefits.

8.4 Conclusions

The overarching aim of this thesis was to provide a multidimensional evaluation of acute and long-term exercise interventions for PwP assessing their effects on participants' physical function, cognition, and biomarker levels (BDNF and pro-BDNF). First, the methodological considerations presented in Chapter 3 set the field of future studies intending to measure BDNF and pro-BDNF in serum, plasma, and saliva. Subsequently, the studies presented in this thesis are able to show the benefits of exercise for PwP in different settings: (1) long-term: after taking part in a MM community-based exercise programme for up to 3 years, an exercising group of participants experienced improvements in walking capacity, functional mobility, and upper limb strength over time, and also perceived that the MM class could positively impact their physical fitness, psychological well-being, and quality of life; (2) long-term: unlike non-active PwP, the exercising group was able to significantly improve both their physical and cognitive function, as well as their BDNF levels; (3) acute: preliminary results suggest that cycling and its combination with cognitively challenging tasks might enhance participant's cognition, however, further research is needed to clarify these findings with a bigger sample size; (4) online: the transition of the community-based exercise class towards an online setting provides a feasible, useful and safe option for PwP to sustain their participation in the MM programme when in-person exercise classes are not available (or used as a supplement). Overall, the studies in this thesis support the notion that exercise, particularly performed in the long-term, can slow down PD progression and enhance neuroprotective mechanisms.

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Appendices

Appendix A

School of Sport & Exercise Sciences (SSES)

REQUEST FOR AMENDMENT TO RESEARCH ETHICS

School of Sport & Exercise Sciences (SSES)
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Original Ethics Reference No.:

Prop04_2016-2017

Date of request: 23.09.2017

Title of research project: Parkinson's Disease Exercise Class

Name of person making request: Dr Steve Meadows & Dr Glen Davison

Details of proposed amendment(s):

This is an ongoing project so notifying SSES ethics that extension until September 2018.

1. **Anna Ferrusola-Pastrana has joined the research team as a new PhD student so need to add her name to the project allowing her access to participant personal information, data collection, data analysis and reporting / publication purposes.**
2. **Anonymised data will be stored in a Dropbox folder. Personal identifiable information will be removed, the Excel spreadsheet will also be password protected and access to Dropbox folder will be limited to Dr Steve Meadows, Dr Glen Davison and Anna Ferrusola-Pastrana.**
3. **Undergraduate students will be volunteering during the exercise class sessions, assisting with data collection on health parameters (height, weight, waist circumference, resting blood pressure and heart rate) and functional capacity testing (6-minute walk test, 1-minute sit-to-stands, timed-up-and-go and bilateral grip strength. They will have access to anonymised data and will report the findings in a poster presentation and thesis. Undergraduate students aligned to this project for 2017 – 2018 are: Tomide Daniel, Ellie Jamieson and Emily Barron.**

Reason for amendment:

Ongoing project with new personnel.

Approval granted: **YES**

Reason for approval being denied:

Signed by SSES REAG Chair: Louis Passfield ----------

Print name: **Louis Passfield----------**

Date of approval: **6/10/2017----------**

Appendix B

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 61_2017_18

Date: 15th December 2017

Dear Anna Pastrana,

Re: Evaluation of Parkinson's Disease exercise class.

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 61_2017_18) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

Appendix C

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 63_2018_19

Date: 19th March 2019

Dear Anna Pastrana,

Re: Physical Function, Biomarkers Levels and Cognitive Function in Healthy Older Adults and People with Parkinson's Disease: A Longitudinal Observational Study

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 63_2018_19) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

Appendix D

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference:

Prop 45_2018_19

Date: 26th February 2019

Dear Chris Fullerton,

Re: Effects of a multimodal exercise programme for people with PD: A focus group study

I am delighted to confirm that SSES REAG has approved your research study (REF No. Prop 45_2018_19) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Louis Passfield

(Chair SSES REAG)

Appendix E

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference: Prop 28_2019_20

Date: 09.01.20

Dear Anna,

Re: Ethics Review - Prop 28_2019_20 - A Biomarker Approach for Exercise in Parkinson's

I am delighted to confirm that SSES REAG has approved your research study (Prop 28_2019_20) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Karen Hambly

(Chair SSES REAG)

Appendix F

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent at Medway
Chatham Maritime
Kent
ME4 4AG

Ethics Reference: 1_2020_21

Date: 3 November 2020

Dear Anna,

Re: Suitability, usefulness and effects of online delivery of multi-modal exercise for people with Parkinson's disease: A focus group study

I am delighted to confirm that SSES REAG has approved your research study (REF No. 1 2020_21) and you are now permitted to recruit participants and commence your research.

If you need to amend any aspect of your research, please ensure you inform SSES REAG by completing a request for amendment form and submitting all revised paperwork (e.g. participant information sheet, questionnaires).

If there should happen to be any adverse event during your study, please also ensure SSES REAG is kept informed.

I hope your study is successful.

With kind regards,

Karen Hambly

(Chair SSES REAG)

Appendix G

Participant's full version of the flow chart including all the information between assessments 4th, 7th and 10th. Flow chart created with BioRender.com.