



**CAN INHALING HYDROGEN GAS OR DRINKING HYDROGEN RICH WATER
IMPROVE SYMPTOMS OF PARKINSON'S?**

This thesis is presented for the Degree of Doctor of Philosophy at the University of Kent

by

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Abstract

The purpose of this thesis was to investigate the effects of Hydrogen (H₂) on motor and non-motor symptoms in people with Parkinson's (PwP), and to investigate the potential mechanisms of H₂ through measurements of markers of inflammation, neuroinflammation and antioxidant capacity.

H₂ has some demonstrated neuroprotective effects in animal models of Parkinson's disease (PD), suggesting benefits for PwP but research has reported opposing findings. There is an imperative need to slow progression of PD as there is no known cure and there are exponential increases in diagnoses in part due to ageing and increased awareness of PD. Therapeutic interventions which may help reduce motor and non-motor symptoms of PD should be explored, and H₂ has demonstrated that it is safe to consume, has no side effects and is well tolerated. However, optimal route of administration, dose, and timing of H₂ has yet to be established, and as PD is an incredibly complex and heterogeneous disease, this may contribute to difficulty in establishing if H₂ can improve health outcomes for PwP.

A pilot study (Study 1) in healthy participants first aimed to assess if the timings of measures and acceptability of an intervention were feasible for a future proposed study (Study 2) in people with Parkinson's (PwP) and helped refine clinical practices. Measures were the Flanker Compatibility Task to assess cognition, Purdue Pegboard Test to measure manual dexterity and the Profile of Mood State – Short Form to assess mood. This study demonstrated the intervention was well-tolerated with no adverse effects reported.

The protocol in Study 2 followed the same design as Study 1 and was the first known study to assess the effects of an acute H₂ inhalation period in PwP. Measures were the same as Study 1, with the addition of the MDS-Unified Parkinson's Disease Rating Scale 3.15 to measure postural tremor and blood samples to measure total antioxidant capacity (TAC). This preliminary study showed H₂ increased TAC.

Subsequently, a study with one participant which assessed a chronic inhalation period was then carried out. Measures were the same as Study 2 with the addition of the Movement Disorder Society – Non-Motor Rating Scale, Timed Up and Go Test and the six-minute walk test with tremor not being assessed. It was found that H₂ had no reported side effects, reduced systolic blood pressure, increased walking distance, and increased TAC.

Study 4 measured H₂ concentration of hydrogen rich water (HRW) and dissipation rate. This study demonstrated that if participants did not drink HRW immediately after production, then H₂ concentration would still be maintained by 90 % after one hour, and approx. 80 % after four hours, which then led to the final study of the thesis.

The final study in this thesis explored drinking HRW in PwP and if this affected motor and non-symptoms and biomarkers of inflammation, neuroinflammation and oxidative stress (as well as TAC). This preliminary study demonstrated that H₂ increased IL-6 and TAC but did not affect functional movement, or motor or non-motor symptoms of PD.

In summary, this thesis provides preliminary evidence that H₂ increases total antioxidant capacity (TAC) in PwP, however, it does not affect motor and non-motor symptoms, which may prove beneficial over time for PwP in helping to mediate their symptoms.

Declaration

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

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Scientific Output

Conference communication

Chapter 4 – Study 2:

Dargan, K., Davison, G., Meadows, S. (2023) The effects of hydrogen gas inhalation on motor and non-motor symptoms in people with Parkinson's. British Association of Sports and Exercise Medicine 2023 Annual Conference, Manchester, UK. October 2023. Poster presentation.

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Abbreviations

°C	Degrees Celsius
4-HNE	4-Hydroxynonenal
6MWT	6-Minute Walk Test
6-OHDA	6-Hydroxydopamine
8-OHdG	8—hydroxy-2'-deoxyguanine
8-OH-G	Oxidized guanine
A-syn	Alpha - synuclein
ADL	Activities of Daily Living
AF	Atrial Fibrillation
ANG	Anger
ANOVA	Analysis of Variance
ART	Average Reaction Time
ATP	Adenosine Triphosphate
B&A	Bland & Altman
BBB	Blood Brain Barrier
BDNF	Brain Derived Neurotrophic Factor
BMC ISRCTN	BMC International Standard Randomised Controlled Trial Number
BMI	Body Mass Index
BP	Blood Pressure
Cm	Centimetre
CNS	Central Nervous System
CON	Confusion

CRP	C-reactive protein
CSE	Congruency sequeunce effect
CSF	Cerebrospinal Fluid
CSP	Chair Stand Performance
CTE	Chronic traumatic encephalopathy
CVD	Cardiovascular Disease
DBS	Deep Brain Stimulation
DEP	Depression
DBP	Diastolic Blood Pressure
DBS	Deep brain stimulation
DH	Dissolved Hydrogen
DiAcSpd	N1, N8-diacetylspermidine
DJ-1	Protein deglycase DJ-1
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ENS	Enteric Nervous System
ERA	Esteem-related affect
ES	Effect size
F	Degrees of Freedom
FAT	Fatigue
FOF	Fear of falling
FW	Free-water (imaging)
g	Grams
GSH	Glutathione

H&Y	Hoehn & Yahr Scale
H	Hour(s)
H ⁺	Hydrogen ion
H ₂	Hydrogen
Hg	Mercury
Hr	Hour(s)
HRW	Hydrogen rich water
HSP70	Heat Shock Protein 70
HYD	Hydrogen intervention
HYD1	Hydrogen intervention 1 st phase
HYD2	Hydrogen intervention 2 nd phase
IL-1 β	Interleukin-1 Beta
IL-6	Interleukin-6
IL-10	Interleukin-10
IMB	Information-Motivation-Behaviour
IV	Intravenous
kg	Kilogram
Lab	Laboratory
LB	Lewy Body
LBD	Lewy Body Disease
L-DOPA	Levodopa
μ L	Microlitre
MAO-B	Monoamine oxidase-B
MDS	Movement Disorder Society

MDS-NMS	Movement Disorder Society Non-Motor Symptoms
MDS-UPDRS	Movement Disorder Society – Unified Parkinson’s Disease Rating Scale
Mg	Magnesium
mg	Milligram
min	Minutes
mL	Millilitres
mm	Millimetre
mmol/L ⁻¹	Millimoles per litre
ms	Millisecond
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Multiple Sclerosis
MVPA	Moderate to Vigorous Physical Activity
NADH	Nicotinamide adenine dinucleotide hydrogen chain
NaOH	Sodium Hydroxide
NF-KB	Nuclear factor kappa B
nM-EDL	Non-Motor Aspects of Experiences of Daily Living
NO	Nitric oxide
·OH	Hydroxyl radicals
O ₂	Oxygen
OH	Orthostatic Hypotension
OH-	Hydroxide ions
ONOO-	Peroxynitrite
p	Significance
η ²	Partial Eta Squared

PA	Physical Activity
PAR-Q	Physical Activity Readiness-Questionnaire
PBM	Photobiomodulation
PCAS	Post-cardiac arrest syndrome
PD	Parkinson's disease
PDQ-8	Parkinson's disease questionnaire - 8
PDQ-39	Parkinson's disease questionnaire - 39
PEM	Proton-exchange membrane
PLA	Placebo intervention
PLA1	Placebo intervention 1 st phase
PLA2	Placebo intervention 2 nd phase
PNS	Peripheral Nervous System
POMS-SF	Profile of Mood States – Short Form
PPM	Parts per million
PPP	Platelet poor plasma
PPT	Purdue Pegboard Test
PUK	Parkinson's United Kingdom
PwP	People with Parkinson's
QoL	Quality of Life
R+L+B	Right + Left + Both
RA	Rheumatoid Arthritis
RCF	Relative Centrifugal Force
REAG	Research Ethics Advisory Group
RM	Repeated Measures

ROS	Reactive Oxygen Species
RPM	Rotations per minute
RT	Reaction Time
SBP	Systolic Blood Pressure
SD	Standard Deviation
SIBO	Small intestinal bacterial overgrowth
SN	Substantia Nigra
SNCA	Synuclein Alpha
SNpc	Substantia Nigra pars compacta
SSED	Single Subject Experimental Design
SSES	School of Sport and Exercise Sciences
SSES REAG	School of Sport and Exercise Sciences Research Advisory Group
TAC	Total antioxidant capacity
TBI	Traumatic Brain Injury
TDS	Total dissolved solids
TGF- β	Transforming growth factor - beta
TEN	Tension
TMD	Total Mood Disturbance
TNF- α	Tumor Necrosis Factor - alpha
TUG	Timed Up and Go Test
UPDRS	Unified Parkinson's Disease Rating Scale
UPSIT	University of Pennsylvania Smell Identification Test
VIG	Vigour
YOPD	Young Onset Parkinson's Disease

Chapter 1. Literature review

There is currently no cure for Parkinson's disease (PD), and use of therapeutic interventions - which aid in the reduction of motor and non-motor symptoms of PD - should be explored. Hydrogen (H₂) can cross the blood-brain barrier (BBB), has no known side effects and does not alter or interfere with other drugs.

Research in PD and H₂ was first carried out fifteen years ago in animal models of PD (Fujita, Seike, Yamakawa, et al. 2009; Fu et al. 2009). A few years later this research was first translated into people with Parkinson's (PwP) (Yoritaka et al. 2013) with promising results that H₂ improved motor and non-motor symptoms. The mechanism by which H₂ follows is yet to be fully understood, with research relying on the theory that H₂ reduces oxidative stress (Ohsawa et al. 2007), and subsequent improvements in PD symptoms are due to a reduction in oxidative stress. More broadly, H₂ research has used different dosages, different timings, and different routes of administration, all while measuring different biomarkers. These differences make comparison between different studies difficult.

This literature review discusses PD, H₂ and synthesises research in H₂ and PD. It gives an overview of the history of PD, the neurobiology of PD, factors which may increase susceptibility to PD, PD symptoms, management of PD, and potential PD biomarkers. Hydrogen will then be discussed; routes of administration, mechanisms of action of H₂, and then there will be a review of the research literature in PD and H₂.

1.1. Introduction to Parkinson's disease

If Parkinson's disease (PD) were transmissible, it would be labelled a pandemic (Allen 2016). PD is the second most common neurodegenerative disease in the world, there is no known cure for PD, and cases globally are expected to more than double in the next 20 years (Dorsey and Bloem 2018). In the UK, around 153,000 people are living with PD, and two people are diagnosed with PD every hour (Parkinson's UK, 2025).

PD was first formally described over 200 years ago in work entitled "An Essay on the Shaking Palsy" (Parkinson 2002). This is considered the foundational work in identifying and describing symptoms of what is now recognised as PD. However, symptoms of PD were described as early as the 17th century, with descriptions of tremor, and descriptions of

quickening gait were described slightly later in the 18th century (Goetz 2011). The term “Maladie de Parkinson” was later coined in the mid-19th century (which translates to “Parkinson’s disease” in English) (Charcot 1872). This work also differentiated PD from other diseases particularly multiple sclerosis (MS), this is important as the aetiology of MS, and the subsequent pharmacological management, are very different to PD. Work was undertaken by various researchers from then on until the seminal work of Greenfield and Bosanquet (1953) who detailed PD and description of brain stem lesions. Hoehn and Yahr (1967) based their PD staging system on this research, and these stages are still in use today to help assess PD severity in clinical practice and research. Levodopa as a therapeutic agent for PD was first introduced in the late 1960s/early 1970s in response to the understanding that people with Parkinson’s (PwP) had significantly reduced levels of dopamine. Levodopa is dopamine’s precursor, and is still the first line drug in the management of motor symptoms of PD.

1.2. The neurobiology of Parkinson’s disease

PD is a multifactorial, progressive, extraordinarily complex disease (Dorszewska et al. 2021), and is still not yet fully understood. This proves problematic in diagnosing cases of PD and in PD research, as symptoms can be wide ranging, and there is no biomarker for PD. PD is observed as either familial, whereby there is a genetic link, or sporadic whereby environmental factors are involved in the development of PD.

Whether familial or sporadic in nature, there is a loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) area of the midbrain in PD, which leads to a reduction in dopamine (see Figure 1.1). Dopamine is a neurotransmitter which is responsible for various functions including movement, motivation, executive functions, and mood regulation. Alongside dopaminergic neuron loss, alpha-synuclein (α -syn) aggregation is observed in the SNpc in PwP, and α -syn aggregation leads to Lewy body pathology, however, α -syn aggregates have been found in other areas of the central nervous system (CNS) and peripheral nervous system (PNS). α -syn aggregates cause microglia to activate, which therefore induces an immune response, resulting in neurodegeneration (Klegeris et al. 2008). As α -syn aggregates have been found in the PNS, this fits nicely with the ‘dual hit’ hypothesis (Hawkes, Del Tredici and Braak 2009) which states that PD is first established in the enteric nervous system (ENS), and then progresses to the central nervous system (CNS) (Houser and Tansey 2017). This has been

demonstrated by injection of preformed fibrillar α -syn (induction of α -syn) into the gut of mice, which has then travelled to areas of the brain including the SN (Kim et al. 2019). This theory also links in with impaired olfaction (sense of smell) as an early PD non-motor symptom which often precedes a PD diagnosis by years (Fullard, Morley and Duda 2017). The loss of dopaminergic neurons is the end point of the disease pathology, and what precedes this cascade in loss of non-symptom function is yet to be fully elucidated and understood.

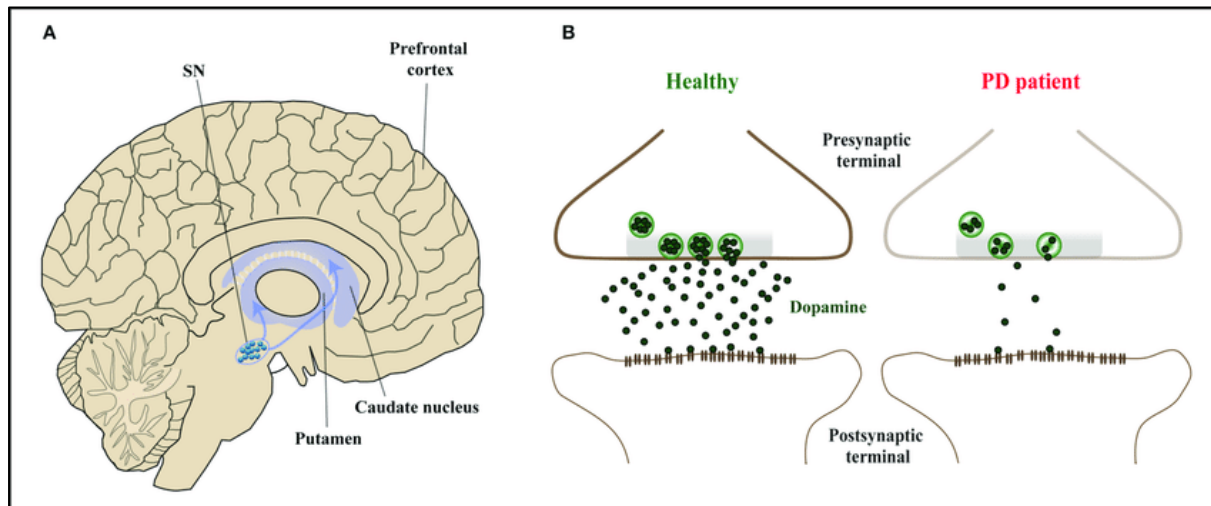


Figure 1.1. Substantia nigra and dopamine. (A) The substantia nigra (SN) area of the brain where dopaminergic neurons are lost in PD. (B) In PD patients' dopamine is reduced compared to healthy controls. Image adapted from (Bridi and Hirth 2018).

Braak *et al.* (2003) attempted to sub type sporadic PD using a six-step staging system which was based on Lewy body (LB) pathology (Table 1.1). The study examined 168 brains by autopsy (female = 79, male = 89). This theory states that PD travels from the brain stem (medulla oblongata and pontine tegmentum) to the midbrain (including the SNpc), then the mesocortex and finally, the neocortex. In 2009 the dual hit hypothesis was postulated and subsequently reinforced this staging system. This staging system theory is highly controversial as it is based on Lewy body pathology as opposed to neuronal loss, and many cases of PD do not follow this staging system. Braak's staging system may be more suited to young onset PD or longer durations of disease.

Table 1.1. Symptoms associated with Braak (2003) stages of Parkinson's disease.

Braak Stage	Symptoms
1 and 2 (initial stages)	Autonomic and olfactory disturbances (premotor symptoms)
3 and 4 (intermediate stages)	Sleep and motor disturbances (motor symptoms)
5 and 6 (final stages)	Emotional and cognitive disturbances

Braak stages demonstrate symptoms associated with area of brain affected by PD. Stage 1 and 2 are initial stages, followed by 3 and 4 as intermediate stages, and finally, stages 5 and 6. Many cases of PD do not follow this staging system, and may be more suited to a young onset PD.

1.3. Factors which may increase susceptibility to Parkinson's disease

Genetic factors, environmental factors, and previous traumatic brain injury have all been linked to increased susceptibility to PD, and a complicated interaction between these factors exists.

1.3.1. Genetic Factors

The first gene to be implicated in PD was the SNCA gene (Polymeropoulos et al. 1997) - an autosomal dominant gene – which encodes the α -syn protein and has been extensively researched. Approximately 100 genes have been linked to increased susceptibility to PD - with a large proportion of genes still to be detected (Cristina et al. 2020) - only one in ten PwP carry disease causing genetic mutations (Cerri, Mus and Blandini 2019), however, and there is a complicated interaction between genetic factors and environmental factors. Other autosomal dominant genes are LRRK2 and VPS35. The Gly2019Ser mutation of the LRRK2 gene occurs mostly in people from European, Jewish, and North African descent. Other LRRK2 mutations affect other populations: the Asn1437Asp is commonly found in people from China, and

1122020Thr in Japanese populations (Kluss, Mamais and Cookson 2019). These genetic factors should be considered in PD research, as different geographical locations may yield different results. Another interesting point is that LRRK2 is also implicated in Crohn's disease, an inflammatory bowel disease, and the gut has been suspected to have been involved in PD.

With the discovery of more and more genes, and the theory that the disease may start elsewhere other than the central nervous system (CNS), would suggest that PD is not a single disease entity. This limits treatment options for PwP and may skew research outcomes as research often relies on the notion that PD is homogenous rather than heterogeneous. While PD genes exist, most cases of PD are idiopathic.

1.3.2. Mitochondrial dysfunction

Mitochondrial dysfunction plays a prominent role in PD pathogenesis in familial and a subset of sporadic PD with different mechanisms of mitochondrial dysfunction in autosomal dominant and autosomal recessive PD, and the same for sporadic PD (Brent J. Ryan et al. 2015). Mitochondrial dysfunction leads to increased rates of oxidative stress, which impairs several cellular pathways. Mitochondrial oxidative stress in dopamine neurons increases the accumulation of 4-hydroxynonenal (4- HNE) which is the most abundant and cytotoxic lipid peroxidation product, and increased levels of 4- HNE have been found in PD. Oxidative stress will be discussed later in the literature review. PD genes such as PARK2 (Dehay et al. 2013) (which encodes Parkin) and PARK7 (Heo et al. 2012) (which encodes DJ-1) play a role in mitochondrial homeostasis, and α -syn binds to the outer mitochondrial membrane.

1.3.3. Environmental factors

Pesticides

The discovery of environmental toxins being implicated in PD occurred by chance when a group of researchers (Langston et al. 1983) found that participants who had had MPTP (found in synthetic heroin) exposure, developed Parkinsonism. Paraquat, a toxic herbicide, is chemically like MPTP, and paraquat exposure has been demonstrated to increase rates of PD

(Tanner et al. 2009), as paraquat impacts complex I of the mitochondria respiratory (the first enzyme of the respiratory chain) which oxidises nicotinamide adenine dinucleotide hydrogen chain (NADH) (Schneider Medeiros et al. 2020) which in turn increases oxidative stress. Paraquat is one of the most widely used herbicides in agriculture, however some countries have banned its use, including China, Brazil, the EU and the UK (although the UK still produces and exports it).

Metals

Metal exposure occurs from occupations, pollution, medications, and mercury dental restorations (Raj et al. 2021). Research into heavy metal exposure and PD is still in its infancy and some people will be more sensitive to the effects of heavy metal exposure than others. Iron (Fe) levels are high in the SN area in the brain of PwP (Griffiths et al. 1999) and there have been promising results in treating PD with Fe chelators (a treatment for Fe overload) (Martin-Bastida et al. 2017), however, while these results showed a trend for Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) and Quality of Life (QoL) scores, these did not show a significant effect. The participants in this study were diagnosed with early onset PD (diagnosed with PD under the age of 50), it would be worth repeating this study with participants who were in the later stages of PD. During early stages of PD, Fe may offer a neuroprotective effect, however, during the later stages of PD, increased Fe may cause large amounts of Reactive Oxygen Species (ROS) (Gaasch et al. 2007) which in turn contributes to neurodegeneration.

Gut microbiome

Gastrointestinal (GI) issues often precede a PD diagnosis by years (Cersosimo et al. 2013). GI issues are one of the most common non-motor symptoms (NMS) in PD, and drastically impact QoL. The gut-brain axis has long been established, and the gut microbiota helps to regulate neurotransmitters including dopamine. Small intestinal bacterial overgrowth (SIBO) has been reported to worsen motor function in PwP (Tan et al. 2014). SIBO is a driver of symptoms such as diarrhoea, abdominal pain, and bloating (Saffouri et al. 2019).

Viruses

Viruses can cause an autoimmune response through different mechanisms (Leta et al. 2022) and various viruses have been associated with PD including Hepatitis C (Yaow et al. 2023) Epstein-Barr virus, and SARS-CoV-2 (Boika et al. 2021). The ‘micro competition model’ (Polansky and Lori 2021) describes how a dormant viral infection in the central nervous system or the peripheral nervous system can cause mitochondrial dysfunction and α -syn accumulation due to a reduction in the availability of the GABP p300 and cbp complex, which promote gene transcription. This theory leads nicely into ageing, as disease rates increase with age, and advancing age reduces immune system efficiency.

Ageing

There is an ageing population which leads to increased rates of disease. Ageing increases rates of oxidative stress, neuroinflammation and mitochondrial dysfunction, all of which have been implicated in PD. Ageing is described as the biggest risk factor for PD development (Reeve, Simcox, Turnbull 2014; Pang et al. 2019). It is worth noting that a small percentage of people present with PD symptoms before the age of 60 (~5%), and these cases are thought to be due to genetic mutations in one of the myriads of genes associated with PD as discussed previously (Gasser, Hardy and Mizuno 2011). Physical activity (PA) is a determinant of successful ageing, and PA has been shown to slow down the progression of PD (van der Kolk et al. 2019). Most older people do not achieve recommended levels of activity at 150 min per week (Boulton, Horne and Todd 2018) despite the documented health benefits associated with PA including improved cognitive function, important in PD. It is known that from age 40, there is an accumulation of amyloid beta ($A\beta$), which is implicated in Alzheimer’s disease. However, there is conflicting evidence whether $A\beta$ contributes to the cognitive decline in PwP (Gomperts et al. 2013; Melzer et al. 2019).

Interventions which mimic the cascade of immune functions of exercise or allow a person to exercise for longer periods of time, at higher intensities and recover quicker from a bout of exercise would be beneficial, particularly to groups of people which may not be able to achieve recommended levels of exercise.

Biological sex

Evidence shows that PD differs between males and females (Cerri, Mus and Blandini 2019) and the ratio of males to females with PD, is approx. 1.4:1 (Parkinson's UK, 2025), however, it has been reported that in Asia, the ratio is lower (Abbas, Xu, and Tan, 2017) which possibly is caused by environmental factors. Much of the research in H₂ and PwP has been carried out in Japan (Yoritaka et al., 2013; Yoritaka et al., 2018; Hirayama et al., 2018; Yoritaka et al., 2021) and there is a known genetic variant (Il22020Thr) in Japanese populations (Kluss, Mamais and Cookson, 2019). with the clinical phenotype drastically differing between males and females, for example tremor is more prevalent in women than men as a first presenting symptom (Haaxma et al. 2007). Historically, female rodents were omitted in pre-clinical research (Landis et al. 2012), due to fluctuating hormones involved in the menstrual cycle, therefore Levodopa - as a dopamine replacement - would not have originally been tested in females. Sex differences occur in brain function and brain structure, and microglia (CNS 'immune cells') have been reported to be more abundant in male brains compared to female brains (Guneykaya et al. 2018). Oestrogen mitigates microglia immune function (Vegeto et al. 2001) and inflammatory response which may be an explanation as to why females are diagnosed with PD post-menopausal. PD phenotypes vary, and females with PD are less likely to develop cognitive impairments than males (Iwaki *et al.* 2021).

1.3.4. Traumatic brain injury

Traumatic brain injury (TBI) has long been established as a risk factor for developing neurodegenerative disease. The potential mechanism of TBI and subsequent neurodegeneration is thought to be due in part to a decrease in dopamine transporters in the SN area of the brain of mice (Impellizzeri *et al.* 2016), neuroinflammation, and α -syn misfolding (Delic et al. 2020). Chronic traumatic encephalopathy (CTE) has been diagnosed in many contact sport athletes, CTE is a neurodegenerative disease, and Parkinsonism and Lewy body disease (LBD) are associated with CTE (Adams et al. 2018), thereby increasing evidence that repeated head trauma may lead to PD.

1.4. Parkinson's disease symptoms

Symptoms of PD are categorised as motor and non-motor symptoms. Progression of symptoms are inevitable, despite Levodopa treatment. Non-motor symptoms often precede motor symptoms by some years, and when motor symptoms of PD begin emerging, it is estimated that there is a reduction of around 80% of dopaminergic cells in the nigrostriatal system.

Conventional attempts at sub-typing PD have relied on motor symptoms such as tremor and postural instability and gait disorder. While this sub-typing is welcomed to help classify patients, these traditional subtypes fail to include non-motor symptoms. Worsening of symptoms is not linear, and not everybody will experience the same non-motor symptoms given the heterogeneity of PD.

1.3.5. Motor symptoms of Parkinson's disease

Motor symptoms of PD include tremor, rigidity, bradykinesia (slowness of movement), postural instability (impaired balance), and gait dysfunction (Litvan et al. 2003). Not all PwP will experience these same symptoms, and females will tend to present with tremor more so than males (Haaxma et al. 2007) as previously discussed. These motor symptoms in turn can impact manual dexterity and hand grip making activities of daily living difficult.

Motor symptoms of PD may lead to falls or fear of falling (FOF), with two thirds of PwP falling at least once annually (Paul et al. 2013). FOF may cause PwP to limit their movement and this will therefore increase their PD symptoms (Chaudhuri et al. 2011).

The Hoehn and Yahr (1967) (Table 1.2) staging system is still used today in clinical practice and research to classify PwP according to their motor symptoms. It assesses bilateral, unilateral, balance and level of disability, and can be used globally, allowing a certain amount of comparison from different geographical locations to be compared. However, it does not capture the heterogeneity of disease, or non-motor symptoms that may be experienced. While there is a modified H&Y staging system (Goetz et al. 2004), this has not yet been validated.

Table 1.2. Symptoms associated with Hoehn and Yahr (H&Y) staging system and modified H&Y staging system.

<i>H&Y scale (Hoehn and Yahr, 1967)</i>	<i>Symptoms</i>	<i>Modified H&Y scale (Goetz et al. 2004)</i>	<i>Symptoms</i>
1	Unilateral only	1	Unilateral only
		1.5	Unilateral with axial involvement
2	Bilateral (no impairment in balance)	2	Bilateral (no impairment in balance)
		2.5	Mild bilateral disease with recovery on pull test
3	As stage 2 with balance and stability affected	3	As stage 2 with balance and stability affected
4	Severe disability - Able to stand and walk unaided	4	Severe disability - Able to stand and walk unaided
5	Severe disability – needs a wheelchair	5	Severe disability – needs a wheelchair

The table shows the additional two stages included in the modified H&Y stage.

1.4.2. Non-motor symptoms of Parkinson's disease

NMS of PD often precede motor symptoms of PD by many years (Berg et al. 2015) which often go undetected, and not diagnosed properly as PD (Figure 1.2). Non-motor symptoms of PD are varied across individuals and can drastically impair QoL. NMS can be quantified using validated questionnaires such as the MDS-NMS (Chaudhuri 2019) which is useful in both a clinical setting to assess the severity of PD, and a research setting. Some more common NMS include loss of smell depression, anxiety and apathy, sleep disorders, pain, incontinence, cognitive dysfunction (Lotanker et al. 2017). Attempts at sub-typing PD have relied on motor symptoms and do not include non-motor symptoms. Along with these symptoms, PwP may have altered handwriting and speech impairments (speech may become softer).

Hyposmia (decreased sense of smell) usually precedes a PD diagnosis by many years and is not often reported as a symptom by patients as they would not link this to PD. The University of Pennsylvania Smell Identification Test (UPSIT) is a reliable and accurate olfactory test and is the most widely used test to measure PD olfactory deficits (Campabadal et al. 2019). It has been suggested that hyposmia could be used as a biomarker, and the UPSIT could capture this, however, the UPSIT has different versions as the test is thought to have American cultural bias, different UPSITS may be geographically tied and therefore not replicable in other areas of the world. Lin et al. (2023) were the first group to demonstrate that hyposmia may predict motor symptom progression in PwP, a Chinese version of the UPSIT was used in the study cohort, which may limit its application to other geographical locations. Loss of smell is an ambiguous symptom and can be linked to other conditions such as SARS-Covid, Influenza and Sinusitis.

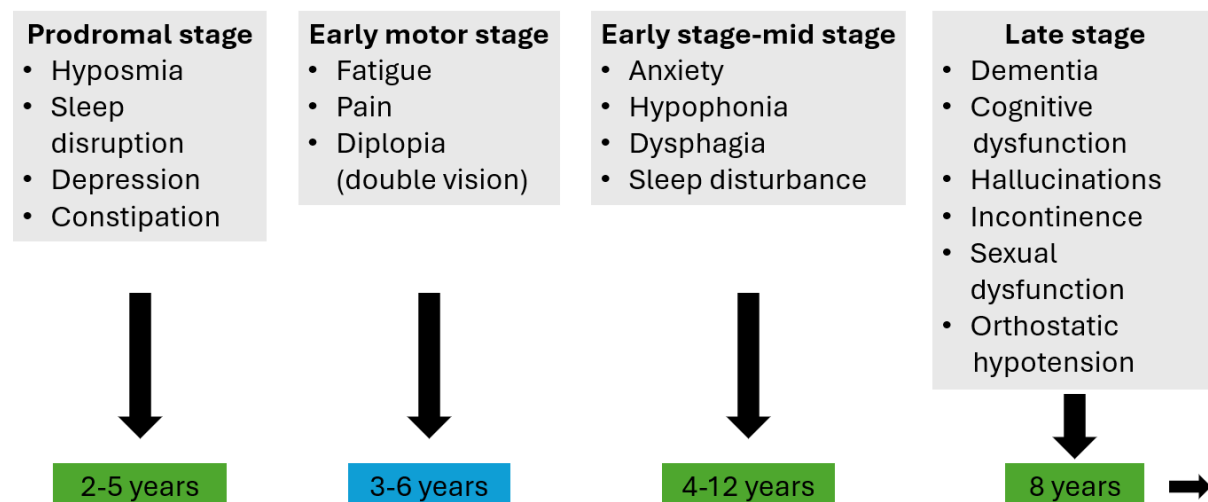


Figure 1.2. PD progression based on non-motor symptoms. The prodromal stage reflects symptoms which are usually insidious and present before the motor symptoms onset. It is worth noting that the development of these non-motor symptoms will vary between PwP. Image based on Schapira, Chaudhuri and Jenner (2017).

1.4.3. Outcome measures in PD research

The Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz et al. 2008) is used both clinically and in research. The MDS-UPDRS seeks to evaluate

various aspects of PD including non-motor and motor experiences of daily living, and is a subjective measure of the symptoms of PD. The MDS-UPDRS has four parts; part one addresses non-motor functions of PD, part two addresses motor functions of PD, part three is carried out by an examiner and tests for motor functions, and part four addresses motor complications such as turning in bed. It is apparent that in PD research, usually not the full MDS-UPDRS is used, and various sections are chosen in different studies which makes it difficult to compare results from studies. This could be that the MDS-UPDRS is fairly time exhaustive and requires training to administer correctly. The MDS-UPDRS is a useful subjective measure of how PD is affecting a person's life; the higher the number scored on the MDS-UPDRS, the more PD is affecting a person's life. Other validated questionnaires which measure QoL are available and include The Older People's Quality of Life Questionnaire-35 (OPQOL-35), and its shorter scale The Older People's Quality of Life Questionnaire-brief (OPQoL-brief), which are both used in older person's research including nursing home populations (Haugan et al. 2020) however, this is not specific to PD. The Parkinson's Disease Questionnaire (PDQ-39) (Peto et al. 1995) and the subsequent shorter version The Parkinson's Disease Questionnaire (PDQ-8) are validated tools for the assessment of QoL in PwP. While the MDS-UPDRS remains the gold standard for measuring disease progression, there are limitations which particularly impact trials of disease modification such as floor effect and it also fails to capture the complexity and heterogeneity of PD (Mari and Mestre 2022).

1.4. Potential Parkinson's disease biomarkers

One of the difficulties in diagnosing PD is there are no specific disease biomarkers, this also poses a challenge to researchers as there is uncertainty of what to measure and makes comparison of studies difficult if the same intervention has been investigated but different biomarkers have been reported. Biomarkers aid in following disease progression, are objectively measured and are used to measure normal biological processes, pathology of disease and can be used to evaluate responses to a therapeutic intervention (Biomarkers Definitions Working Group 2001). Research is ongoing to elucidate PD biomarkers, this would aid in early diagnosis and reduce diagnostic errors. Diagnostic errors can affect between 10 to 30% of people diagnosed with PD, and consequently patients are reclassified clinically (Drotár *et al.* 2016). Biomarkers can be analysed through cerebrospinal fluid (CSF), blood, and saliva samples. CSF is the gold-standard in obtaining biomarkers in neurodegenerative disease (Chan

et al. 2019) but the invasive nature of this procedure limits their application, therefore peripheral fluid biomarkers (such as blood markers) are more accessible in a research setting. While there are no specific biomarkers for PD diagnosis (Raghunathan, Turajane, Wong, 2022), biomarkers which are responsible for inflammation and oxidative stress can be measured, thus providing an insight into whether an intervention may help with the reduction of PD symptoms. Given the heterogenous nature of PD and variability of motor and cognitive symptoms in PD, it is difficult to establish a single biomarker. If biomarkers could be accepted, this would lead to different classifications under the umbrella term ‘Parkinson’s’ and would allow for different treatments to be prescribed.

There are many theories which support the decline of dopaminergic neurons and the consequential aggregation of alpha-synuclein (α -syn) proteins. Misfolding of α -syn has long been established as the cause of PD but the aetiology of this is still yet to be fully understood. Measuring plasma α -syn levels could be a diagnostic measure for PD as >99% of α -syn is found in erythrocytes (Barbour *et al.* 2008), however, this does present a problem as residual erythrocytes during sample collection and processing can result in increased plasma α -syn levels. This could be a possibility of why studies have reported conflicting findings (Duran *et al.* 2010; Foulds *et al.* 2011) and there are also differences in analysis, which could cause variations in sensitivity.

1.5. Pharmacological and non-pharmacological management of Parkinson’s disease

1.6.1. Pharmacological management

There is no cure and no disease modifying treatments available for PD (Paolini Paoletti, Gaetani and Parnetti 2020). Levodopa is currently one of most important drug therapies in earlier cases of PD and helps to reduce the severity of PD symptoms. Levodopa can cross the blood-brain barrier and aids in facilitation of dopamine biosynthesis; however, Levodopa can become less effective the longer it is administered. L-Tyrosine is the precursor to Levodopa, which have an almost identical chemical structure to each other except for a cation hydroxyl group (HO) on carbon 3 of the L-DOPA aromatic ring (Figure 1.3). Levodopa causes many side effects including the ‘on/off’ effect which can reduce QoL (Rodríguez-Violante *et al.* 2018), these fluctuations are seen in between doses and refer to when medication is working

well “on” (symptoms are less noticeable) and when medication effects start wearing off and symptoms become more noticeable. Carbidopa is given in addition to Levodopa as it aids in reduction of the feeling of nausea caused by Levodopa, as well as inhibiting the extracerebral decarboxylation of Levodopa, which allows more dopamine to reach the brain (Safe 2017). Other treatments for PD include Dopamine agonists and Monoamine oxidase-B (MAO-B) inhibitors. As with most medication, these treatments all carry side-effects, and dopamine agonists carry more adverse events such as excessive day time sleepiness and hallucinations than levodopa and MAO-B inhibitors (NICE 2017). Dopaminergic treatments have no effect on other aspects of PD that are not related to dopamine deficits.

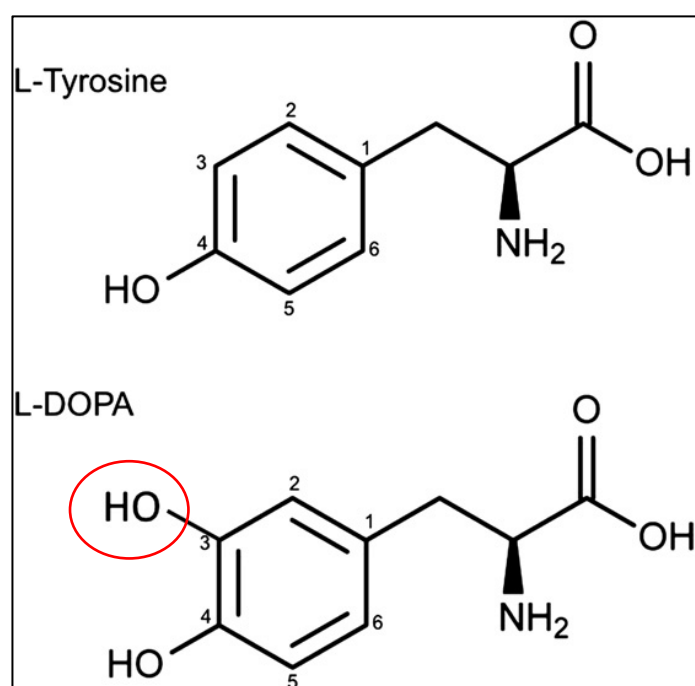


Figure 1.3. L-Tyrosine and L-DOPA chemical structures. L-Tyrosine is the precursor to L-DOPA, which have an almost identical chemical structure to each other except for a cation hydroxyl group (HO) (circled red) on carbon 3 of the L-DOPA aromatic ring. Image adapted from (Chan *et al.* 2012)

1.6.2. Non-pharmacological management of Parkinson's disease

PwP are treated within a multi-disciplinary team and non-pharmacological management of symptoms are incorporated across all stages of the disease, this can be from a specialist nurse, physiotherapist, occupational therapist, speech and language therapist and a nutritionist. There are wide ranging NMS and PwP may find various non-pharmacological treatments work better for them, this could be due to different aetiologies of PD. Complementary therapies such as Tai Chi (Li *et al.* 2012) and massage therapy (Angelopoulou *et al.* 2020) may be beneficial for PwP, although there is scant evidence to support this. PwP may report a 'quiet voice', and a speech and language therapist can assist with this, as well as dysphagia.

1.6. Hydrogen

It was in the mid-1970s when the therapeutic application of Hydrogen (H_2) first made an appearance in scientific literature, where it was shown to reduce skin tumours in animal models (Dole, Wilson and Fife, 1975). A few years later, similar findings were reported (Roberts *et al.* 1978). There was a gap in research, until landmark work in 2007 demonstrated that H_2 in vitro and in vivo protected against oxidative stress (Ohsawa *et al.* 2007). Since the re-emergence of H_2 research in 2007, there has been a flurry of H_2 research in both animal models and humans, however H_2 studies in PwP are limited and report different findings. H_2 is the smallest gas molecule and diffuses through cell membranes and into mitochondria (Sano and Tamura 2021). H_2 is the gas which forms when two hydrogen atoms bond together and become a hydrogen molecule consisting of two protons and two electrons. There are different methods of consuming H_2 , inhalation of gas and drinking water are the most ubiquitous which have different advantages and disadvantages (Table 1.3).

1.6.1. Hydrogen rich water

Hydrogen rich water (HRW) involves using electrolysis (using a proton exchange membrane) to separate H_2 and oxygen (O_2) or dissolving H_2 tablets in water. Many PwP report urinary urgency, and an increase in intake of fluids may not be the best way to consume H_2 . While HRW is portable, it may limit the physical activity of PwP - or their social interaction away from their home - if they are concerned about accessing a toilet. Also, larger HRW bottles may

be heavy, and this may be a problem for someone carrying the water around. HRW bottles are simpler to use than a H₂ gas inhalation machine, as the bottles just need to be filled up and a button pressed. Whereas H₂ gas inhalation machines are heavy, non-portable and require regular maintenance. It has been shown that approx. 40% of consumed H₂ in HRW is used by the body (Shimouchi *et al.* 2012).

1.6.2. Hydrogen gas

Hydrogen gas (H₂ gas) is administered at approximately 2-4% H₂, as H₂ is extremely flammable at increased levels. It has been demonstrated that H₂ inhalation, as opposed to drinking HRW or injection of H₂ rich saline, maintains a H₂ concentration for at least one hour post cessation in blood (Liu *et al.* 2014). H₂ does not disturb metabolic oxidation-reduction reactions or reactive oxygen species (ROS) mediated cell signalling.

A recent study (Sano, Shirakawa, *et al.* 2020) explored H₂ concentrations and arterial blood saturations in pigs. However, it was a very small study, and there was no control condition. However, H₂ concentrations and arterial blood saturations varied in the pigs, with the lowest weight pig having lower H₂ concentrations and arterial blood saturations than the other two pigs; this could be related to body weight. Arterial blood H₂ saturation was found to be approximately 2% H₂ after three hours of H₂ gas inhalation in people with Post-cardiac arrest syndrome (PCAS) (Tomoyoshi *et al.* 2020), which is lower than the pig study, as this study reported that arterial blood H₂ concentration was 6-11%. It is worth noting that researchers have found it difficult to maintain high levels of concentration of H₂ within the body due to its rapid diffusion (Kobayashi *et al.* 2020).

The most effective way of taking H₂ into circulating blood is to fully exhale, then inhale 100% H₂ as much as possible, and hold your breath for as long as possible (Sano, Ichihara, *et al.* 2020), however it would become tiresome to breathe like this.

Table 1.3. Advantages and disadvantages of hydrogen rich water and hydrogen gas

<i>Route of administration</i>	<i>Advantages</i>	<i>Disadvantages</i>
<i>Hydrogen Rich Water (using PEM* bottle)</i>	Portable Fairly cheap to buy	May increase urinary urgency and urination (particularly important if there is restricted toilet access) Only 40% uptake of H₂ into blood (Shimouchi <i>et al.</i> 2012) Bottle needs charging as it is battery operated and the battery needs charging periodically.
<i>Hydrogen Gas</i>	Larger tissue saturation than water (Liu <i>et al.</i> 2014) Will not increase need to urinate	Equipment requires maintenance May increase time spent sedentary as needs mains electricity More time consuming than taking regular drinks of water May be prohibited when travelling (e.g. plane and ferry) Machine is expensive

*Proton Exchange Membrane (PEM) separates H₂ and O₂ which therefore allows water to become H₂ saturated.

1.7. Potential mechanisms of action of H₂

The mechanism of action of H₂ is not yet fully understood, however, it has been proposed that H₂ has antioxidant and anti-inflammatory properties (Ohsawa, 2007). Optimal administration dose range of H₂ is yet to be elucidated, and dose ranges of HRW and H₂ gas have varied in literature. H₂ gas needs to be delivered at below 4% due to its highly flammable nature, however studies involving animals have reported higher concentrations. H₂ gas has been delivered at 1%, 2%, 4% (Ohsawa *et al.* 2007), with 66.7% concentration given to rats (Cui *et*

al. 2016). HRW doses in PwP have been reported as 1.6 ppm (Yoritaka *et al.* 2013) and 10 ppm (calculated from reported 5mM concentration and molar mass of hydrogen as 2.01568g/mole) (Yoritaka *et al.* 2018). Oxidative stress and inflammation are inextricably linked, however, the antioxidant and anti-inflammatory effects of H₂ will be discussed separately below.

1.7.1. *Anti-inflammatory Effects of H₂*

H₂ has been shown to reduce inflammation by downregulating pro-inflammatory cytokines and upregulating anti-inflammatory cytokines (Li *et al.* 2021). Inflammation is an essential aspect of the body's defence mechanism, particularly in the case of acute inflammation, however, chronic inflammation can be detrimental to health and has been linked to a wide range of chronic diseases. There is also an established link between sustained inflammation and neurodegeneration (Heneka *et al.* 2015). While directly impacting pro- and anti-inflammatory cytokines, H₂ may also inhibit inflammatory pathways including the Nuclear factor kappa B (NF-κB) pathway (Hirayama *et al.*, 2018), which are also found in microglia, neurons and astrocytes. Two major pathways are involved in the activation of the NF-κB, canonical and noncanonical (alternative) pathways. Canonical and noncanonical have different signalling mechanisms but both regulate inflammation and immune response (Sun 2017). The canonical NF-κB pathway responds to diverse stimuli, whereas the noncanonical NF-κB responds to a specific group of stimuli. Dysregulated NF-κB is involved in various acute or chronic inflammatory diseases including neurodegenerative diseases (Tak and Firestein 2001), however, the role of NF-κB is yet to be fully elucidated in these diseases.

M1 macrophages produce pro-inflammatory cytokines such as Interleukin-1β (IL-1β) (Wisitpongpun, Potup and Usuwanthim 2022) and tumour necrosis factor-α (TNF-α) (Lu *et al.* 2018), which play a central role in the initiation and propagation of inflammation. M2 macrophages produce anti-inflammatory cytokines such as Interleukin – 10 (IL-10) and transforming growth factor beta (TGF-β) (He and Carter, 2015) which play a role in the resolution of inflammation. While targeting the NF-κB pathway may seem like a useful approach to anti-inflammatory therapies, NF-κB also regulates the anti-inflammatory cascade of events. Glutamatergic neurons have been observed to show constitutive NF-κB expression. Depleted glutathione (GSH) levels have been demonstrated in the SN of post-mortem brains

from individuals with PD (Sian *et al.* 1994) and GSH is the most important antioxidant defence which protects normal cell function (Bjørklund *et al.* 2021).

Positive effects of H₂ on microglia have been demonstrated in animal models (Chu *et al.* 2019) and in vitro (Ning *et al.* 2018). Inhalation of H₂ has been demonstrated to promote microglia polarisation from M1 phenotype to M2 phenotype in a rat model of TBI (Guo *et al.* 2020). M1-like microglia secrete pro-inflammatory cytokines such as IL-1 β and TNF- α which have been implicated in non-motor symptoms of PD (Wang *et al.* 2016).

A recent paper (Liu *et al.* 2021) demonstrated that H₂ water triggered GSH synthesis. GSH supplementation has been explored within a PD population, however, oral GSH has low bioavailability, which therefore affects the amount of GSH delivered to central nervous system tissues. Intravenous (IV) GSH has been explored as a possible way of administering large amounts of GSH, to bypass this. It was demonstrated that there was improvement in motor symptoms after GSH. Strengths of this study (Mischley *et al.* 2017) were that it was one clinician which administered GSH, and it was double blinded. H₂ gas inhalation is non-invasive, and may trigger GSH synthesis, this could be an alternative to GSH IV. However, it is difficult to administer IV GSH due to cost, inconvenience, and side effects of an IV. GSH can be delivered intranasally, however, some participants withdrew due to being affected by the intranasal GSH.

A case study nicely illustrates the response to IV GSH by a PD patient (Otto, Magerus and Langland 2018). A maximum improvement one hour following the IV was discovered, followed by a plateau and regression until the following dose. However, nutritional interventions (including nutritional supplements) were initiated at the same time as starting the IV GSH, so it is not clear which intervention yielded the results. Reduced levels of GSH promote microglia activation and trigger a cytokine response (Aoyama 2021), and there is a high level of GSH concentration in microglia more than in any other cells of the brain such as astrocytes (Lawson *et al.* 1990).

1.8.2. Antioxidant Effects of H₂

Like inflammation, oxidative stress is both essential and detrimental to humans (Pizzino *et al.* 2017). Oxidative stress is caused when an increase of Reactive Oxygen Species (ROS) (a by-product of aerobic metabolism) outweighs antioxidant capacity, which causes molecular damage to cells (Schieber and Chandel 2014). However, low levels of ROS are fundamental in maintaining homeostasis including regulation of immune cell metabolism, immune cell activation, cell differentiation and proliferation, and apoptosis (elimination of unwanted cells). Increasing age impairs the ability to protect the body against ROS, which implicates oxidative stress in diseases associated with ageing (Reddy 2023), and PD rates increase with age.

H₂ has been described as a safe antioxidant to help treat disease (Zhang *et al.* 2018), and it seems obvious to treat diseases which are related to oxidative stress with antioxidants, but clinical studies have failed to evaluate the effects of antioxidants (Rojas-Solé *et al.* 2024) due in part to varying study methodologies. However, Vitamin C and Vitamin E have approximately 100 times greater antioxidant effect than H₂ (Iida *et al.* 2016), Vitamin C is water soluble, allowing the body to eliminate unwanted levels, Vitamin E is fat soluble which can sometimes cause toxicity if ingested in large doses. The influential work of Ohsawa *et al.* (2007) demonstrated H₂ selectively scavenged hydroxyl radicals ($\cdot\text{OH}$) which are the most reactive free radicals, and an abundance of free radicals induce oxidative stress. Researchers basing their theory that H₂ reduces oxidative stress have demonstrated opposing findings. These studies have measured DNA markers for oxidative stress such as 8-hydroxydeoxyguanosine (8-OHdG) (Hirayama *et al.* 2018), as opposed to precursors of oxidative stress e.g. $\cdot\text{OH}$. If researchers report that H₂ improves outcomes (Timón *et al.* 2021), often the conclusion is this is due to the antioxidant effects of H₂, rather than measuring oxidative stress markers.

While H₂ may have a lower antioxidant effect than Vitamin C and Vitamin E, it is safe to consume and has shown no adverse effects in animals (Cole *et al.* 2019). H₂ is usually delivered at 2-4% as higher concentrations are combustible and safety standards regarding high concentrations of H₂ used in deep-diving gases to aid in prevention of sickness have long been established (Fontanari *et al.*, 2000).

There are four studies to date which have investigated H₂ and PwP (Table 1.4), which are summarised over the page.

Table 1.4. Summary of studies in H₂ and PwP

Paper	H&Y stage	Number of participants	Route of administration	Methods	Study design	Measurements	Results
Yoritaka et al. 2013, Japan	2.1 ± 0.2	18 (female = 11, male = 7) however 1 person was removed therefore 17 participants were included in analysis	HRW	1000 ml water daily for 48 weeks	Placebo, randomised, double blind, parallel group	MDS-UPDRS	Significant effect of H ₂ reported - improvement of Total MDS scores in H ₂ group.
Yoritaka et al. 2018, Japan	2.1 ± 0.6	178 (female = 93, male = 85)	HRW	1000 ml water daily for 72 weeks	Placebo, randomised, double blind, parallel group	MDS-UPDRS, PDQ-39	No significant effect reported
Hirayama et al. 2018, Japan	2.3 ± 0.8	20 (biological sex not reported)	Gas	10 min, twice daily for 4 weeks, then repeat again	Placebo, randomised, double blind, cross over	MDS-UPDRS, OSIT-J, Urine 8-OHdG	Significant effect of H ₂ - 8-OHdG increased in H ₂ group
Yoritaka et al. 2021, Japan	2.5 ± 0.5	20 (biological sex not reported)	Gas	1 hr, twice daily for 16 weeks	Placebo, randomised, double blind parallel group	MDS-UPDRS, PDQ-39, DiAcSpd, Urine 8-OHdG	No significant effect reported

H&Y stage = Hoehn & Yahr (1967), represented as mean ± standard deviation. HRW = hydrogen rich water, MDS-UPDRS = Movement Disorder Society - Unified Parkinson's Disease Rating Scale, PDQ-39 = Parkinson's Disease Questionnaire-39, 8-OHdG = 8-hydroxy-2'deoxyguanosine, DiAcSpd = N1, N8-diacetylspermidine

Most studies in H₂ and PD have been carried out in animal models, with limited studies in PwP. Encouraging results found from early studies in animal models of PD did not translate seamlessly into H₂ and PwP studies, as opposing findings have been reported (Yoritaka *et al.* 2021; Hirayama *et al.* 2018).

Animal models are frequently used in PD research, two of the most common chemically induced models of PD in rats are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA). 6-OHDA is a more robust model than MPTP and 6-OHDA promotes more dopaminergic cell loss that cannot be prevented by neuroprotective methodologies (Armentero *et al.* 2008). PD in rats only represent dopaminergic neuron loss, therefore cannot be used to investigate neurodegeneration mechanism or disease pathology progression. Thus, a novel intervention for PD may give varying results in an animal model rather than a PwP.

The first studies in H₂ and PD were published in 2009 (Fu *et al.* 2009; Fujita, Seike, Yutsudo, *et al.* 2009), and were carried out in a 6-OHDA model and a MPTP model of PD respectively. These studies worked on the premise that PD is caused by oxidative stress, and oxidative stress is a known driver of PD. Both studies demonstrated that H₂ water reduced the loss of dopaminergic fibres more than placebo water. The first known study in PwP and H₂ (Yoritaka *et al.* 2013) showed promising results as it demonstrated a statistically significant difference in UPDRS scores at week 48 in the H₂ treated group compared to the placebo group ($p=0.046$) where the H₂ group had greater changes than the placebo group. This study was relatively small with a group of 17 participants (age in years: 60.1 ± 10.6). A larger study (Yoritaka *et al.* 2018) (178 participants), published five years later, showed no significant changes between the H₂ treated group and placebo group at 72 weeks, despite using the same study protocol, however some participants in the study were not taking Levodopa. These patients would have been in the earlier stages of PD, H₂ may only work in the mid stages of PD. Measurements were also taken 48 weeks after baseline, and no significant changes were reported either, no oxidative stress biomarkers reported in both studies. Previous studies have analysed oxidative stress in animal models of PD after H₂ consumption, thus showing that other potential mechanisms of action of H₂ may exist. Both studies used a placebo controlled, randomised parallel-group design. It may have been of merit to use a cross over design which aids in reducing participant variation.

Studies have suggested that H₂ may slow down the progression of PD, but the mechanism has yet to be elucidated (Wang *et al.* 2021). However, H₂ is known to attenuate oxidative stress. Oxidative stress has long been implicated in the aetiology of PD (Basu, Je and Kim 2015) and is caused by an increase of ROS which is a by-product of oxygen metabolism and adenosine triphosphate (ATP) production. When ROS outweighs antioxidant capacity, ROS causes molecular damage to cells. It has been suggested that H₂ gas regulates protein heme oxygenase-1 (HO-1) and nuclear-related factor-2 (Nrf-2) which control mitochondrial activity (Zhang *et al.* 2020) and mitochondrial dysfunction has been shown to play a crucial role in the pathophysiology of PD (Grimm and Eckert 2017). It has also been demonstrated that there is a bi-directional relationship between α -syn and mitochondrial dysfunction (Di Maio *et al.* 2016). Dopaminergic neurons have a low mitochondrial mass compared with other neuronal cells (Olguin *et al.* 2015) which makes the brain more susceptible to mitochondrial dysfunction as neurons have high energy requirements due to excitability and survival (Mamelak 2018) and high energy requirements are dependent on mitochondria. Most PD-associated genes play a role in mitochondrial homeostasis (Brent J Ryan *et al.* 2015) and mitochondrial dysfunction has also been described as central to the pathogenesis of sporadic as well as familial PD (Rocha, de Miranda and Sanders 2017).

The first study in H₂ gas inhalation and PwP (Hirayama *et al.*, 2018) reported significant increases of urinary 8-OHdG levels (an oxidative stress marker) after H₂ gas inhalation, compared to a placebo. 8-OHdG levels were higher in the H₂ arm than the placebo arm at baseline. A strength of this study is that it was a randomised cross over study. The inhalation was short (ten minutes per day, twice a day) for four weeks. While oxidative stress is a known driver of PD, it was theorised in the paper that induced low levels of oxidative stress by inhalation of H₂ gas have been shown to activate the nuclear-related factor-2 (Nrf-2) signalling pathway, which ultimately evokes an anti-oxidative defence system (Murakami, Ito and Ohsawa 2017; Xie *et al.* 2020) and Nrf-2 function is suppressed in PwP (Yang *et al.* 2014). While increase of an oxidative stress marker in PwP through inhalation of H₂ gas may seem counterintuitive – as oxidative stress is a driver of PD – low levels of oxidative stress may be beneficial due to the cascade of events which follow it. Findings from an earlier study (Ito *et al.* 2012) also concluded that intermittent H₂ gas inhalation was more beneficial than prolonged inhalation of H₂ gas. The study examined H₂ water, H₂ gas and lactulose in PwP and an animal model of 6-OHDA-induced with Parkinson's disease, and the effect on end-alveolar breath H₂ concentrations. This simple test is a validated measure by which to measure H₂ concentrations.

The study showed that intermittent H₂ gas inhalation seemed to mimic the results of *ad libitum* administration of H₂ water. However, there are opposing findings to the timings of H₂ gas inhalation, which makes it difficult to ascertain which is more beneficial: short bouts (10 min) of H₂ gas inhalation or longer bouts (1 h) of H₂ gas inhalation. In 2021 (Yoritaka et al. 2021) a pilot study examined a much longer period of inhalation in PwP; one hour, twice a day for 16 weeks. This study found it difficult for participants to adhere and comply to the study protocol, with 25% of participants (5) failing to inhale half of what was requested by the research team, and therefore the results from these participants were not included in the analysis. The study was a placebo controlled, randomised, double-blind and parallel group. Ages of the placebo and hydrogen gas groups were similar (mean placebo = 66.4 years, mean hydrogen = 65.9 years), however no females were included in the hydrogen group and five females were included in the placebo group, given that females and males exhibit different symptoms, this may have affected the results. The study showed that H₂ was safe to inhale but had no beneficial effects in PwP. It also demonstrated that it may be difficult to have compliance with study protocol when the inhalation time expected is one hour, twice a day for four months.

During an 18-hour continuation H₂ gas inhalation in five patients with PCAS (Tomoyoshi et al. 2020), urinary 8-OHdG levels decreased in three patients, increased in one patient, and remained somewhat stable with one patient over a 24-hour period. Arterial H₂ concentrations were measured, and it was found they reached approximately 2% during H₂ gas inhalation but were washed out at six hours after discontinuation of gas inhalation. The patients in this study were aged between 20 and 80 years old and 60% were male. This poses the question of how long a participant should be inhaling H₂ gas to see positive physiological effects. It is worth noting that if PwP were inhaling H₂ gas for large amounts of time, this would cause them to be sedentary for extended periods of time, and physical activity has been reported to be incredibly beneficial for attenuation of PD symptoms (van der Kolk et al. 2019). A H₂ gas inhalation protocol needs to find the right balance to aid in reduction of symptoms of PD, without promoting sedentary behaviour. Studies which have focused on HRW consumption would not encounter this problem as much, given that drinking water can be done anywhere. Although HRW needs to be consumed in a brief period as H₂ dissipates, so participants need to have access to a toilet, and if people were in the later stages of PD, they would possibly be reliant on another person to help them in going to the toilet. Or they may be suffering with incontinence, which often encourages people to avoid consuming liquids. It could be that larger amounts of H₂ can be consumed with HRW, without promoting sedentary behaviour if the PwP

was in the earlier stages of their disease. The opposing findings of these studies suggest that short bouts of H₂ gas inhalation may be more beneficial than longer periods spent inhaling H₂ gas. This is also reiterated by a study which examined longer periods of inhaling H₂ gas (Yoritaka et al. 2021). This was the second study from a group which were the first to publish a study into H₂ and PwP. The study explored PwP inhaling H₂ gas for one hour, twice a day for 16 weeks. The group did not find any significant difference in 8-OHdG levels, which is suggestive that shorter bouts of H₂ gas may have a more beneficial effect than longer bouts of inhalation if measuring that oxidative stress marker. However, given the focus of 8-OHdG in H₂ literature, studies have found 8-OHdG reduced significantly (Shibayama et al. 2020) or there was no difference in levels after a H₂ protocol (Zhang et al. 2018), which seems consistent with results found in a PD population. These studies were in exercising males (Shibayama et al. 2020) and asthmatic mice (Zhang et al. 2018), while one study investigated airway disease and one was in exercise, the differences in 8-OHdG demonstrate that oxidative stress may not be the only underlying mechanism of H₂. 8-OHdG is a relatively easy marker to obtain from urine as it does not require training in clinical practices such as phlebotomy, and if relying on a repeated measures design, there is an almost guaranteed success at collecting urine at each time point.

It is worth mentioning that a study which evaluated concurrent photobiomodulation (PBM) (red light therapy delivered onto the head to help prevent neurodegeneration) and H₂ water in 18 participants who were at H&Y stage 2 and 3 (Hong et al. 2021) over a period of two weeks in PwP demonstrated significant improvements to UPDRS scores, however there was no control in the study, so it is unclear whether the improvements were from the PBM or the H₂ water. An improvement to this study would be to have four groups: a control group, a PBM group, a H₂ water group and a PBM and H₂ water group. While this study shows promise with PBM and H₂, there is not enough evidence to suggest that H₂ water reduces non-motor and motor symptoms of PD to run a study which examines if two therapies improve UPDRS scores.

As PD rates are predicted to rise exponentially in the next 20 years, and there is no cure and no disease modifying treatments available, it is important that safe and side-effect-free therapeutic applications should be explored to help reduce the severity of symptoms in PwP.

1.8. Summary

This research proposes to assess effects of H₂ on motor and non-motor symptoms in PwP. There is still much to learn how H₂ aids in the prevention and management of disease, and this research aims to elucidate some of the potential physiological mechanisms of H₂. Promising findings in animal models of PD and H₂ have not translated into PwP studies, potentially due to the difficulty in investigating the mechanism of neurodegeneration or the pathology of disease progression in animal models. The four studies which have researched H₂ and PwP have been conducted in Japan, which may yield different results to a UK population due to PD genetic variants found in people from Japan. Out of these four studies, two studies assessed biomarkers of oxidative stress, working on the premise that H₂ selectively scavenged ·OH, and these two studies have reported opposing findings. If this research demonstrates that H₂ can improve motor and non-motor symptoms of PD, and potentially elucidate mechanisms of action this would add to the therapeutic treatments available to PwP. H₂ has no reported adverse effects, scavenges ·OH and peroxynitrite (ONOO⁻) and could potentially target signalling pathways involved in inflammation and oxidative stress. Depending on the delivery mechanism of H₂, it can be relatively cheap to administer, without much maintenance needed regarding HRW. H₂ gas is more expensive, and equipment needs to be maintained, but does have a higher tissue saturation than HRW, but does require the user to be sedentary during inhalation therapy. H₂ administration needs to be convenient and effective, and a recent study in chronic H₂ inhalation (Yoritaka et al. 2021) demonstrated poor adherence and compliance to the study protocol, potentially impacting on results. Studies designed to assess motor and non-motor symptoms of PD in people, as well as attempting to elucidate the mechanism of action of H₂ are needed to bring clarity to the field. Studies are needed to help PwP make informed decisions about expensive treatments which may not benefit them.

1.10. Thesis aims

The aims of this thesis were to examine if H₂ impacted motor symptoms and non-motor symptoms of PD through various validated methodologies and to investigate the potential mechanism of action of H₂ in PwP.

Chapter 2. General Methods

This chapter will detail the methodology used in two or more thesis chapters. Methodologies used in only one chapter are detailed in the appropriate chapter.

2.1. Ethical approval

All four studies were granted ethical approval by the University of Kent School of Sport and Exercise Sciences Research Ethics Advisory Group (SSES REAG) (Table 2.1). Studies 2, 3 and 4 were prospectively registered with the BMC ISRCTN registry. Permission to use International Parkinson and Movement Disorder Society (MDS) rating scales of the Unified Parkinson's Disorder Rating Scale (UPDRS) and Non-Motor Rating Scale (NMS) was granted from the MDS Scientific Programs Coordinator.

Table 2.1. Ethical approval details.

<i>Study Title</i>	<i>SSES REAG Number</i>	<i>ISRCTN number</i>	<i>Authorised to Use Materials Owned by the MDS</i>
Study 1. The effects of hydrogen gas in healthy participants – a Pilot study	25_20_21		
Study 2. The acute effects of hydrogen gas in people with Parkinson's	26_20_21	13183500	Permission granted for MDS-UPDRS in June 2022
Study 3. The chronic effects of hydrogen gas in people with Parkinson's – a case study	21_20_23	46799043	Permission granted for MDS-NMS in January 2023
Study 4. The chronic effects of hydrogen rich water in people with Parkinson's	23_20_23	17961308	Permission granted for MDS-NMS in January 2023

Study title with corresponding SSES REAG Number, ISRCTN number and MDS authorisation date if applicable.

2.2. Participants

Study 1

Please see Chapter 3.3.2. for participant criteria.

Studies 2, 3 and 4

Inclusion criteria

All participants must have a diagnosis of Parkinson's Disease (PD) by a Neurologist or Geriatrician. It was required that participants be at stage 1, 2 or 3 on the Hoehn and Yahr (H&Y) scale. Participants could be male or female. Participants needed to be able to attend the School of Sport and Exercise Sciences (SSES) lab due to the nature of the study (taking blood samples etc). It was required that all participants' PD had been stable over the two months prior to study commencement.

Exclusion criteria

Participants whose PD was not controlled. Participants who were at stage 4 and stage 5 of the H&Y scale. Participants who could not consent for themselves.

2.2.1. Participant recruitment (Study 2 and 4)

Study 2 and 4 were shared with the Parkinson's UK (PUK) Research Support Network (RSN). Posters were placed on community noticeboards around the Canterbury area, and the Parkinson's Centre for Integrated Therapy (Canterbury, Kent, UK) for Study 4. PUK groups were contacted after PUK had shared the research with their supporters, and the researcher was invited to present their research to attendees.

Study 3 required contact with participants from Study 2 as an open call for a chronic study.

2.2.2. Participant consent

All participants gave written informed consent and completed a medical history form before participation in the study. Where applicable (Study 3 and Study 4), participants completed a Physical Activity Readiness Questionnaire (PAR-Q) to check their suitability for participation in the functional movement tests.

2.3. Anthropometric measurements

2.3.1. Height

Height was recorded using a Stadiometer (model 213, Seca, Birmingham, UK). Participants were asked to remove their shoes; height was recorded in centimetres at the end of an inhalation and with the head in the Frankfort horizontal plane.

2.3.2. Weight

Weight was taken using a mechanical floor scale (model 761, Seca, Birmingham, UK) with the participant not wearing shoes.

2.4. Blood pressure (BP)

The participant was seated in an upright position, a sphygmomanometer (DuraShock DS54, Welch Allyn, New York), an adult cuff (Flexiport Adult Cuff Size 11, Welch Allyn, New York, USA), and a stethoscope (Classic 111 Monitoring Stethoscope, Littmann, Massachusetts, USA) were used to take BP. Participants remained seated for approximately 10 minutes (min) before measurement commenced. The participant was asked to not speak while BP was being taken.

2.5. Gas machine

2.5.1. Hydrogen and placebo gas machine

The Osmio Infinity Hydroxy Gas/Water Production Electrolysis System (HYD) (Osmio Solutions Ltd, Cliffe, Kent, UK) was reported to generate up to 50 litres per hour of mixed hydrogen (H_2) and oxygen (O_2) gases (approximately 830 millilitre (mL) per min of 66% H_2 and 33% O_2), which when breathed equates to approximately 2-4% H_2 . The HYD machine had a timer situated at the back of the machine which logged operation time, the placebo machine had a timer but was found to not measure time accurately as the timer did not change from 8 mins throughout the study (the timer did not move throughout operation). When this was noticed by the researcher, the timer was covered by a piece of paper, so it was not visible. The humidifier filtered out any sodium hydroxide (NaOH) residue, however, during study 1, it was noticed that the placebo and HYD humidifiers did not have the same appearance (the HYD machine appeared to have ‘condensation’) therefore in study 2 the humidifier was covered with paper so there was no difference seen between the HYD and placebo machines. The placebo machine had the same appearance as the HYD machine, but an electrolysis electrode was disconnected, therefore, the participant was breathing just ‘air’. Flow rate of both machines was equally matched, so that no difference would be detected by the participant.

The NaOH solution for both the HYD and placebo machines was made as per the manufacturer’s guidelines. Both machines were correctly maintained and stored at an ambient temperature when not in use.

O_2 concentration of the HYD machine was measured at 32.3% (Servoflex MiniHF 5200 gas analyser, Servomex Group Ltd, Crowborough, UK), it was not possible to measure H_2 gas concentration. Given that O_2 concentration was measured at 32.3%, it was assumed that the H_2 concentration would be $\sim 67.7\%$, which agreed with the manufacturer’s claims.

2.5.2. *Gas machine water*

The gas machine requires pure water (i.e. measuring 0 total dissolved solids (TDS) – or as close as possible to 0 TDS) to run efficiently. As an example, tap water in Canterbury, UK contained around 225 parts per million (ppm) TDS (Hommix Water TDS-3 and Temperature Meter, Enfield, UK). Lab water (Milli-Q Direct Water Purification System, Merck Life Sciences Ltd, Watford, UK) was used in the machine.

2.5.3. *Nasal cannulas*

Gas was breathed through a 210 centimetre (cm) nasal cannula (Osmio Solutions Ltd, Cliffe, Kent, UK), (see Figure 2.1 for cannula placement) and a new nasal cannula was used for each breathing period and then disposed of in clinical waste. The nasal cannula was placed on the machine by the researcher, then the cannula ‘prongs’ were placed in the participant’s nostrils, it was then tightened enough that the nasal cannula would not move, but comfortable enough for the participant to wear for the breathing period. In Study 3, as the participant had taken the machine home, the participant completed this themselves after demonstration by the researcher. In study 1 and study 2 the cannula was checked periodically throughout the breathing period to ensure that it was still secured. If participants needed a break from breathing (to use the toilet for example), the time which the participant was away from the machine was noted.

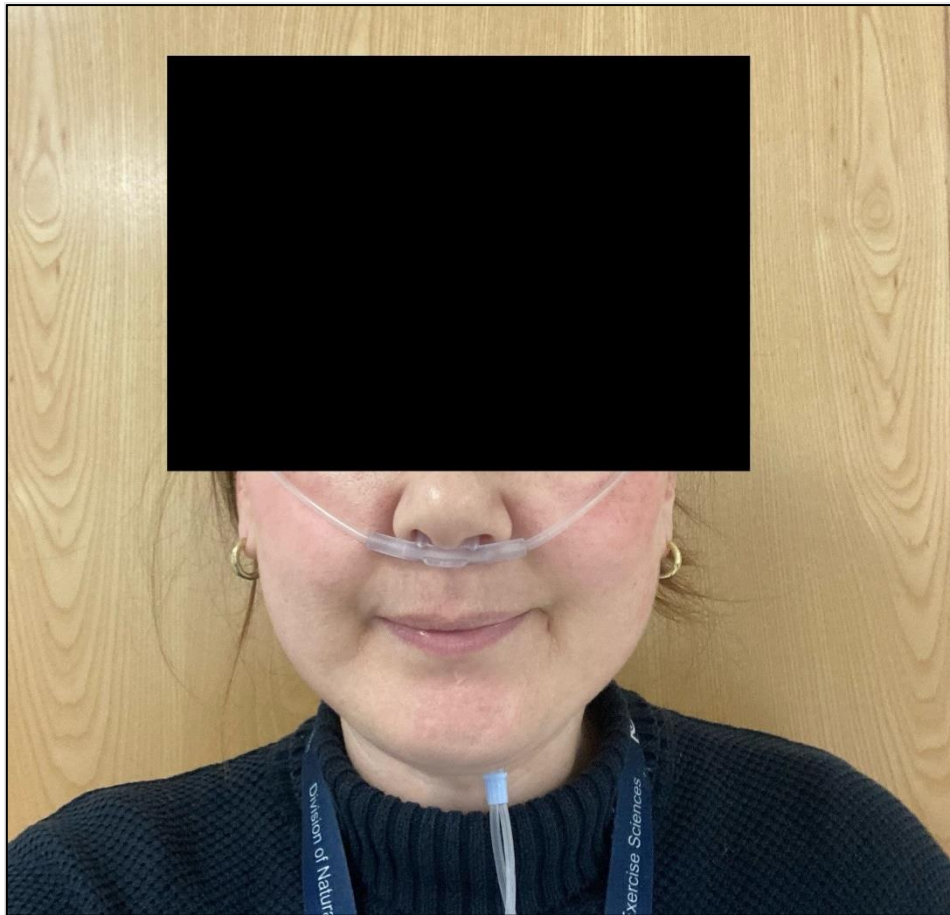


Figure 2.1. Cannula placement demonstration

2.6. Blood collection

2.6.1. Venous blood collection

Venous blood samples were obtained from the antecubital vein of each participant, after being located using a tourniquet. Disposable gloves were worn throughout the procedure. Skin was prepared using a small 70% alcohol pre-injection swab (Medisave UK Ltd, Dorset, UK). A 0.8 x 25 mm multiple sample blood collection needle (BD Vacutainer™) was used for the procedure, and then blood was collected using vacutainers (BD Vacutainer™). Samples were centrifuged at 1500 relative centrifugal force (RCF) at 4°C for 10 min (Eppendorf UK Ltd, Stevenage, UK). Heparin sample tubes were processed as soon as possible after collection. Serum samples were allowed to clot at room temperature for one hour before being centrifuged at 1500 relative centrifugal force (RCF) at 4°C for 10 min. Serum and plasma were collected and pipetted into 1.5 ml aliquots and stored in –80°C until analysis.

2.6.2. Platelet poor plasma

Aliquoted plasma from venous blood collection was further centrifuged (Accuspin Micro 17 R, Fisher Scientific, Loughborough, UK) at 13,300 rpm at 4°C for 10 min. 0.5ml of plasma was pipetted into a further aliquot and stored in –80°C until analysis.

2.6.3. Finger prick blood collection

Fingertip capillary blood was obtained in microvettes (Microvette 300 Lithium Heparin, Sarstedt Ltd, Leicester, UK) and then centrifuged (Accuspin Micro 17 R, Fisher Scientific, Loughborough, UK) at 13,300 rpm at 4°C for 10 min.

2.6.4. Sample analysis

Samples were allowed to thaw at room temperature. Working standards, samples (after thawing) and reagents were prepared as per manufacturer's guidelines, and assay procedure was followed according to manufacturer instructions for each individual assay (including the calculation of results). Calculation of results was carried out by averaging the duplicate readings for each standard and sample (if assayed in duplicate). The average zero standard was subtracted from averaged readings. A standard curve was generated for each set of sample assays (Microsoft Excel, Microsoft Corp. Washington, USA) Absorbance, Luminescence or Chemiluminescence was determined using a microplate reader (BMG Labtech FLUOstar Omega, Ortenberg, Germany). Standards and samples were assayed in duplicate where possible. Please see further chapters for more detail on which analysis were used.

2.7. Profile of Mood States- Short Form

The Profile of Mood States – Short Form (POMS-SF) (Grove, 1992) has been used in previous research in people with Parkinson's (PwP) (Leentjens et al., 2006) and consists of 40 adjectives that measure mood in the moment: tension (TEN), anger (ANG), fatigue (FAT), depression (DEP), esteem-related affect (ERA), vigour (VIG) and confusion (CON). Participants were asked to use a 5-point Likert scale (0 = not at all, 1 = a little, 2 = moderate, 3 = quite a lot, 4 =

extremely), to review a list of 40 words (feelings) and rank the degree to which they are experiencing that feeling at that moment. High vigour-activity scores reflected good moods or emotions, whereas low scores in the other subscales reflected a good mood or emotion, and the total mood disturbance (TMD) score was calculated by summing the totals for the negative sub-scales and then subtracting the totals for the positive subscales:

$$\text{TMD} = [\text{TEN} + \text{DEP} + \text{ANG} + \text{FAT} + \text{CON}] - [\text{VIG} + \text{ERA}]$$

A constant of 100 was added to the TMD formula to eliminate negative scores. Higher numeric values of the TMD score indicated a greater degree of mood disturbance.

2.8. Flanker compatibility task

Dopamine loss and pathology within the white matter are associated with cognitive slowing and impact reductions in executive control on the Stroop test (Verhaeghen, 2011). The Flanker Compatibility Task is like the Stroop and was administered using the Psych Lab 101 application (Presentation, Neurobehavioural Systems, Inc, California, USA) on an iPad (9th Generation, Version 16.6.1, Apple. Inc., California, USA) (see Figure 2.2 for participant task set up) which measured 250.6mm in length and 174.1mm in width, the screen size measured 259mm diagonally.



Figure 2.2. Flanker compatibility task set up

Inhibition is defined as an executive function, and the Flanker is a visual attention-based task and explores the lack of inhibition related to the difficulty in suppressing interference by incongruent stimuli. Each participant was given a verbal explanation of the task by the researcher before commencement of the task, and within the app there was a built-in practise which lasted approximately 20 seconds and there were four stimuli in the practise that did not count towards the scoring. The participant would be asked whether they would prefer the tablet to be flat on the table, or upright. This was to ensure the participant was comfortable and could clearly see the screen (due to ceiling lights being reflected on the screen) and this positioning of the iPad was maintained consistent across all visits for each participant. The researcher stood out of sight of the participant to reduce distractions. The participant completed 64 trials: 32

with a shape (compatible and incompatible shape), 32 without a shape (no shape). The screen went blank in between stimuli for approximately 1.5 seconds, and then a random stimulus (compatible, incompatible and no shape) would be shown. Each experiment would last approximately three and a half minutes. Reaction time and accuracy were recorded for each trial.

Flanker test instructions and screenshots are shown in Figure 2.3, 2.4 and 2.5: if the shape in the red circle was a square, the participant pressed the left-hand side of the screen, if the shape in the red circle was a diamond, the participant pressed the right-hand side of the screen. The shape outside of the circles served as ‘distraction’ stimulation that might require the employment of response inhibition (incompatible) or not (compatible).

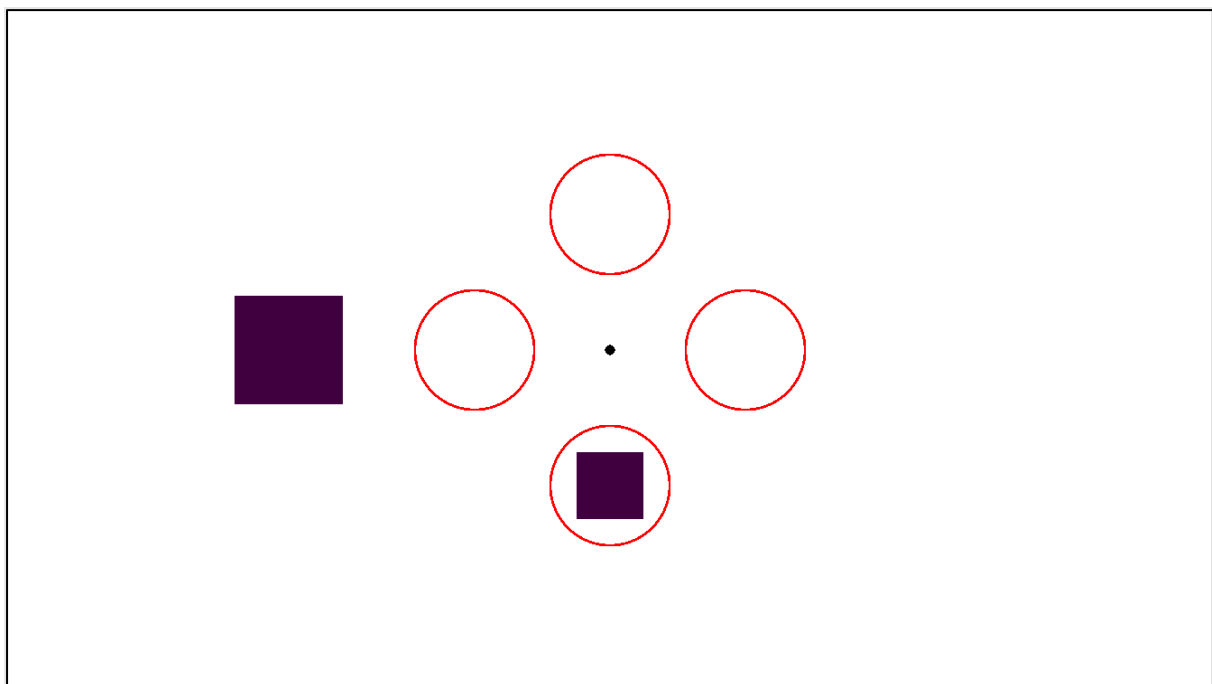


Figure 2.3. Screenshot from the Flanker compatibility task type ‘compatible’

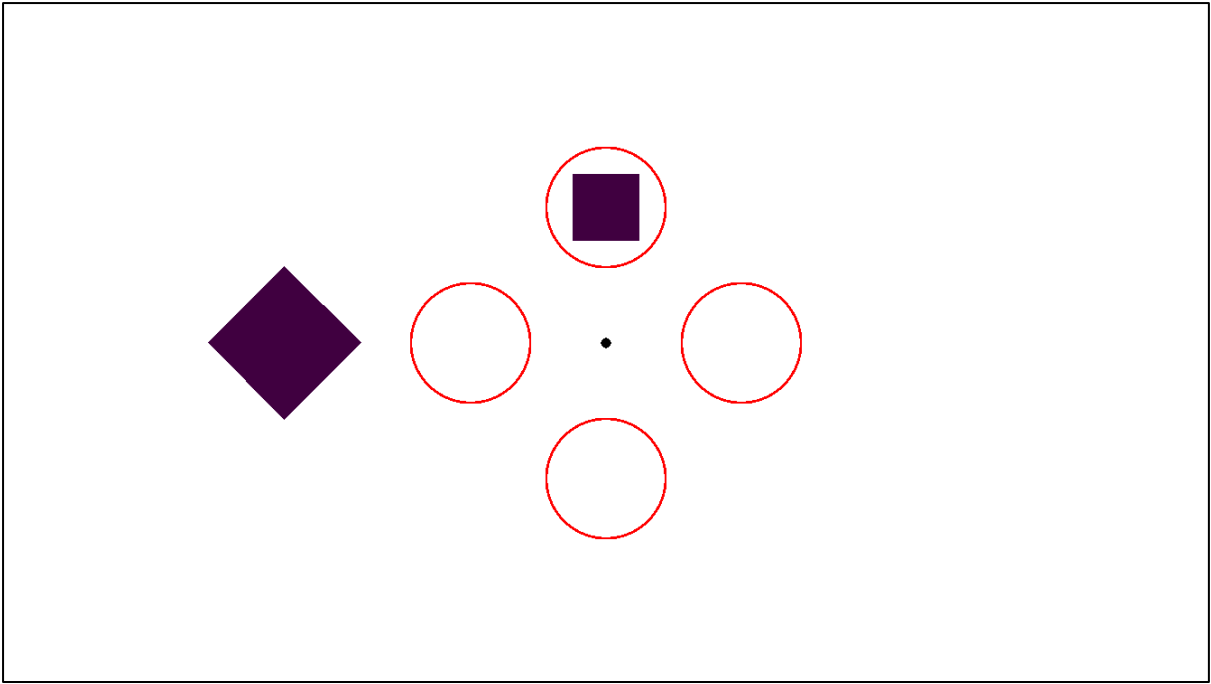


Figure 2.4. Screenshot from the Flanker compatibility task type 'incompatible'

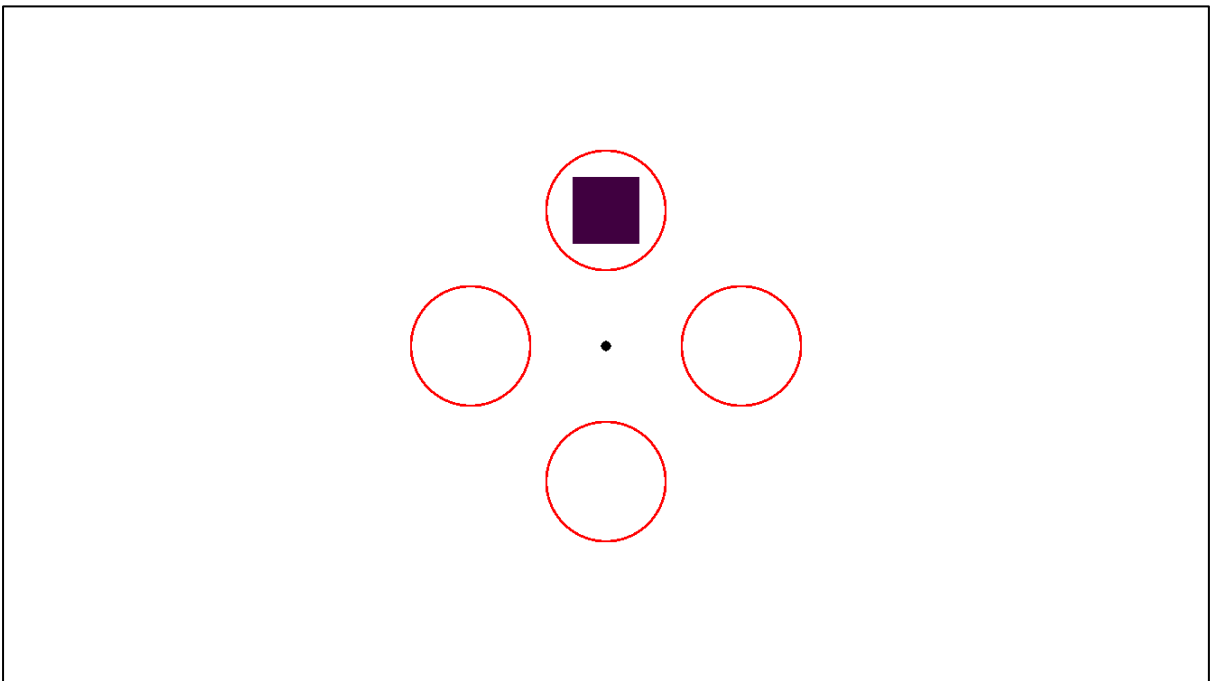


Figure 2.5. Screenshot from the Flanker compatibility task type 'none'

2.9. Purdue Pegboard Test

The Purdue Pegboard Test (PPT) (Tiffin & Asher, 1948) (Figure 2.6) is a simple functional assessment which measures manual dexterity and visuomotor co-ordination (Desrosiers et al., 1995). Over a predetermined time, the participant must insert as many pins (or complete an ‘assembly’) as possible into the board for each of the four tasks, the number of inserted pins or parts represents the score.

Study standardisation protocol of PPT

The PPT task was standardised for each participant using a standardised script, and encouragement was not given to the participant. The PPT would take around 15 mins to complete for each participant, although some participants needed more clarification on the different tasks. Each task was demonstrated by the researcher, and then the participant was asked to practise. Tasks were carried out three times (and the mean determined) for each task in accordance with standard protocol, this is to mitigate any learning effect.

The tasks would be in the same order for each participant; right hand, then left hand (or vice versa if the participant was left-handed dominant), followed by right and left hand together, and then the assembly. The assembly was completed on the right-hand row of the holes on the PPT if the participant was right-handed, and on the left side if the participant was left-handed.



Figure 2.6. The Purdue Pegboard Test and pins (demonstrating both hands together).

2.10. Movement Disorder Society Non-Motor Rating Scale (MDS-NMS)

The MDS-NMS (Chaudhuri, 2019) is used to assess frequency and severity of 13 non-motor domains. Non-motor symptoms included: depression, anxiety, psychosis, impulse control, cognition, orthostatic hypotension, urinary (urgency and frequency), sexual (drive and arousal), gastrointestinal (drooling, swallowing, nausea, constipation), sleep and wakefulness, pain, and

other symptoms such as weight loss. Subscale total is the sum of all item totals for that subscale. The item total is calculated by frequency multiplied by severity. The MDS – NMS Total Score is the sum of all subscales. Care was taken by the researcher asking these questions (as many questions were sensitive in nature), and participants were advised they did not need to answer questions if they did not want to or could stop the questionnaire at any point. At no time was anybody else present during these questions. Participants were advised to see their GP, Neurologist or PD Nurse if they reported symptoms such as urinary urgency, depression, anxiety etc, which the participant may not have attributed to PD.

2.11. Functional movement

2.11.1. Timed Up and Go Test

The Timed Up and Go Test (TUG) assessed functional mobility. The TUG was demonstrated to each participant by the researcher. Each participant performed this wearing their regular footwear, and they were allowed to use their walking aid if they had one. The participant sat on a solid plastic chair with a height of 40 cm, and walked to a 3-meter line, a cone was placed 50 cm away from the 3 m line (at 2.5m) to ensure that the distance covered was 3 m (Figure 2.7), and then the participant sat back down again. Once sat down, the timer was stopped. Each participant completed the TUG three times, and the mean was calculated and recorded.

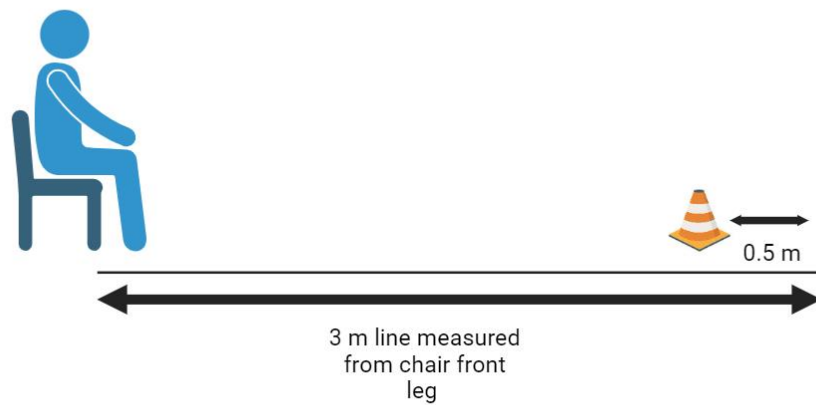


Figure 2.7. Timed Up and Go Test set up

2.11.2. Six-minute Walk test

The six-minute walk test (6MWT) is a sub-maximal test where participants are instructed to walk as far as possible in six min. A 10-metre track was used due to space limitation, with cones placed 50 cm before each end of the track. Each metre was marked with masking tape. Participants were allowed to stop or slow down, if they stopped before the end of the six min, they were encouraged to resume the test as soon as they were able. Walking aids were allowed to be used if required. A standardised script was used for each participant. The timer began when the participant started walking after a prompt from the researcher. A handheld analogue tally counter (DK Tools Ltd, Middlesex, UK) was ‘clicked’ after each ten metres was completed. At six min, the participant was told to stop, and the nearest meter was noted, and total distance recorded. Both the TUG and 6MWT are highly reliable measures when used in PwP (Steffen & Seney, 2008).

2.12. Accelerometer

An actigraph with a triaxial accelerometer (GENEActiv, Activinsights Ltd, Kimbolton, UK) was worn around the participants' waists for the duration of the study (Chapter 5 and 7), except the 1-week 'washout' period to measure sleep, sedentary time, and activity levels. Recall of behaviour has been demonstrated to decline with age (Barnett et al., 2016) therefore it was decided to not use a recall questionnaire such as the International Physical Activity Questionnaire and use accelerometry which requires no user input or memory recall.

Participants were asked to wear the accelerometer 24 hours a day for the duration of each study period (28 days), apart from bathing/swimming due to the waist belt becoming wet, and therefore uncomfortable for the participant to wear. Participants were also advised to wear the belt while sleeping. Typically, accelerometers are worn on the non-dominant wrist, however it has been demonstrated that discrimination of sedentary behaviour is excellent and discrimination of MVPA behaviour is good when the accelerometer is worn on the waist in older adults (Duncan et al., 2020). The accelerometers were set to collect data at 20 Hz to allow for a 30-day wear period without charging (each wear period was 28 days). Data was downloaded using GENEActiv Software (version 3.3) set at 30 second epochs. Data was then analysed using R Studio (Version 4.3.2) using Activinsights GENEActiv R Markdowns of physical activity. A valid day was defined as at least 14 hours of wear. If daily non-wear exceeded 10 hours, this day was discarded from analysis. A seven-day wear period has been shown to reliably estimate habitual physical activity in adults (Miguelles et al., 2017).

Chapter 3. The effects of hydrogen gas inhalation in healthy participants – a Pilot study

3.1. ABSTRACT

Introduction: Hydrogen (H₂) has demonstrated health benefits in both animal models and humans. H₂ may improve the motor and non-motor symptoms of PD, however the acute effects of a H₂ intervention in people with Parkinson's (PwP) have yet to be explored. **Aim:** This pilot study in healthy participants aimed to assess if timings of measures and acceptability of the intervention were feasible for a future planned study in PwP and to help refine clinical practices. **Methods:** Eight healthy participants (male = 7, female = 1; age = 35 ± 11 years; height = 180.1 ± 7.5 cm; body mass = 79.8 ± 10.3 kg) inhaled hydrogen gas (HYD) and placebo gas (PLA) for two hours, one week apart. Participants were randomised to which gas they inhaled first. Cognition, mood and manual dexterity tests were taken at baseline, 1 hour and at 2 hours post- inhalation. **Results:** There was an increase in the amount of pins placed in the PPT Assembly from baseline to 2 h (HYD = 4%, PLA = 13%), a reduction of POMS-SF from baseline to 2 h subscales of tension (HYD = 47%, PLA = 42%), TMD (HYD = 3%, PLA = 5%), Tension (HYD = 47%, PLA = 42%), Anger (HYD scored 0 at 2 h, PLA = 58%) and Confusion (HYD = 38%, PLA = 38%), no other mood subscale was found to have a significant effect of time. There was a reduction in reaction time in the Flanker Compatibility Task from baseline to 2 h for 'Incompatible' (HYD = 13%, PLA = 29%) and 'None' (HYD = 4%, PLA = 21%) tasks. **Conclusion:** HYD gas was safely administered and could be a feasible intervention for PwP.

3.2. INTRODUCTION

H₂ consumption has demonstrated health benefits in both animal models and humans (Zhang et al., 2021; Timón et al., 2021; Chu et al., 2019). H₂ gas inhalation and PwP research has focussed on chronic consumption (Hirayama, Ito, Minato, Yoritaka, LeBaron, et al., 2018; Yoritaka et al., 2021), and no known study has yet examined the effects of an acute H₂ consumption period in PwP. H₂ gas inhalation yields a larger tissue saturation than hydrogen rich water (HRW) and subsequent H₂ concentrations are maintained post cessation for approx. 60 min (Liu et al., 2014). It is anticipated that there will be no adverse reaction due to previously shown safety (Cole *et al.* 2019, pp.133–138; Yoritaka et al., 2021). Compliance issues have been reported in previous chronic H₂ inhalation and PwP research (Yoritaka et al., 2021), where 25% of participants failed to inhale gas twice a day for one hour for 16 weeks and the study

revealed there was no beneficial effects in PwP, however dose response and mechanism of action of H₂ have yet to be elucidated. As the proposed study will be an acute inhalation protocol, it is anticipated there will be high compliance to the protocol as the researcher will always be present, except when blood samples will be centrifuged.

This pilot study intends to precede a future proposed study in H₂ and PwP. Pilot studies do not test hypotheses about intervention effects, but rather future study feasibility which allows refinement and modification of subsequent studies. Pilot studies allow researchers to refine clinical practices, such as venous blood sampling, which increases rigour and reproducibility in further studies (Lancaster et al., 2004).

H₂ gas needs to be delivered at 2-3% due to its flammable nature even at low concentrations. There is limited evidence on the dose-response relationship, which would give an indication of the potency and its ceiling effect. Gas inhalation has increased bioavailability compared to oral administration as it bypasses the digestive tract, and while H₂ is the smallest gas molecule, facilitating its diffusion through the blood-brain barrier (BBB), this also has disadvantages, as H₂ can rapidly diffuse from the body (Kobayashi et al., 2020).

Studies investigating the effects of H₂ gas inhalation in PwP (Hirayama, Ito, Minato, Yoritaka, Lebaron, et al., 2018; Yoritaka et al., 2021) have relied on the Movement Disorder Society- Unified Parkinson's Disease Rating Scale (UPDRS) (Goetz et al., 2008) which evaluates motor function and characterises extent and burden of disease. While the UPDRS is the gold standard for measuring disease progression, it would not capture the effects of an acute response. The utilisation of the UPDRS in H₂ studies allows for comparison of findings, but the UPDRS fails to capture the complexity and heterogenicity of PD (Mari & Mestre, 2022). The Profile of Mood States-Short Form (POMS-SF) (Grove, 1992) will therefore be used, as this can also be used in a disease-free population. The POMS-SF is a validated and reliable assessment of an individual's mood and has been used to assess the effectiveness of therapeutic interventions (Lopresti et al., 2019; Lynch et al., 2019).

The purpose of this pilot study in healthy participants was to test the feasibility of the study design, to determine if the intervention would be practical and tolerated by participants (Lancaster et al., 2004), if randomisation and blinding was properly executed, and highlight any necessary modifications needed to conduct a similar study in PwP. This study proposes to assess markers (Cognition, mood, and manual dexterity) which are important in contributing

to the effects that may occur in PwP and will help to understand if the timings selected for the measurements are appropriate or need adjustment. The researcher felt it was better to do this in healthy volunteers in the preliminary stages.

3.3. METHODS

3.3.1 Ethical approval

The study was granted ethical approval by the University of Kent's School of Sport and Exercise Sciences (SSES) Research Ethics Advisory Group, SSES REAG Number: 25_20_21.

3.3.2 Participants

Inclusion criteria

All participants were aged 18+ years and male (n=7) or female (n=1). Participants were required to attend the SSES lab for approximately 3 hours each visit. Participants were healthy, not taking any medication and were not diagnosed with any chronic illnesses or disease.

Exclusion criteria

Participants could not be on any medication or diagnosed with any chronic illness or disease. Participants who had drank or bathed in hydrogen rich water, inhaled hydrogen gas, or consumed hydrogen supplements within the past week. Participants aged under 18 years. Participants who may have been pregnant or breastfeeding. Participants who could not consent for themselves. Participants who had any signs or symptoms of Covid-19, or who were suffering from a cold or viral infection on the day of data collection.

Participant consent

All participants gave written informed consent and completed a medical history questionnaire before participation in the study.

3.3.3 Experimental design

Participants attended the SSES Research Laboratory on two occasions (at a similar time of day), approximately one week apart. Participants were randomised whether they had hydrogen (HYD) gas inhalation HYD or placebo (PLA) gas inhalation first. Initially the study compromised inhaling gas for four hours, however, after one participant, it was found that this amount of physical inactivity, being unable to consume food, and attempting five venous blood draws were uncomfortable for the participant. The inhalation period was reduced to two hours, and venous blood draws were reduced from five to three. Participants were sat down for the entirety of the study and could not consume food or drink anything other than water. A schematic of the experimental visits can be seen in figure 3.1.

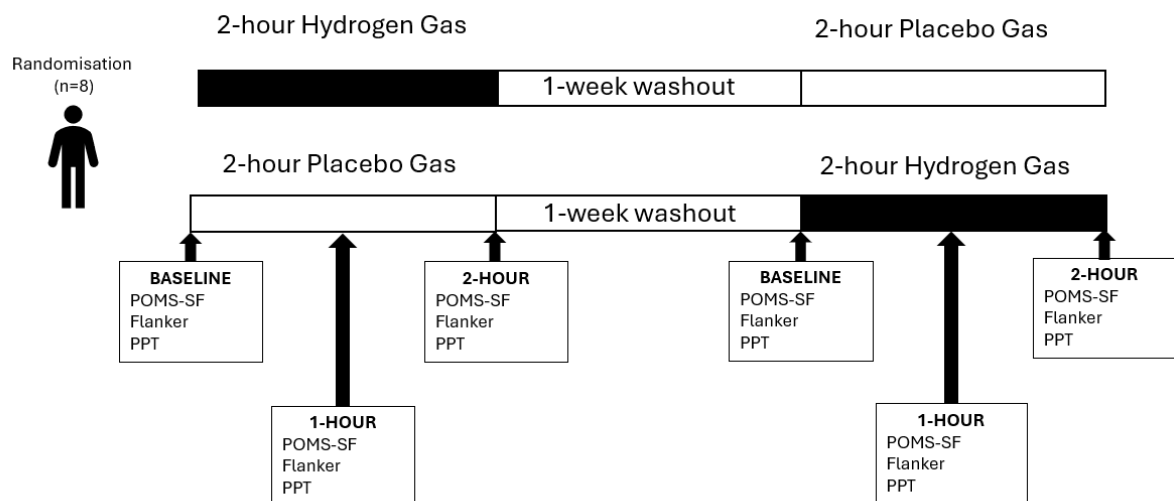


Figure 3.1. Schematic design of the study. Participants were randomised into inhaling HYD or PLA first. Measures were taken at baseline, after 1 hour inhalation and after 2 hours inhalation with a one-week washout period. (POMS-SF = Profile of Mood States – Short Form, Flanker = Flanker Compatibility Task, PPT = Purdue Pegboard Test).

Hydrogen gas machine

Gas inhalation

Participants either inhaled HYD or PLA gas (Osmio Solutions Ltd, Cliffe, Kent, UK) for two hours using a new cannula at each visit. Randomisation was carried out using randomization.com. A timer was set when the cannula had been fitted, and gas machine humidifiers were bubbling. The humidifier filtered sodium hydroxide (NaOH) residue, and during the study, it was noticed that the HYD and PLA humidifiers did not have the same appearance (one machine appeared to have ‘condensation’, whereas the other machine did not have the same appearance). After completion of the study, and de-blinding, it was found that the ‘condensation’ appearance was the HYD machine. In subsequent studies, the humidifier was covered so that no there was no difference in appearance.

Please see General Methods chapter 2.5.1. for further information on the hydrogen machine including O₂ concentration and water content.

Nasal cannula

Gas was inhaled through a 210 centimetre (cm) nasal cannula (Osmio Solutions Ltd, Cliffe, Kent, UK), and a new nasal cannula was used for each inhalation period and then disposed in clinical waste. Proper nasal cannula placement was observed periodically to ensure that it was in place.

Please see General Methods chapter 2.5.3. for further information on nasal cannulas including how the nasal cannulas were placed on the participant.

Measures

Measures were taken at baseline, after 1 hour inhalation and after 2 hours inhalation. Measures were taken in the same order at each timepoint; venous blood samples, POMS-SF, Flanker compatibility task, Purdue Pegboard Test (PPT).

Anthropometric measurements

Height and weight were measured, and BMI calculated at the first visit. Please see General Methods chapter 2.31. and 2.3.2.

Profile of Mood States – Short Form (POMS-SF) (Grove, 1992)

Please see General Methods chapter 2.7. for further information on this.

Flanker compatibility task (Presentation, Neurobehavioural Systems, Inc, California, USA)

Please see General Methods chapter 2.8. for further information on this.

Purdue Pegboard Test (PPT) (Tiffin & Asher, 1948)

Please see General Methods chapter 2.9. for further information on this.

Statistical analysis

Assumptions of normal distribution of data were checked using the Shapiro-Wilk test (and Z scores for skewness and kurtosis for confirmation). Data that were not normally distributed were transformed prior to analysis to normalise the data distribution. The following transformations were attempted, and the method that provided the best normalisation (based on Z-scores) was used to transform these data for analysis: log, square root, cube root, power (x cubed), inverse and arcsine, Log was found to provide the best normalisation for data Where the assumption of sphericity was violated (determined by Mauchly's test), the Greenhouse-Geiser adjustment was applied to the degrees of freedom. Post hoc Bonferroni correction was carried out to identify the location of differences when a main effect for time was observed. Outliers in the data were identified by box plot. Non extreme outliers were identified as not greater than three times the interquartile range from a quartile, while extreme outliers were

identified as greater than three times the interquartile range from a quartile (Tukey, 1977). If outliers were identified by box plot, these were both included and excluded in the data analysis. Statistical significance level was set at $P < 0.05$, analysis was performed with IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA).

3.4. RESULTS

Participant characteristics are presented in Table 3.1

Table 3.1. Participant characteristics.

<i>Variable</i>	<i>Mean $\pm$ SD</i>	<i>Minimum value</i>	<i>Maximum value</i>
Age (years)	35 $\pm$ 11	19	57
Height (cm)	180.1 $\pm$ 7.5	164	189
Body Mass (kg)	79.8 $\pm$ 10.3	63	100
BMI (kg/m²)	24.7 $\pm$ 2.4	22	29

Eight healthy participants were initially recruited (male= 7, female = 1). The first participant was removed from the statistical analysis as they had completed the four-hour inhalation period and did not want to repeat the study again, therefore male = 6, female = 1 formed the final sample.

3.4.1. FLANKER COMPATIBILITY TASK – ACCURACY

Mean and standard deviation results to Flanker Compatibility Task – Accuracy are presented in Figure 3.2

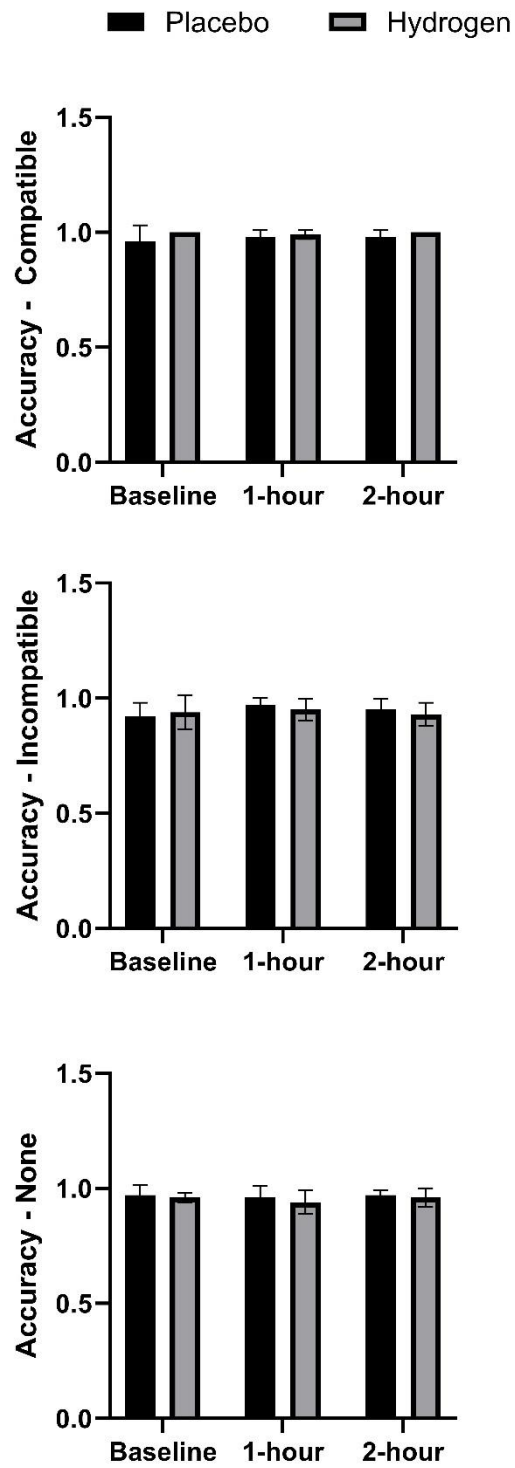


Figure 3.2. Flanker Compatibility Task Accuracy scores. Results show accuracy response to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli), with a maximum score of 1. Data are presented as mean $\pm$ standard deviation.

Accuracy – Compatible

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the Accuracy – Compatible subscale, ($F(1,6) = 3.488$, $p = 0.111$, $\eta^2 = 0.368$), or main effect of time ($F(2,12) = 0.192$, $p = 0.828$, $\eta^2 = 0.031$). There was no significant condition x time interaction ($F(2,12) = 0.669$, $p = 0.530$, $\eta^2 = 0.100$).

Accuracy – Incompatible

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participants' Accuracy - Incompatible, ($F(1,6) = 0.162$, $p = 0.701$, $\eta^2 = 0.026$), or significant main effect of time ($F(1.165, 6.990) = 0.779$, $p = 0.427$, $\eta^2 = 0.115$). There was no significant condition x time interaction ($F(2,12) = 1.279$, $p = 0.314$, $\eta^2 = 0.176$).

Accuracy – None

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participants' Accuracy - None, ($F(1,6) = 2.717$, $p = 0.150$, $\eta^2 = 0.312$), or significant main effect of time ($F(2,12) = 0.307$, $p = 0.741$, $\eta^2 = 0.049$). There was no significant condition x time interaction ($F(2,12) = 0.254$, $p = 0.780$, $\eta^2 = 0.041$).

3.4.2. FLANKER COMPATIBILITY TASK – REACTION TIME

Mean and standard deviation results to Flanker Compatibility Task – Reaction Time are presented in Figure 3.3

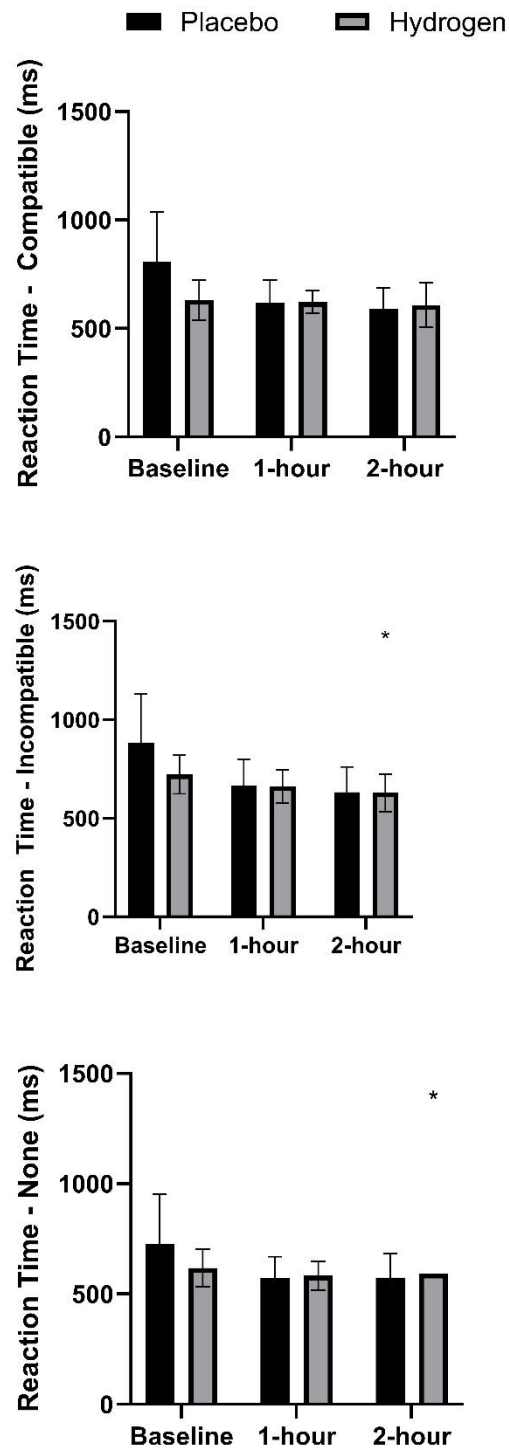


Figure 3.3. Flanker Compatibility Task Reaction Time. Results show reaction time to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli). Data is shown in milliseconds and presented as mean $\pm$ standard deviation. *Significant main effect of time.

Average Reaction Time – Compatible

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participants' average reaction time – compatible ($F(1,6) = 1.904$, $p = 0.217$, $\eta^2 = 0.241$), or significant main effect of time ($F(2,12) = 7.185$, $p = 0.009$, $\eta^2 = 0.545$). There was no significant condition x time interaction ($F(1.137, 6.824) = 3.793$, $p = 0.091$, $\eta^2 = 0.387$).

Average Reaction Time – Incompatible

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participants' ART-IC ($F(1,6) = 1.274$, $p = 0.302$, $\eta^2 = 0.175$), or significant condition x time interaction ($F(2,12) = 1.782$, $p = 0.210$, $\eta^2 = 0.229$). There was a significant main effect of time ($F(2,12) = 17.466$, $p < 0.001$, $\eta^2 = 0.744$), post-hoc analysis with Bonferroni correction showed values at 1 h (663 ms) were significantly faster ($p = 0.046$) than baseline (803 ms). Values at 2 h (631 ms) were significantly faster ($p = 0.001$) than baseline (803 ms).

Average Reaction Time – None

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participants' ART-None ($F(1,6) = 0.528$, $p = 0.495$, $\eta^2 = 0.081$), or significant condition x time interaction ($F(1.108, 6.649) = 2.130$, $p = 0.191$, $\eta^2 = 0.262$). There was a significant main effect of time ($F(2,12) = 6.001$, $p = 0.016$, $\eta^2 = 0.500$), post-hoc analysis with Bonferroni correction showed values at 2 h (584 ms) were significantly faster ($p = 0.024$) than baseline (671 ms).

3.4.2. PROFILE OF MOOD STATES

Mean and standard deviation results of Profile of Mood States short form are presented in Table 3.2

Table 3.2. Profile of Mood States short form (POMS)

<i>Subscale</i>	<i>Condition</i>	<i>Baseline</i>	<i>1-hour</i>	<i>2-hours</i>	<i>% change from baseline to 1 h</i>	<i>% change from baseline to 2 h</i>	<i>N</i>
Total Mood Disturbance (TMD)	HYD	89.86 ± 5.55	86.71 ± 5.589	87 ± 3.512	96	97	7
	PLA	95.86 ± 8.726	93.57 ± 8.522	91 ± 6.831	98	95	7
Tension	HYD	2.43 ± 1.718	1.86 ± 2.116	1.29 ± 1.976	77	53	7
	PLA	4.43 ± 3.259	3.43 ± 2.936	2.57 ± 2.573	77	58	7
Anger	HYD	0.71 ± 1.496	0.29 ± 0.488	0.0 ± 0.0	41	-	7
	PLA	1.71 ± 2.43	0.86 ± 1.574	0.71 ± 1.113	50	42	7
Fatigue	HYD	4.86 ± 2.12	3.29 ± 2.5	3.57 ± 2.23	68	76	7
	PLA	5.29 ± 4.11	4.29 ± 3.45	3.57 ± 2.82	81	67	7
Depression	HYD	1.14 ± 1.22	0.14 ± 0.38	0.43 ± 0.535	12	38	7
	PLA	2 ± 2.309	2.29 ± 2.87	1.43 ± 1.9	115	72	7
Esteem-related affect (ERA)	HYD	16 ± 3.74	15 ± 5.22	14.57 ± 4.72	94	91	7
	PLA	14.14 ± 4.85	14.29 ± 4.536	14.71 ± 4.461	101	104	7
Vigour	HYD	6.43 ± 5.62	5.43 ± 4.72	5 ± 4.08	84	78	7
	PLA	6.29 ± 4.72	6.14 ± 5.05	5.86 ± 4.49	98	93	7
Confusion	HYD	3 ± 2.3	2.29 ± 2.29	1.86 ± 1.864	76	62	7
	PLA	3 ± 3.109	2.43 ± 2.68	1.86 ± 2.04	81	62	7

Data are presented as mean ± standard deviation.

POMS TMD

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS TMD subscale, ($F(1,6) = 2.358$, $p = 0.176$, $\eta^2 = 0.282$), or condition x time interaction ($F(2,12) = 0.852$, $p = 0.451$, $\eta^2 = 0.124$). There was a significant main effect of time ($F(2,12) = 4.443$, $p = 0.036$, $\eta^2 = 0.425$), post-hoc analysis with Bonferroni correction showed no significant main effect between any time point.

Mean and standard deviation results to POMS - TMD – Accuracy are presented in Figure 3.4

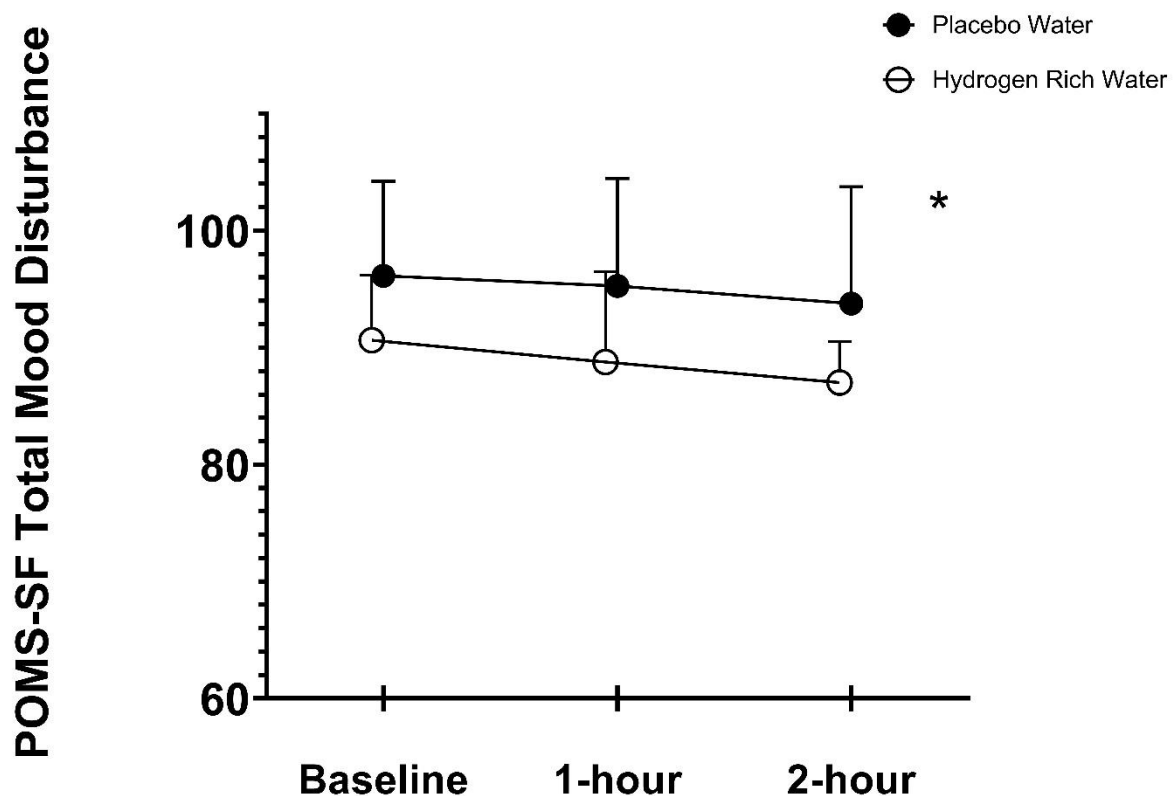


Figure 3.4. The Profile of Mood States-Short Form (POMS-SF). Total Mood Disturbance is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: $TMD = [TEN+DEP+ANG+FAT+CON] - [VIG+ERA]$. Data are presented as mean $\pm$ standard deviation, *significant main effect of time with placebo scoring higher at all time points.

POMS Tension

One outlier in the data was identified by box plot, this was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile).

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Tension subscale ($F(1,6) = 3.631, p = 0.105, \eta^2 = 0.377$), or condition x time interaction ($F(2,12) = 0.385, p = 0.688, \eta^2 = 0.060$). There was a significant main effect of time ($F(2,12) = 8.946, p = 0.004, \eta^2 = 0.599$). Post-hoc analysis with Bonferroni correction showed values at 2 h (2) were significantly lower ($p = 0.036$) than baseline (3), and values at 2 h (2) were significantly lower ($p = 0.047$) than 1 h (3).

POMS Anger

Two outliers in the data were identified by box plot. One outlier was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile), while one outlier was removed as it was considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for POMS-Anger, the first set of results include the extreme outlier in the data analysis, the second set of results exclude the extreme outlier.

Outlier included:

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Anger subscale ($F(1,6) = 1.343, p = 0.291, \eta^2 = 0.183$), or condition x time interaction ($F(1.039,12) = 0.176, p = 0.698, \eta^2 = 0.029$). There was a significant main effect of time on ($F(2,12) = 4.944, p = 0.027, \eta^2 = 0.452$), post hoc analysis with Bonferroni correction did not show any significant main effect between any time points.

Outlier excluded:

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Anger subscale ($F(1,5) = 2.841, p = 0.153, \eta^2 = 0.362$). There was no significant main effect of time ($F(2,10) = 3.182, p = 0.085, \eta^2 = 0.389$), or condition x time interaction ($F(1.107, 5.533) = 3.028, p = 0.136, \eta^2 = 0.377$).

POMS Fatigue

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Fatigue subscale ($F(1,6) = 0.265, p = 0.625, \eta^2 = 0.042$). There was no significant main effect of time ($F(2,12) = 3.466, p = 0.065, \eta^2 = 0.366$), or condition x time interaction ($F(2,12) = 0.665, p = 0.532, \eta^2 = 0.100$).

POMS Depression

Two outliers in the data were identified by box plot. One outlier was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile), while one outlier was removed as it was considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for POMS-Depression, the first set of results include the extreme outlier in the data analysis, the second set of results exclude the extreme outlier.

Outlier included:

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Depression subscale ($F(1,6) = 2.973, p = 0.135, \eta^2 = 0.331$). There was no significant main effect of time ($F(2,12) = 0.946, p = 0.416, \eta^2 = 0.136$), or significant condition x time interaction ($F(2,12) = 1.864, p = 0.197, \eta^2 = 0.237$).

Outlier excluded:

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Depression subscale ($F(1,6) = 2.317$, $p = 0.188$, $\eta^2 = 0.317$). There was no significant main effect of time ($F(2,12) = 0.094$, $p = 0.911$, $\eta^2 = 0.019$), or condition x time interaction ($F(2,12) = 2.047$, $p = 0.180$, $\eta^2 = 0.290$).

POMS ERA

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS ERA subscale ($F(1,6) = 0.969$, $p = 0.363$, $\eta^2 = 0.139$). There was no significant main effect of time ($F(2,12) = 0.418$, $p = 0.668$, $\eta^2 = 0.065$), or condition x time interaction ($F(2,12) = 1.629$, $p = 0.237$, $\eta^2 = 0.214$).

POMS Vigour

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the POMS Vigour subscale ($F(1,6) = 0.131$, $p = 0.730$, $\eta^2 = 0.021$). There was no significant main effect of time ($F(2,12) = 2.481$, $p = 0.125$, $\eta^2 = 0.293$), or condition x time interaction ($F(2,12) = 0.445$, $p = 0.651$, $\eta^2 = 0.069$).

POMS Confusion

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition the POMS Concentration subscale ($F(1,6) = 0.003$, $p = 0.955$, $\eta^2 = 0.001$), or significant condition x time interaction ($F(1.133, 6.798) = 0.031$, $p = 0.891$, $\eta^2 = 0.005$). There was a significant main effect of time ($F(2,12) = 10.070$, $p = 0.003$, $\eta^2 = 0.627$), post hoc analysis with Bonferroni correction showed no difference between any time point.

3.4.3. PURDUE PEGBOARD TEST

Mean and standard deviation for PPT are presented in Table 3.3

Table 3.3. Descriptive statistics for Purdue Pegboard Test (PPT)

<i>PPT</i>	<i>Condition</i>	<i>Baseline</i>	<i>1-hour</i>	<i>2-hour</i>	<i>% change from baseline to 1 h</i>	<i>% change from baseline to 2 h</i>	<i>N</i>
Right Hand	HYD	16.07 ± 2.05	16.35 ± 2.37	17.2 ± 2.16	102	107	7
	PLA	15.05 ± 3.19	16.14 ± 2.83	16.71 ± 2.36	107	111	7
Left Hand	HYD	14.21 ± 2.23	15.35 ± 2.18	15.04 ± 1.91	108	106	7
	PLA	13.94 ± 2.93	15.14 ± 2.25	15.24 ± 1.84	109	109	7
Both	HYD	11.95 ± 2.38	12.21 ± 2.11	12.97 ± 1.83	102	109	7
	PLA	11.52 ± 2.49	12.18 ± 2.23	12.87 ± 2.21	106	112	7
(R+L+B)	HYD	42.35 ± 5.89	44.02 ± 6.16	44.92 ± 5.86	104	106	7
	PLA	40.57 ± 8.24	43.57 ± 6.82	44.72 ± 6.33	107	110	7
Assembly	HYD	40.74 ± 9.35	42.20 ± 8.45	42.50 ± 6.89	104	104	7
	PLA	39.35 ± 8.59	42.64 ± 9.44	44.58 ± 8.24	108	113	7

Data shows number of pegs placed in holes using the Purdue Pegboard Test (PPT). Data are presented as mean ± standard deviation.

PPT Right Hand

Two outliers in the data were identified by box plot, these were included in the data analysis as they were not considered extreme (not greater than three times the interquartile range from a quartile).

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the PPT Right Hand task, ($F(1,6) = 0.549$, $p = 0.487$, $\eta^2 = 0.084$),

or condition x time interaction ($F(2,12) = 2.599$, $p = 0.115$, $\eta^2 = 0.302$). There was a significant main effect of time ($F(2,12) = 14.820$, $p < 0.001$, $\eta^2 = 0.712$), post hoc analysis with Bonferroni correction showed values at 2 h (17 pins) were significantly higher ($p = 0.001$) than at baseline (16 pins).

PPT Left Hand

One outlier in the data was identified by box plot, this was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile).

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the PPT Left Hand task, ($F(1,6) = 0.022$, $p = 0.886$, $\eta^2 = 0.004$), or condition x time interaction ($F(2,12) = 0.161$, $p = 0.853$, $\eta^2 = 0.026$). There was a significant main effect of time ($F(2,12) = 9.556$, $p = 0.003$, $\eta^2 = 0.614$), post-hoc analysis with Bonferroni correction showed values at 1 h (15 pins) were significantly higher ($p = 0.005$) than at baseline (14 pins).

PPT Both Hands

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the PPT Both Hands task, ($F(1,6) = 0.168$, $p = 0.696$, $\eta^2 = 0.027$), or condition x time interaction ($F(2,12) = 0.235$, $p = 0.794$, $\eta^2 = 0.038$). There was a significant main effect of time ($F(2,12) = 8.129$, $p = 0.006$, $\eta^2 = 0.575$), post hoc analysis with Bonferroni correction showed values at 2 h (13 pins) were significantly higher ($p = 0.011$) than at baseline (12 pins).

PPT Right + Left + Both (R+L+B)

Two outliers in the data were identified by box plot, these were included in the data analysis as they were not considered extreme (not greater than three times the interquartile range from a quartile).

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the PPT-R+L+B combined total, ($F(1,6) = 0.217$, $p = 0.658$, $\eta^2 = 0.035$), or condition x time interaction ($F(1.174, 7.045) = 0.837$, $p = 0.410$, $\eta^2 = 0.122$).

There was a significant main effect of time ($F(2,12) = 42.310$, $p < 0.001$, $\eta^2 = 0.876$), post-hoc analysis with Bonferroni correction showed values at 1 h (44 pins) were significantly higher ($p = 0.002$) than at baseline (41 pins). Values at 2 h (45 pins) were significantly higher ($p = 0.001$) than at baseline (41 pins). It also showed that values at 2 h (45 pins) were significantly higher than at 1 h (44 pins).

PPT Assembly

One outlier in the data was identified by box plot, this was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile).

The results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on the PPT Assembly task, ($F(1,6) = 0.022$, $p = 0.888$, $\eta^2 = 0.004$), or condition x time interaction ($F(2,12) = 2.414$, $p = 0.131$, $\eta^2 = 0.287$).

There was a significant main effect of time ($F(2,12) = 8.066$, $p = 0.006$, $\eta^2 = 0.573$), post hoc analysis with Bonferroni correction showed values at 2 hr (44 pins) were significantly ($p = 0.007$) higher than baseline (40 pins).

Mean and standard deviation results for PPT are presented in Figure 3.5

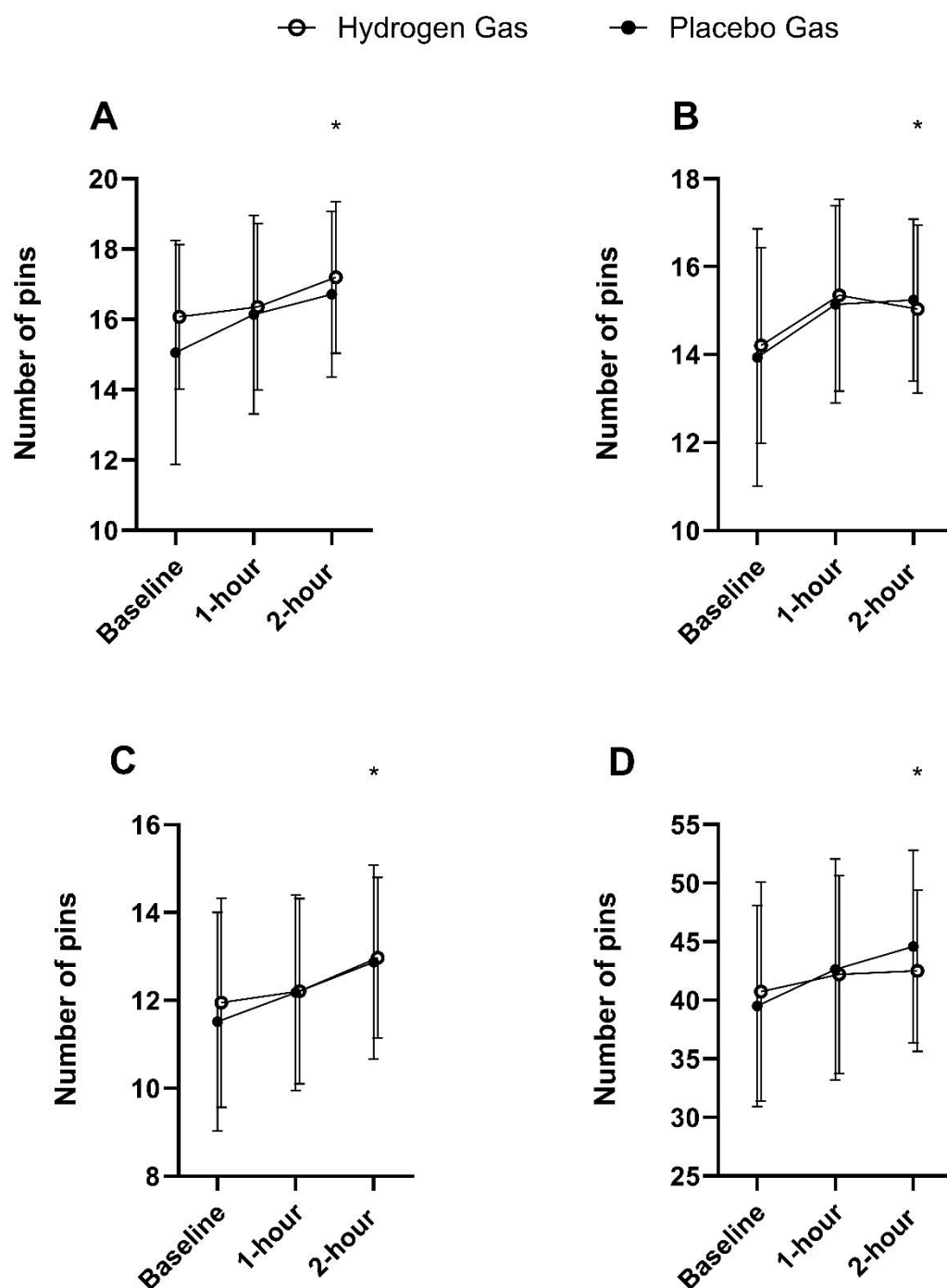


Figure 3.5. Purdue Pegboard Test scores. A = Right hand. B = Left hand. C = Both hands. D = Assembly. Data represented as mean $\pm$ standard deviation. * Significant main effect of time for each test.

3.5. DISCUSSION

The results of this pilot study demonstrated that a two-hour H₂ intervention was safe and practical, but did not improve mood, cognition, or manual dexterity in healthy participants. These results are consistent with the findings of research in H₂ gas in healthy adults (Cole et al., 2021) where no clinically detectable changes in neurological function, attention, fine motor skills or co-ordination were found. Cole et al. (2021) used a much longer inhalation period (participants were randomised into either 24, 48, or 72 hours), had a similar participant number (n=8), participants had a similar age (18-30 years old), with a 50:50 male: female split. While there was no significant change in the battery of tests undertaken by the participants in the current study, this may yield different results in a clinical population cohort, such as Parkinson's, due to the multi-faceted nature of the disease.

However, the aim of this study in healthy participants was to understand if the study design was feasible, if the intervention could be tolerated by participants (Lancaster et al., 2004), and if any modification was necessary for a study in PwP.

Mood

H₂ did not yield any significant main effect on any POMS-SF subscale or Total Mood Disturbance. However, Total Mood Disturbance reduced over time from baseline to 2 hours (HYD = 3%, PLA = 5%), as well as Tension (HYD = 47%, PLA = 42%), Anger (HYD = 100%, PLA = 58%), Confusion (HYD = 38%, PLA = 38%), Fatigue (HYD = 24%, PLA = 33%), Depression (HYD = 62%, PLA = 28%), and Vigour (HYD = 22%, PLA = 7%), Esteem-related effect scores from baseline to 2 hours increased for PLA (4%) and decreased for HYD (9%). The Tension subscale indicates whether the participant feels tense, on-edge, uneasy, nervous and these words may be associated with feeling anxious about the study. This was noted and the subsequent PwP study proposes to include a familiarisation visit.

Reaction time and accuracy

Reaction time

Participants had a faster average reaction time over time when completing an incompatible stimuli task and no stimuli task, there was no difference when completing a compatible stimuli task. When completing a no stimuli task, there was a 13 % reduction in average reaction time from 671 ms at baseline to 584 ms at 2 h and when completing an incompatible stimuli task there was a reduction of 22 % of average reaction time from 803 ms at baseline to 631 ms at 2 h, regardless of whether the participant was inhaling PLA or HYD gas. A faster reaction time demonstrates an increased facilitation of executive function (Wu et al., 2023), and results from the current study are typical compared to reported performance in the literature; performance is slower and more error-prone on incompatible trials than on compatible tasks (Bausenhart et al., 2021; Eriksen & Eriksen, 1974). Exercise improves executive function in younger and older people (with executive function being slower at the baseline than younger people) (Chandran et al., 2023; Benzing et al., 2018) however, in the present study all participants were sedentary for two hours, the faster reaction time could potentially be due to a learning effect. To help mitigate this, there could be a repeat of the Flanker compatibility task (Flanker), however, it was found that each Flanker took around three and half minutes to complete and repeating the task would increase the time spent on the Flanker. With this specific Flanker (Presentation, Neurobehavioural Systems, Inc, California, USA) there is no breakdown given on responses to individual stimuli, or which stimuli preceded a trial. The congruency sequence effect (CSE) is the type of congruency on the prior stimuli influences current trial performance, and as there is no individual breakdown for each stimuli given, the CSE cannot be investigated in the current study. The Inhibition Deficit in Older Age theory (Hasher & Zacks, 1988) suggests that inhibition is impaired with advancing age, therefore participants should have a faster reaction time than PwP (who are generally older).

Accuracy

H₂ inhalation did not affect participants accuracy in the Flanker for any task: incompatible, compatible, or no stimuli despite research suggesting that performance is more accurate with a

compatible task rather than an incompatible task (Stins et al., 2007) which is termed the ‘flanker congruency effect’. There was no improvement over time with either condition.

Manual dexterity

There was a significant main effect of time on participants’ scores, despite each of the PPT tasks being carried out three times (and the mean determined) for each task in accordance with the standard protocol, to mitigate any learning effect. The PPT is a neuropsychological test that measures visuomotor co-ordination and manual dexterity (Desrosiers et al., 1995; Tiffin & Asher, 1948), and controls have previously demonstrated a better performance compared to PwP (Růžicka et al., 2016; Proud et al., 2020) in the PPT.

Norms for PPT scoring for the age group 15-40 years rely on work published in 1986, and adults aged 50 plus rely on work published approximately 30 years ago (Agnew et al., 1988; Desrosiers et al., 1995). As the use of smartphones, gaming and typewriting are different now to what they were over 30 years ago, these norms may vary in different populations. Work has been undertaken to try and update these norms (Lindstrom-Hazel & VanderVlies Veenstra, 2015; Stijic et al., 2023) but new validated and reliable norms have yet to be formally published alongside the PPT. The PPT has been demonstrated to require more cognitive speed and attention control than other dexterity tests (Strauss, 2006) therefore, despite the relatively lengthy administration of the PPT (approx. 25 minutes in the current study due to repeating each test), the PPT may suit a PD population and be able to identify not just impaired manual dexterity, but also impaired cognition.

Limitations

One female took part in the study; however, the study did not control for which phase of the menstrual cycle they were in. Future research in H₂ which includes females who have a menstrual cycle should account for this. This is due to a decline in oestrogen (particularly Oestradiol) impacting mood and cognition (Russell et al., 2019) and effects of H₂ in females may rely on changes – and a complex interplay - in oestrogen, oestrogen receptor beta (Erβ), and brain-derived neurotrophic factor (BNDF) (Hou et al., 2018).

No adverse symptoms and signs were collected formally; however, no participants reported any adverse signs and symptoms, except for one participant who completed the longer inhalation period and found it to be uncomfortable (due to inactivity, not being able to consume food etc). The mean age of the participants in the study was 35 years old, while there was no upper age limit for the study, the study attracted younger participants. Older participants may have different needs, which would be like PwP.

There was no familiarisation visit implemented into the study which may have affected scores, causing a 'learning effect' in the Flanker and the PPT.

The first participant did not want to repeat one arm of the study (this was originally four hours), and their subsequent second arm of the study was two hours; therefore, this participant was not included in the results.

3.6. CONCLUSION

In conclusion, the current study demonstrated that a H₂ gas inhalation is safe and tolerable, and the study design was feasible, however a familiarisation visit would benefit participants in further studies, potentially reducing the learning effect in the Flanker and the PPT. The familiarisation should allow participants to complete a Flanker, a PPT, a POMS-SF – and become accustomed to the vocabulary used in the POMS-SF. A nasal cannula should be placed on the participant and the machine switched on to allow the participant to breathe in air. While the current study showed no significant main effect of condition improving mood, cognition, or manual dexterity in healthy participants, it has demonstrated that this study is potentially feasible in a PwP population.

Randomisation was properly executed, but blinding of the machines was an issue due to the differences in visible 'condensation' in the humidifiers. It was decided that in the next study the humidifiers would be covered, so that participants and the researcher would be blinded to the machine.

Chapter 4. The acute effects of hydrogen gas inhalation in people with Parkinson's

4.1. ABSTRACT

Introduction: Inhaling hydrogen (H₂) has proposed neuroprotective effects in animal models of Parkinson's disease (PD), suggesting potential benefits for people with Parkinson's (PwP). However, the acute effects of inhaling H₂ gas in PwP have not yet been investigated. This study examined whether an acute H₂ gas inhalation period influenced motor and non-motor symptoms, and antioxidant capacity in PwP. **Methods:** A randomised, double blind, placebo controlled, cross-over design was utilised in ten PwP (male = 7, female = 3, age = 69 ± 8 years, age disease onset = 63 ± 10 years, H&Y stage = 1.8 ± 0.92 height = 173.1 ± 9.16 cm, weight = 77.6 ± 10.3 kg, BMI = 25.9). Treatment order was randomised; participants inhaled H₂ gas (HYD: 67% hydrogen, 33% oxygen) or placebo gas (PLA: composition as atmospheric air), for 2 h at two visits, with trials separated by one week. Measures were taken at baseline, as well as at 1 h, and 2 h into the inhalation period. Assessments were blood samples measured for total antioxidant capacity, Profile of Mood States – Short Form (POMS-SF; for mood assessment), Postural Tremor (assessed by MDS-Unified Parkinson's Disease Rating Scale 3.15), the Flanker Compatibility Task (for cognitive function), and the Purdue Pegboard Test (for fingertip dexterity and gross movement of hand, fingers, and arm). **Results:** Two-way repeated measures analysis of variance (ANOVA) revealed a significant effect of time for Flanker Reaction Time 'Compatible' ($p = <0.001$), 'Incompatible' ($p = 0.006$), and 'None' ($p = 0.007$). However, no effects of condition or condition × time interaction were observed for other measures. **Conclusion:** An acute dose of HYD did not appear to affect motor or non-motor symptoms in PwP, however, seemed to increase antioxidant capacity in a small sample of PwP. Repeated cognitive training over a 2 h period improved executive function in PwP, probably due to familiarisation effects rather than a true chronic improvement.

4.2. INTRODUCTION

If Parkinson's disease (PD) were transmissible, it would be labelled a pandemic (Allen 2016, pp.5–9). PD is the second most common neurodegenerative disease in the world, there is no known cure or disease modifying drugs available for PD, and cases globally are expected to more than double in the next 20 years (Dorsey and Bloem 2018, pp.9–10). In the UK, around 153,000 are living with PD, and two people are diagnosed with PD every hour (Parkinson's UK, 2025). It is important that therapeutic applications which aid in the reduction of PD

symptoms are explored, which would ultimately improve health outcomes and quality of life for people with Parkinson's (PwP). Interventions which do not impact other drugs, have side-effects, and are well tolerated would be especially beneficial to those affected by PD.

Hydrogen (H₂) gas is safe for consumption and has shown no adverse effects in animals (Cole *et al.* 2019, pp.133–138) or PwP (Yoritaka *et al.* 2021, pp 4767-4770). Safety standards regarding high concentrations of H₂ used in deep-diving gases to prevent sickness have long been established (Fontanari *et al.* 2000, pp.325–328). However, more research is needed to determine if H₂ improves symptoms of PD and to explore the potential mechanisms of H₂ effects in people with Parkinson's (PwP). Despite ongoing research into H₂, the exact mechanism of action has not yet been fully elucidated, though evidence suggests that H₂ reduces inflammation and oxidative stress (Oshawa *et al.*, 2007).

A challenge in PD research is the lack of biomarkers, which are crucial for tracking disease progression and evaluating responses to therapeutic interventions (Biomarkers Definitions Working Group, 2001). Therefore, biomarkers which are responsible for inflammation, neuroinflammation and, oxidative stress could serve as substitute biomarkers providing insight into the disease such as antioxidant capacity.

PD is a multifaceted, highly heterogeneous disease characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) area of the midbrain, leading to a reduction in dopamine. This loss of dopaminergic neurons represents the end point of PD pathology, and what precedes this cascade of loss is yet to be fully elucidated. Factors such as genetic predisposition, environmental factors, and previous traumatic brain injury increase susceptibility to PD. PD is slightly more prevalent in males than females, and females may respond to treatments differently, with a higher mortality rate, and experience more rapid disease progression (Dahodwala *et al.*, 2018). Tremor dominant PD tends to progress more slowly, but it can be less responsive to Levodopa, potentially leading to Levodopa-induced dyskinesia, which occurs when Levodopa overstimulates dopamine receptors. Deep-brain stimulation may be a surgical treatment for Levodopa-induced dyskinesia.

Translation of the MDS-UPDRS into an acute intervention study may prove difficult as it asks the patient “over the past week have you...”. Mood and motivation can be drastically impacted by PD, categorised into hypodopaminergic symptoms (such as depression, apathy, and anxiety) and hyperdopaminergic (impulsivity) (van der Velden *et al.*, 2018). Therefore,

capturing participant's current mood in acute intervention studies may be beneficial by using different validated questionnaires.

H₂ water has demonstrated beneficial effects on depressive-like behaviour in mice, possibly due to attenuation of interleukin-1 β (IL-1 β) (a pro-inflammatory cytokine and key mediator of neuroinflammation) and reactive oxygen species (ROS) production (Zhang et al., 2016) both implicated in PwP (Soke et al., 2021; Hong et al., 2021).

Intermittent H₂ gas inhalation (inhaling 2% H₂ for 15 min, then room air for 45 min repeated for 12 hours a day) has been shown to be more beneficial - in that it protected 4 out of 6 rats from PD - than prolonged inhalation (continuous administration of 2% H₂ gas) in an animal model of PD (Ito *et al.* 2012, p.15). However, conflicting findings of length of time spent inhaling H₂ make it challenging to determine which is more beneficial: shorter or longer bouts of inhalation. Validated measures should be taken at various times during experiments to determine when benefits occur. As the H₂ machine requires mains electricity, which necessitates sedentary behaviour, it would be beneficial to investigate whether shorter periods of breathing yield similar benefits, allowing participants to remain physically active and to breathe H₂ gas during their 'normal' sitting periods. However, despite the sedentary nature of breathing H₂, it has a larger tissue saturation than H₂ water (Liu et al., 2014), making it a potentially more viable option for PwP who may be in the later stages of disease.

The current study explored the acute effects of breathing H₂ gas (2-3% hydrogen) as previous studies have focused on chronic breathing in PwP (Hirayama et al., 2019; Yoritaka et al., 2021) yielding opposing findings. Results from a previous pilot study (Chapter 3) demonstrated that a 2 h gas breathing was safe, tolerable, and feasible. A familiarisation visit may be beneficial for participants to become accustomed to the study's procedures and tasks.

Promising results have been observed in studies investigating H₂ and PD, but effects are inconsistent, necessitating further evidence to understand the effects on both motor and non-motor symptoms of PD, particularly in humans rather than animal models, as an animal model of PD does not represent the whole picture of PD due to the heterogeneity of the disease, therefore this may give varying results.

It is hypothesised an acute dose of H₂ will positively affect tremor, mood, executive function, manual dexterity and increase total antioxidant capacity in PwP.

4.3. METHODS

4.3.1. Ethical approval

Ethical approval was granted by the University of Kent's School of Sport and Exercise Sciences (SSES) Research Ethics Advisory Group, SSES REAG Number: 26_20_21, and was prospectively registered with the BMC ISRCTN registry, ISRCTN number: 13183500.

Permission to use the International Parkinson and Movement Disorder Society (MDS) 3.15 (postural tremor of the hands) was granted by the MDS Scientific Programs Co-ordinator.

4.3.2. Participants

Inclusion criteria

Male and female participants were included and all had a PD diagnosis by a Neurologist or Geriatrician. All participants were at stage 1-3 on the Hoehn and Yahr (H&Y) scale. Participants had to be able to attend the School of Sport and Exercise Sciences (SSES) Lab and sit for a period of two hours. Participant's medication was not changed and it was required that all participants' PD had been stable over the two months prior to study commencement.

Exclusion criteria

Participants whose PD was not controlled. Participants in stage 4 or 5 of the H&Y scale. Participants who could not consent for themselves.

Participant consent

All participants gave written informed consent and completed a medical history questionnaire before study participation.

Recruitment

Recruitment was carried out through Parkinson's UK (PUK) 'Take part in research', in which PUK send out invitations to research to its members. Posters were placed in public spaces in Canterbury, UK such as community notice boards, and social media posts were created.

4.3.3. Experimental design

Participants attended the SSES Research Laboratory on three occasions. The first visit was a familiarisation visit. All tests were demonstrated to the participants and all participants completed each task once. Any questions that the participant had were answered. Two visits to the Lab were then completed (at a similar time of day for individual participants, due to individual requests from participants feeling 'better' at different points in the day, and thus accommodating this.), approximately one week apart. Participants were randomised to receive H₂ (HYD) or placebo (PLA) gas at their first visit with the breathing period set at two hours. Participants were sat down for the entirety of the visit/assessment and were asked not to consume food or drink anything other than water during the study period. A schematic of the experimental visits can be seen in figure 4.1 (over page).

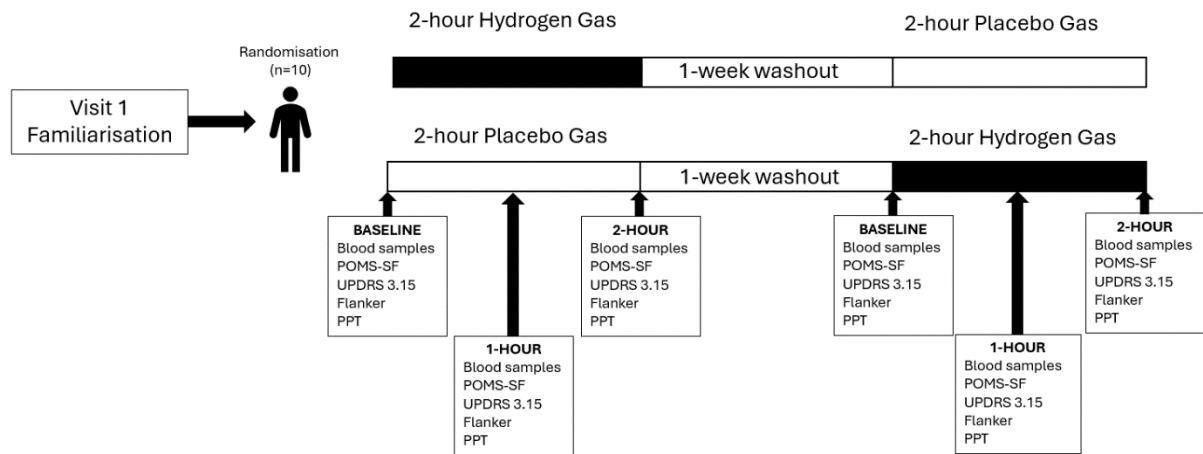


Figure 4.1. Schematic design of the study. Participants attended the SSES Lab for a familiarisation visit, then were randomised into breathing HYD first or PLA first, followed by a one-week washout period, and then inhaled either HYD or PLA for two hours. Measures were taken at baseline, after 1 hour breathing and after 2 hours breathing. POMS-SF = Profile of Mood States – Short Form, UPDRS = Unified Parkinson’s Disease Rating Scale, Flanker = Flanker Compatibility Task, PPT = Purdue Pegboard Test.

4.3.4. Gas machine

Gas

A timer was set when the cannula had been fitted, and machine humidifiers were bubbling. Please see General Methods Chapter 2.5 for further information on the machine including gas concentration and water content.

Nasal cannula

Gas was inhaled through a 210 centimetre (cm) nasal cannula (Osmio Solutions Ltd, Cliffe, Kent, UK), and a new nasal cannula was used for each breathing period and then disposed in clinical waste. Proper nasal cannula placement was observed periodically to ensure that it was in place.

Please see General Methods Chapter 2.5.3. for further information on nasal cannulas including how the nasal cannulas were placed on the participant.

4.3.5. Measures

Measures were taken at baseline, after 1 hour breathing and after 2 hours breathing. Measures were taken in the same order at each timepoint; venous blood samples, POMS-SF, MDS-UPDRS 3.15 Postural Tremor of Hands, Flanker Compatibility Task, Purdue Pegboard Test.

Anthropometric measurements

Height and weight were measured, and BMI calculated at the first visit. Please see General Methods Chapter 2.3. for further information on this.

Blood samples

A 6 ml serum and a 6 ml heparin blood sample were taken by a trained phlebotomist using standard venepuncture procedure for measurement of Total Antioxidant Capacity. Plasma antioxidant capacity was determined by a chemiluminescence assay kit (ABEL Antioxidant Test Kit for Hydroxyl Radicals with Pholasin, Knight Scientific Ltd, Plymouth, UK) in accordance with manufacturer instructions.

Profile of Mood States – Short Form (POMS-SF) (Grove, 1992)

Please see General Methods Chapter 2.7. for further information on this.

Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS 3.15 – Postural Tremor of Hands)

The MDS-UPDRS 3.15 was used to quantify postural tremor of hands. This measured all tremor, including re-emergent rest tremor. Participants were asked to stand and stretch their

arms out in front of their body, with their palms down. Fingers were not touching, and each hand was observed separately for 10 seconds. Tremor was rated at 0 – 4, 0 = normal (no tremor), 1 = slight (tremor is present but less than 1 cm in amplitude), 2 = mild (tremor is at least 1 but less than 3 cm in amplitude), 3 = moderate (tremor is at least 3 but less than 10 cm in amplitude), 4 = severe (tremor is at least 10 cm in amplitude). The highest amplitude per hand was recorded as per protocol.

Flanker Compatibility Task (Presentation, Neurobehavioural Systems, Inc, California, USA)

Please see General Methods Chapter 2.8. for further information on this.

Purdue Pegboard Test (PPT) (Tiffin & Asher, 1948)

Please see General Methods Chapter 2.9. for further information on this.

4.3.6. Statistical analysis

Assumptions of normal distribution of data were checked using the Shapiro-Wilk test (and Z scores for skewness and kurtosis for confirmation). Data which was not normally distributed were transformed prior to analysis to normalise data distribution. The following transformations were attempted, and the method that provided the best normalisation (based on Z-scores) was used to transform these data for analysis: log, square root, cube root, power (x cubed), inverse and arcsine. Log transformation was used. Outliers were assessed by boxplot. Non extreme outliers were identified as not greater than three times the interquartile range from a quartile, while extreme outliers were identified as greater than three times the interquartile range from a quartile (Tukey, 1977). If outliers were identified by box plot, these were both included and excluded in the data analysis. Where the assumption of sphericity was violated (determined by Mauchly's test), the Greenhouse-Geiser adjustment was applied to degrees of freedom. Statistical significance level was set to $P < 0.05$ and analysis was performed with IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA).

4.4. RESULTS

Participant characteristics are presented in Table 4.1

Table 4.1. Participant characteristics (n=10).

Variable	Mean $\pm$ SD	Minimum	Maximum
Age (years)	69 $\pm$ 8	54	80
Sex	Male n=7 Female n=3		
Height (cm)	173.1 $\pm$ 9.16	162	181
Body Mass (kg)	77.6 $\pm$ 10.3	98	57
BMI (kg/m²)	25.83 $\pm$ 2.4	22	31
Age of Disease onset (years)	63 $\pm$ 10	50	78
Disease duration (years)	6 $\pm$ 6	1	13
Levodopa equivalent daily dose (mg)	476 $\pm$ 134	200	600
Hoehn and Yahr stage	1.8 $\pm$ 0.92	1	3

One participant was de novo PD. Data presented as mean $\pm$ standard deviation.

Blood sample collection results are presented in Table 4.2

Table 4.2. Venous blood sample collection

<i>Participant Number</i>	<i>Sex</i>	<i>H&Y Stage</i>	<i>Off gas HYD</i>	<i>Off gas PLA</i>	<i>HYD Baseline</i>	<i>HYD 1-hour</i>	<i>HYD 2-hours</i>	<i>PLA Baseline</i>	<i>PLA 1-hour</i>	<i>PLA 2-hours</i>
1	F	3	0	0						
2	M	1	4 min 20 sec		Y	Y	Y	Y	Y	Y
3	F	1	0	0	Y	Y				
4	M	2	0	8 min	Y	Y	Y	Y	Y	Y
5	M	1	0	0	Y	Y	Y	Y	Y	Y
6	M	1	0	5 min 50 sec						
7	M	3	0	3 min 30 sec						
8	M	1	0	0	Y	Y	Y	Y	Y	Y
9	M	3	0	0	Y			Y	Y	Y
10	F	2	0	0						

A shaded grey square denotes no venous blood sample was collected at that time point. F = female, M = male. **Y** indicates a venous blood sample for that time point was obtained. If venous blood was not collected, this was due to not being able to locate a vein. If the participant was removed from the gas for any reason (toilet break etc) this was timed. Participant 4 was off PLA gas for 8 minutes due to having to evacuate the building because of a fire alarm.

4.4.5 PLASMA TOTAL ANTIOXIDANT CAPACITY

Individual data points are presented for Total Antioxidant Capacity in Figure 4.2

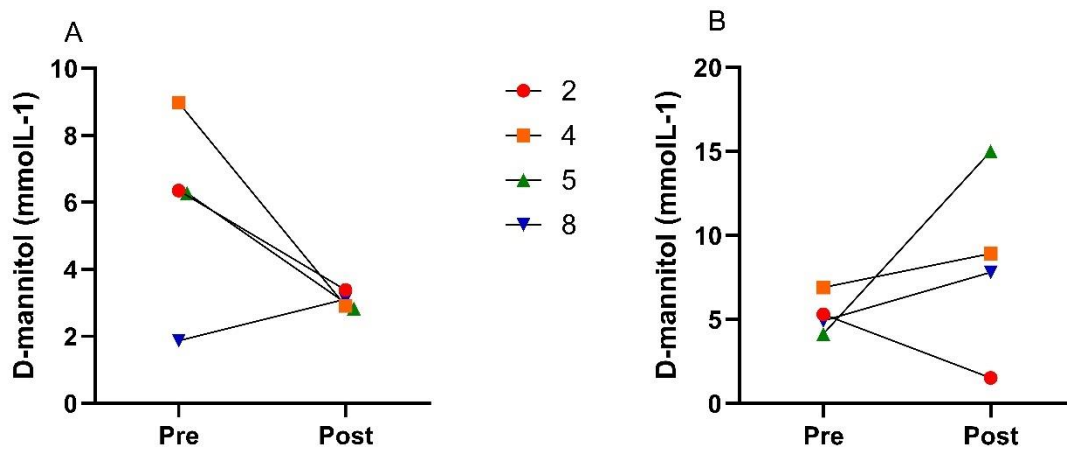


Figure 4.2. Total antioxidant capacity (represented as D-mannitol equivalent units (mmol/L⁻¹) measured in plasma. A = placebo gas, B = hydrogen gas. Numbers 2,4,5,8 refer to participant number.

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on participant's total antioxidant capacity ($F(1,3) = 2.762$, $p = 0.195$, $\eta^2 = 0.479$) or significant main effect of time ($F(1,3) = 0.003$, $p = 0.959$, $\eta^2 = 0.001$). There was no significant condition x time interaction ($F(1,3) = 2.917$, $p = 0.186$, $\eta^2 = 0.493$).

4.4.2. PROFILE OF MOOD STATES – SHORT FORM

Results for the POMS-SF are presented in Table 4.3

Table 4.3. Mood assessed by the Profile of Mood States (Short-Form)

2-way RM ANOVA main effects

<i>Subscale</i>	<i>Condition</i>	<i>Baseline</i>	<i>1-hour</i>	<i>2-hours</i>	<i>p</i> <i>(condition)</i>	<i>p</i> <i>(time)</i>	<i>p</i> <i>(condition</i> <i>x time)</i>
<i>Tension</i>	PLA	2.3±2.7	2.1±2.8	1.3±1.9	0.280	0.052	0.279
	HYD	3.9±4.5	1.7±2.2	2.6±4.9			
<i>Anger</i>	PLA	0.5±1.6	0.9±2.5	0.5±1.6	0.215	0.376	0.702
	HYD	1.2±2.7	1.9±4.2	1.4±3.1			
<i>Fatigue</i>	PLA	2.9±5.9	2.8±5.1	3.1±4.4	0.420	0.995	0.863
	HYD	3.8±5.2	3.8±6.6	3.5±5.8			
<i>Depression</i>	PLA	1.1±2.6	1.2±2.6	0.6±1.3	0.249	0.190	0.580
	HYD	2.3±4.2	1.6±2.9	1.7±4.4			
<i>ERA</i>	PLA	14±4.2	14.2±4.1	13.9±4.6	0.191	0.151	0.440
	HYD	12.8±5.1	14.1±3.7	13.1±4.5			
<i>Vigour</i>	PLA	6±3.2	5.5±2.7	4.6±3.0	0.697	0.173	0.342
	HYD	4.4±3.4	5.8±4.1	5.1±3.3			
<i>Confusion</i>	PLA	2±2	2±2.3	1.7±2.1	0.215	0.376	0.702
	HYD	3.5±4.6	1.9±2.6	3±5.2			
<i>TMD</i>	PLA	88.8±14.3	89.3±14.7	88.7±10.7	0.188	0.669	0.756
	HYD	97.4±23.2	93.7±19.0	96±26			

POMS-SF items are scored 0-4 (0 = not at all, 1 = a little, 2 = moderate, 3 = quite a lot, 4 = extremely.) There are seven subscales. Two items are reversed scores: ashamed and embarrassed (both part of the esteem-related effect (ERA) subscale). Total Mood Disturbance (TMD) is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: $TMD = [Tension + Depression + Anger + Fatigue + Confusion] - [Vigour + ERA]$. Data presented as mean ± standard deviation.

POMS – Tension

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-Tension subscale, ($F(1,9) = 1.319, p = 0.280, \eta^2=0.128$), or significant main effect of time ($F(2,18) = 3.488, p = 0.052, \eta^2=0.279$). There was no significant condition x time interaction ($F(2,18) = 1.371, p = 0.279, \eta^2=0.132$).

POMS – Anger

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-Anger subscale, ($F(1,9) = 1.777, p = 0.215, \eta^2=0.165$), or significant main effect of time ($F(2,18) = 1.033, p = 0.376, \eta^2=0.103$). There was no significant condition x time interaction ($F(1.174, 10.566) = 0.201, p = 0.702, \eta^2=0.022$).

POMS – Fatigue

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-Fatigue subscale, ($F(1,9) = 0.715, p = 0.420, \eta^2=0.074$), or significant main effect of time ($F(2,18) = 0.005, p = 0.995, \eta^2=0.001$). There was no significant condition x time interaction ($F(2,18) = 0.148, p = 0.863, \eta^2=0.016$).

POMS – Depression

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-Depression subscale, ($F(1,9) = 1.518, p = 0.249, \eta^2=0.144$), or significant main effect of time ($F(1.268, 11.410) = 1.955, p = 0.190, \eta^2=0.178$). There was no significant condition x time interaction ($F(1.112, 10.007) = 0.368, p = 0.580, \eta^2=0.039$).

POMS – Esteem-related affect (ERA)

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-ERA subscale, ($F(1,9) = 1.995, p = 0.191, \eta^2=0.181$), or

significant main effect of time ($F(2,18) = 2.105$, $p = 0.151$, $\eta^2=0.190$). There was no significant condition x time interaction ($F(1.258, 11.322) = 0.736$, $p = 0.440$, $\eta^2=0.076$).

POMS – Vigour

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-Vigour subscale, ($F(1,9) = 0.162$, $p = 0.697$, $\eta^2=0.018$), or significant main effect of time ($F(2,18) = 1.936$, $p = 0.173$, $\eta^2=0.177$). There was no significant condition x time interaction ($F(2,18) = 1.138$, $p = 0.342$, $\eta^2=0.112$).

POMS – Confusion

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on POMS-Confusion subscale, ($F(1,9) = 1.777$, $p = 0.215$, $\eta^2=0.165$), or significant main effect of time ($F(2,18) = 1.033$, $p = 0.376$, $\eta^2=0.103$). There was no significant condition x time interaction ($F(1.174, 10.566) = 0.201$, $p = 0.702$, $\eta^2=0.022$).

POMS – Total Mood Disturbance

Log transformed results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the POMS-Total Mood Disturbance subscale, ($F(1,9) = 2.028$, $p = 0.188$, $\eta^2=0.184$), or significant main effect of time ($F(2,18) = 0.411$, $p = 0.669$, $\eta^2=0.044$). There was no significant condition x time interaction ($F(2,18) = 0.284$, $p = 0.756$, $\eta^2=0.031$).

4.4.1. Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS 3.15) – Postural Tremor of Hands

Postural Tremor of Hands - Left hand

Results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participant's postural tremor of left hand, ($F(1,9) = 1.714$, $p = 0.223$, $\eta^2 = 0.160$), or significant main effect of time ($F(2,18) = 1.000$, $p = 0.387$, $\eta^2 = 0.100$). There was no significant condition x time interaction ($F(2,12) = 1.000$, $p = 0.387$, $\eta^2 = 0.100$).

Postural Tremor of Hands -Right hand

No statistics could be carried out on the data due to the nature of the data. At each time point, each participant scored the same score. Please see table 4.4 (over page).

Table 4.4. MDS-UPDRS 3.15 - Postural Tremor of Hands

Results for the MDS-UPDRS 3.15 – Postural Tremor of Hands are presented in Table 4.4.

<i>Participant Number</i>	<i>Sex</i>	<i>Hand</i>	<i>HYD Baseline</i>	<i>HYD 1-hour</i>	<i>HYD 2-hours</i>	<i>PLA Baseline</i>	<i>PLA 1-hour</i>	<i>PLA 2-hours</i>
1	F	Right	2	2	2	1	1	1
		Left	1	1	1	1	1	0
2	M	Right	2	2	2	2	2	2
		Left	1	1	1	1	1	1
3	F	Right	0	0	0	0	0	0
		Left	1	1	1	1	1	1
4	M	Right	1	1	1	1	1	1
		Left	2	2	2	1	1	1
5	M	Right	1	1	1	1	1	1
		Left	1	1	1	1	1	1
6	M	Right	0	0	0	0	0	0
		Left	1	1	1	1	1	1
7	M	Right	0	0	0	0	0	0
		Left	0	0	0	0	0	0
8	M	Right	0	0	0	0	0	0
		Left	0	0	0	0	0	0
9	M	Right	1	1	1	1	1	1
		Left	1	1	1	1	1	1
10	F	Right	0	0	0	0	0	0
		Left	1	1	1	1	1	1

Results show MDS-UPDRS 3.15 postural tremor scores for both hands for all participants at each time point. Fingers were not touching, and each hand was observed separately for 10 seconds. Ratings are 0 – 4, 0 = normal (no tremor), 1 = slight, 2 = mild, 3 = moderate, 4 = severe. The highest amplitude per hand was recorded. No participant had a moderate or severe tremor.

Table 4.5. MDS-UPDRS 3.15 - Postural Tremor of Hands group mean

Results for the MDS-UPDRS 3.15 are presented in Table 4.5

<i>Hand</i>	<i>Condition</i>	<i>Baseline</i>	<i>1-hour</i>	<i>2-hour</i>
<i>Left</i>	PLA	0.9±0.57	0.9±0.57	0.9±0.57
	HYD	0.8±0.42	0.8±0.42	0.7±0.48
<i>Right</i>	PLA	0.7±0.82	0.7±0.82	0.7±0.82
	HYD	0.6±0.7	0.6±0.7	0.6±0.7

Results show MDS-UPDRS 3.15 postural tremor scores for both hands for all participants at each time point. Fingers were not touching, and each hand was observed separately for 10 seconds. Ratings are 0 – 4, 0 = normal (no tremor), 1 = slight, 2 = mild, 3 = moderate, 4 = severe. The highest amplitude per hand was recorded. No participant had a moderate or severe tremor. Data presented as mean ± standard deviation.

4.4.3. FLANKER COMPATIBILITY TASK

Results are presented for the Flanker Compatibility Task Accuracy in Table 4.6

Flanker - Accuracy

Table 4.6. Flanker Compatibility Task Accuracy measurements

2-way RM ANOVA main effects

<i>Accuracy Subscale</i>	<i>Condition</i>	<i>Baseline</i>	<i>1-hour</i>	<i>2-hour</i>	<i>p (condition)</i>	<i>p (time)</i>	<i>p (condition x time)</i>
<i>Compatible</i>	PLA	0.99±0.02	0.95±0.06	0.98±0.02	0.351	0.168	0.151
	HYD	0.97±0.05	0.95±0.07	1±0			
<i>Incompatible</i>	PLA	0.94±0.07	0.98±0.04	0.96±0.06	0.923	0.923	0.158
	HYD	0.96±0.07	0.93±0.07	0.94±0.07			
<i>None</i>	PLA	0.98±0.01	0.96±0.05	0.97±0.04	0.585	0.136	0.833
	HYD	0.96±0.03	0.96±0.06	0.98±0.02			

Results show accuracy responses to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli), with a maximum score of 1 (with extreme outliers removed). Data presented as mean ± standard deviation.

Accuracy - Compatible

Two outliers in the data were identified by box plot, both outliers were removed as they were considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for Accuracy - Compatible, the first set of results include extreme outliers in the data analysis, the second set of results exclude extreme outliers.

Outliers included:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on participants' Accuracy - Compatible, ($F(1,9) = 0.197, p = 0.668, \eta^2 = 0.021$), or significant main effect of time ($F(2,18) = 1.693, p = 0.212, \eta^2 = 0.158$). There was no significant condition x time interaction ($F(2,18) = 0.325, p = 0.727, \eta^2 = 0.035$).

Outliers excluded:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on participants' Accuracy - Compatible, ($F(1,7) = 1.000, p = 0.351, \eta^2 = 0.125$), or significant main effect of time ($F(2,14) = 2.032, p = 0.168, \eta^2 = 0.225$). There was no significant condition x time interaction ($F(2,14) = 2.174, p = 0.151, \eta^2 = 0.237$).

Accuracy - Incompatible

One outlier in the data was identified by box plot, this was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile).

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on participants' Accuracy - Incompatible, ($F(1,9) = 1.054, p = 0.331, \eta^2 = 0.105$), or significant main effect of time ($F(2,18) = 0.080, p = 0.923, \eta^2 = 0.009$). There was no significant condition x time interaction ($F(2,18) = 2.049, p = 0.158, \eta^2 = 0.185$).

Accuracy - None

Three outliers in the data were identified by boxplot. One outlier was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range

from a quartile), while two outliers were removed as they were considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for Accuracy - None, the first set of results include the extreme outliers in the data analysis, the second set of results exclude the extreme outliers.

Outliers included:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on participants' Accuracy - None, ($F(1,9) = 0.000$, $p = 0.994$, $\eta^2 = 0.000$), or significant main effect of time ($F(2,18) = 0.325$, $p = 0.727$, $\eta^2 = 0.035$). There was no significant condition x time interaction ($F(2,18) = 0.100$, $p = 0.905$, $\eta^2 = 0.011$).

Outliers excluded:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on participants' Accuracy - None, ($F(1,7) = 0.327$, $p = 0.585$, $\eta^2 = 0.045$), or significant main effect of time ($F(2,14) = 2.307$, $p = 0.136$, $\eta^2 = 0.248$). There was no significant condition x time interaction ($F(2,14) = 0.185$, $p = 0.833$, $\eta^2 = 0.026$).

Flanker - Reaction Time

Results for the Flanker Compatibility Task Reaction Time are presented in Table 4.7

Table 4.7. Flanker Compatibility Task Reaction Time measurements

2-way RM ANOVA main effects

<i>Reaction Time Subscale</i>	<i>Condition</i>	<i>Baseline</i>	<i>1-hour</i>	<i>2-hour</i>	<i>p (condition)</i>	<i>p (time)</i>	<i>p (condition x time)</i>
<i>Compatible</i>	PLA	860.04±209.4	713.25±165.63	714.26±165.63	0.308	<0.001*	0.328
	HYD	864.1±318.02	781.42±188.25	734.74±156.40			
<i>Incompatible</i>	PLA	932.14±215.8	760.63±56.34	795.49±169.63	0.227	0.006*	0.863
	HYD	972.07±258.6	824.84±173.09	791.10±107.24			
<i>None</i>	PLA	805.40±212.7	675.67±117.98	677.79±126.94	0.066	0.007*	0.200
	HYD	785.92±211.6	757.10±191.69	707.23±132.9			

Results show reaction time responses to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli), in milliseconds (with extreme outliers removed). *Significant main effect of time. Data presented as mean ± standard deviation.

Results for the Flanker Reaction Time are presented in Table 4.8

Table 4.8. Percentage reduction in average reaction time for HYD and PLA combined (outliers excluded).

<i>Flanker Reaction Time</i>	<i>Baseline (ms)</i>	<i>1 h (ms)</i>	<i>2 h (ms)</i>	<i>% reduction from Baseline to 2 h</i>
Compatible	862	747	724	17
Incompatible	904	758	763	14
None	796	716	693	13

Reaction time responses to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli), in milliseconds (with extreme outliers removed).

Reaction Time - Compatible

Three outliers in the data were identified by box plot. Two outliers were included in the data analysis as they were not considered extreme (not greater than three times the interquartile range from a quartile), while one outlier was removed as it was considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for Reaction Time - Compatible, the first set of results include the extreme outlier in the data analysis, the second set of results exclude the extreme outlier.

Outlier included:

Log transformed results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on Reaction Time Compatible ($F(1,9) = 0.104$, $p = 0.754$, $\eta^2 = 0.011$) or significant condition $\times$ time interaction ($F(2,18) = 0.666$, $p = 0.526$, $\eta^2 = 0.069$). There was a significant main effect of time ($F(2,18) = 5.500$, $p = 0.014$, $\eta^2 = 0.379$), with values at 1 h (785 ms) significantly faster ($p = 0.049$) than baseline (877 ms).

Outlier excluded:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on Reaction Time Compatible ($F(1,8) = 1.185, p = 0.308, \eta^2 = 0.129$) or significant condition x time interaction ($F(2,16) = 1.197, p = 0.328, \eta^2 = 0.130$). There was a significant main effect of time ($F(2,16) = 12.853, p < 0.001, \eta^2 = 0.616$), with values at 1 h (747 ms) significantly faster ($p = 0.009$) than baseline (862 ms) and values at 2 h (724 ms) significantly faster ($p = 0.007$) than at 1 hr (747 ms).

Reaction Time - Incompatible

Two outliers in the data were identified by box plot. Both outliers were removed as they were considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for Reaction Time - Incompatible, the first set of results include the extreme outliers in the data analysis, the second set of results exclude the extreme outliers.

Outliers included:

Log transformed results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on Reaction Time Compatible ($F(1,9) = 0.282, p = 0.608, \eta^2 = 0.030$) or significant condition x time interaction ($F(1.225, 11.025) = 0.280, p = 0.654, \eta^2 = 0.030$). There was a significant main effect of time ($F(2,18) = 7.068, p = 0.005, \eta^2 = 0.440$), with values at 2 h (815 ms) significantly faster ($p = 0.028$) than baseline (942 ms).

Outliers excluded:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on Reaction Time Compatible ($F(1,7) = 1.753, p = 0.227, \eta^2 = 0.200$) or significant condition x time interaction ($F(2,14) = 0.149, p = 0.863, \eta^2 = 0.021$). There was a significant main effect of time ($F(2,14) = 7.669, p = 0.006, \eta^2 = 0.523$), with values at 2 h (763 ms) significantly faster ($p = 0.015$) than baseline (904 ms).

Reaction Time – None

Three outliers in the data were identified by box plot. Two outliers were included in the data analysis as they were not considered extreme (not greater than three times the interquartile range from a quartile), while one outlier was removed as it was considered extreme (greater than three times the interquartile range from a quartile). Two sets of results are reported for Reaction Time - None, the first set of results include the extreme outlier in the data analysis, the second set of results exclude the extreme outlier.

Outlier included:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on Reaction Time none ($F(1,9) = 3.764$, $p = 0.084$, $\eta^2 = 0.295$) or significant condition x time interaction ($F(2,18) = 2.133$, $p = 0.147$, $\eta^2 = 0.192$). There was a significant main effect of time ($F(2,18) = 4.601$, $p = 0.024$, $\eta^2 = 0.338$), however, post hoc analysis with Bonferroni correction did not identify any differences between individual time points.

Outlier excluded:

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on Reaction Time none ($F(1,8) = 4.538$, $p = 0.066$, $\eta^2 = 0.362$) or significant condition x time interaction ($F(2,16) = 1.781$, $p = 0.200$, $\eta^2 = 0.182$). There was a significant main effect of time ($F(2,16) = 6.904$, $p = 0.007$, $\eta^2 = 0.463$), with values at 2 h (693 ms) significantly faster ($p = 0.025$) than baseline (796 ms).

4.4.4. PURDUE PEGBOARD TEST

Results for the Purdue Pegboard Test scores are presented in Figure 4.3

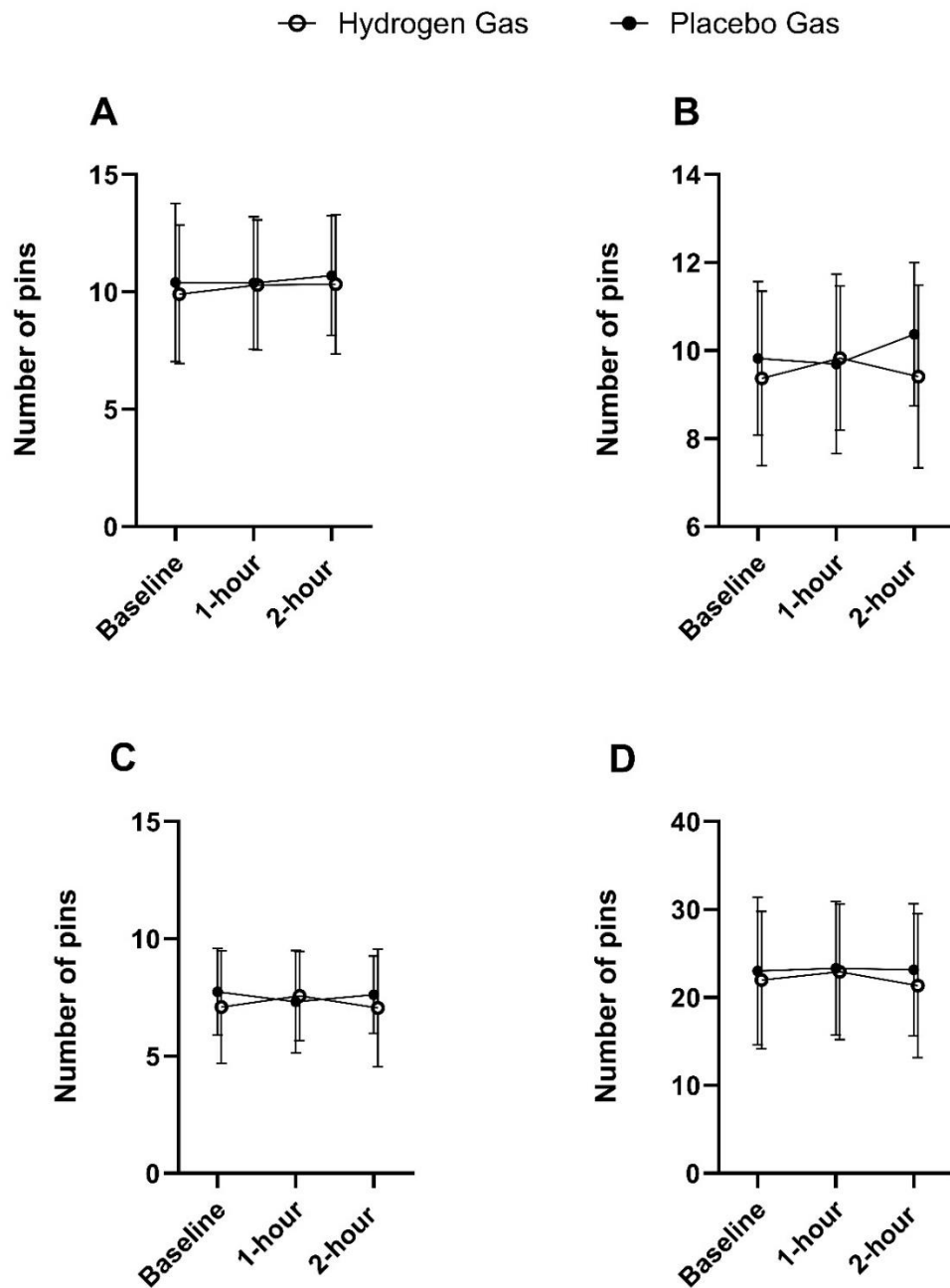


Figure 4.3. Purdue Pegboard Test scores. Right, Left and Both Hands were completed over 30 seconds, assembly was completed over 60 seconds. Each task was completed three times, and mean measures of pins were taken, as per standard protocol. A = Right hand. B = Left hand. C = Both hands. D = Assembly. Data presented as mean $\pm$ standard deviation.

PPT Right Hand

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the PPT Right Hand task ($F(1,9) = 0.536$, $p = 0.483$, $\eta^2 = 0.056$) or significant main effect of time ($F(2,18) = 0.732$, $p = 0.495$, $\eta^2 = 0.075$). There was no significant condition x time interaction ($F(2,18) = 0.241$, $p = 0.788$, $\eta^2 = 0.026$).

PPT Left Hand

One outlier in the data was identified by box plot, this was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile).

Log transformed results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the PPT Left Hand task ($F(1,9) = 1.756$, $p = 0.218$, $\eta^2 = 0.163$) or significant main effect of time ($F(2,18) = 1.422$, $p = 0.267$, $\eta^2 = 0.136$). There was no significant condition x time interaction ($F(2,18) = 2.120$, $p = 0.149$, $\eta^2 = 0.191$).

PPT Both Hands

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the PPT Both Hands task ($F(1,9) = 1.711$, $p = 0.223$, $\eta^2 = 0.160$) or significant main effect of time ($F(2,18) = 0.120$, $p = 0.887$, $\eta^2 = 0.013$). There was no significant condition x time interaction ($F(2,18) = 3.524$, $p = 0.051$, $\eta^2 = 0.281$).

PPT Right + Left + Both

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the total of PPT right + left + both ($F(1,9) = 1.914$, $p = 0.200$, $\eta^2 = 0.175$) or significant main effect of time ($F(2,18) = 0.687$, $p = 0.516$, $\eta^2 = 0.071$). There was no significant condition x time interaction ($F(2,18) = 1.972$, $p = 0.168$, $\eta^2 = 0.180$).

PPT Assembly

Results of the two-way repeated measures ANOVA revealed there was no significant main effect of condition on the PPT Assembly task ($F(1,9) = 1.884$, $p = 0.203$, $\eta^2 = 0.173$) or significant main effect of time ($F(2,18) = 2.782$, $p = 0.089$, $\eta^2 = 0.236$). There was no significant condition x time interaction ($F(2,18) = 0.443$, $p = 0.649$, $\eta^2 = 0.047$).

4.5. DISCUSSION

A 2 h inhalation of H_2 gas did not yield improvements in mood, tremor, cognition or manual dexterity, and total antioxidant capacity was altered among PwP. This is the first known study to investigate the effects of an acute H_2 intervention in PwP. The study included a familiarisation visit which enabled participants to firstly find the Lab (which some participants struggled with on their first visit) and be introduced to the researcher and facilities.

Total antioxidant capacity

HYD did not have a significant effect on participants' total antioxidant capacity (TAC), however, only four participants' samples were analysed for TAC and a large effect was observed ($\eta^2 = 0.479$). HYD increased TAC from baseline by 56 % to post 2-hour breathing. While no significant effect was found, potentially due to the low sample size (from not being able to draw blood from participants), there are large effect sizes for both condition ($\eta^2 = 0.479$) and interaction ($\eta^2 = 0.493$). This data is preliminary in nature, and further research is needed with a larger sample size to confirm that there is an increase in TAC with HYD. Concentration of H_2 increases over the first 30 min of breathing and then maintains the same level of concentration until cessation of breathing (Liu et al., 2014), where it dissipates. Future studies could use more frequent blood sampling, by cannula rather than repeated venepuncture, as this would be more comfortable for the participant. As there is a two-hour gap between the baseline sample and the post sample, this would enable researchers to measure antioxidant capacity more frequently. Previous research in H_2 gas and PwP have used different protocols over different time periods (10 min twice daily and 1 hr twice daily) as dose effect of H_2 remains unknown. If TAC was increased with H_2 , this would suggest that potentially, there could be a slowing down of progression of the disease. Measures of markers

of oxidative stress could have given a more detailed understanding of whether an increase in TAC would reduce oxidative stress.

Mood

HYD did not produce beneficial effects in mood among PwP. No significant main effects were observed for any POMS subscale or POMS Total Mood Disturbance (TMD). However, the POMS-SF was used to evaluate the participant's baseline mood state, and a familiarisation visit should have meant that they were in a more reliable baseline state, which then allows better comparison of mood on PLA and HYD, and to observe if mood affected this. Similarly, no significant main effect of time was found. A familiarisation visit may have contributed to reduced tension among participants, as they became familiar with the testing site, met the researcher, and became accustomed to the breathing machines and battery of tests. Notably, the POMS-SF questionnaire was administered after a blood sample had been collected to reduce participant's anxiety. The POMS-SF questionnaire, despite its reduced number of questions (40), is commonly used in intervention studies and has demonstrated reliability and validity. A recent study (Sistarelli et al., 2023) demonstrated immediate mood improvement after a dance class in PwP using the original 65 question POMS scale, though mood reverted to baseline after 24 hours. Given that HYD gas is considered a non-pharmacological intervention, the lack of mood improvement after two hours suggests a potential cumulative effect on mood following prolonged H₂ consumption. A 2013 study examining the efficacy of H₂ water in PwP demonstrated improved total Unified Parkinson's Disease Rating Scale (UPDRS) scores after 48 weeks of H₂ water consumption (Yoritaka et al., 2013). However, UPDRS Part 1 scores (Non-Motor Aspects of Experiences of Daily Living - nM-EDL) which include depressive mood, anxious mood, and apathy were not reported. Despite using a parallel-group design with nine participants consuming H₂ water (similar to the current study's participant count), this study did not report specific mood-related outcomes.

Postural Tremor

HYD breathing did not mitigate postural tremor in participants' left-hand, with right-hand scores remaining unchanged throughout the study. Two participants exhibited no tremor in either hand at both visits, five had bilateral tremor and three had unilateral tremor. One participant had undergone deep-brain stimulation (DBS), and consistently scored '0' for both

hands at all time points. However, it was noted that if the DBS should ‘switch off’ (sometimes it malfunctioned) then the participant’s tremor would return immediately, and instructions were provided on how to reactivate the DBS. Results from MDS-UPDRS Part 3 were consistent across three studies involving PwP, showing no significant score changes. Findings from the current study align with those of other H₂ and PwP studies (Yoritaka et al., 2013; Yoritaka et al., 2018; Yoritaka et al., 2021) (two on H₂ water and one on H₂ gas), though the current study specifically focussed on postural tremor rather than the entire UPDRS part 3. The largest study (Yoritaka et al., 2018) involving 178 participants, reported no significant difference in UPDRS Part 3 when drinking 1000ml of water for 72 weeks. Thus, an acute intervention of H₂ did not alleviate postural tremor in PwP, however, no participants had a moderate or severe tremor and if participants had a more severe tremor, this may have been better in observing effects.

Flanker Compatibility Task

Participants demonstrated faster reaction times across all Flanker tasks (compatible, incompatible, none) over time, irrespective of intervention. For compatible tasks, average reaction time reduced by 17% at baseline to 2 h. Similarly, for incompatible tasks, average reaction time reduced by 14% at baseline to 2 h, and for no stimuli tasks, there was a 13 % reduction from baseline to 2 h. A familiarisation visit was required for the study, to help eliminate any learning effect from cognitive tests, however, there may have been a learning effect over the two-hour period. A faster reaction time indicates enhanced executive function (Wu et al., 2023), with dopamine playing a crucial role in this process (Monchi et al., 2004). The ‘dopamine overdose’ hypothesis (Gotham et al., 1988) suggests preserved ventral striatum can be overstimulated with dopamine replacement (e.g. Levodopa), leading to limbic system overstimulation, impacting learning and risk taking. A recent study demonstrated that a cognitive training programme reduced freezing of gait (Walton et al., 2018), and another study showed that better Flanker task performance predicted improved mobility tests performance (Gothe et al., 2014). In future studies freezing of gait could be measured using the timed up and go test. While the current study assessed the impact of H₂ in PwP, repeated Flanker tasks may effectively function as cognitive training, explaining the observed faster reaction times irrespective of intervention.

Purdue Pegboard Test

HYD did not enhance scores on the Purdue Pegboard Test (PPT) (Tiffin & Asher, 1948), nor did PPT scores improve over time despite intervention. However, both hands together were found to be close to interaction ($p=0.051$) with a large effect size ($\eta^2=0.281$). Both hands together evaluate bilateral hand tasks (which can reflect impaired activities of daily living such as shoelace tying, doing buttons up). As with TAC, a large effect size suggest that participants performed better on both hands together task, and a larger sample size is needed to confirm this. Approximately 90% of PwP exhibit impaired manual dexterity (Raggi et al., 2011) with no established ‘gold standard’ for assessing manual dexterity in PwP (Proud et al., 2013). PwP typically perform poorer on the PPT compared to healthy controls (Proud et al., 2020), with the PPT being the only mechanical test showing significant differentiation between PwP and controls (Růžička et al., 2016). Although the PPT measures manual dexterity and visuomotor co-ordination (Desrosiers et al., 1995), a potential link exists between PPT performance and cognition (Hinkle & Pontone, 2021). Given the high heterogeneity of PD, further exploration of PPT score in relation to cognition may reveal domain-specific cognitive changes. Recently, PPT performance was predicted using baseline free-water (FW) imaging measures from the basal ganglia for PD (Wilkes et al., 2023) and FW imaging demonstrating reliability and sensitivity in tracking PD progression (Ofori et al., 2015), especially in prodromal and early clinical stages compared to later stages of the disease (Zhou et al., 2023). As the PPT is objective, reliable, non-invasive, and cost effective, it may offer a means to track disease progression and evaluate intervention/treatment outcomes without the need for costly FW imaging.

As with the POMS-SF and Flanker, during the familiarisation visit, participants completed the full battery of PPT Tests (right hand, left hand, both hands, and assembly). This standardisation allowed participants to become familiar with the pins, the researcher’s instructions, and timings of each test. All PPT tests were administered by a single researcher to ensure consistency.

Limitations

The study’s relatively small sample size, potentially hindered generalisation of the results. Larger studies investigating the acute effects of H₂ in PwP might yield different outcomes. TAC in plasma sample size was even smaller ($n=4$) due to difficulty in locating veins.

Samples were only taken at baseline, after one hour and two hours of inhalation, this was to reduce blood draws to three times, to help reduce discomfort from repeated venepuncture. A cannula could have been placed to draw blood more often and thereby help identify when antioxidant capacity started to increase.

It's worth noting that no symptomology data were collected in this study. Given the heterogeneous nature of PD, symptom grouping could have provided valuable insights, especially with a larger participant pool. Additionally, the collection of non-motor symptoms alongside motor symptoms would have offered a more comprehensive understanding of the disease's impact.

4.6. CONCLUSION

In conclusion, acute H₂ gas appeared to increase TAC, however, did not ameliorate tremor, mood, executive function, or manual dexterity in PwP. These findings are preliminary. Repeated cognitive training over a short period improved executive function, despite intervention. Repeated cognitive training can be beneficial in a PD cohort, given that many PwP incur impairment of cognitive function, although this may be a learning effect rather than a chronic change.

Chapter 5. The chronic effects of hydrogen gas inhalation in people with Parkinson's – a case study

5.1. ABSTRACT

Introduction: The purpose of this study was to examine if hydrogen gas (HYD) inhalation affected motor symptoms, non-motor symptoms and antioxidant capacity in a person with Parkinson's (PwP). **Methods:** The study was a single-blind ABAB design in one PwP (age = 78, male, age disease onset = 75, H&Y stage 2, height = 194 cm, weight = 117 kg, BMI = 31.1). The participant inhaled placebo gas (PLA: composition as atmospheric air) daily for 2 weeks, followed by 2 weeks of inhaling hydrogen gas (HYD: 67% hydrogen, 33% oxygen), then 2 weeks of inhaling PLA), followed by a further 2 weeks of inhaling HYD (defined as PLA1, HRW1, PLA2, HRW2 in the study). Total inhalation period was 8 weeks; with 1 h inhaling each day (30 min in the morning and 30 min the evening) during the participant's 'normal' sitting time. Measures were: total antioxidant capacity (TAC) in plasma, blood pressure, Profile of Mood States – Short Form (POMS-SF) (to assess mood), Flanker Compatibility Task (to assess cognitive function), Purdue Pegboard Test (PPT) (to assess fingertip dexterity and gross movement of hand, fingers, and arm), Movement Disorder Society – Non-Motor Rating Scale (measures frequency and severity of 13 non-motor domains), Timed Up and Go Test (TUG) (to assess mobility) and the six-minute walk test (6MWT) (to assess walking capacity). The participant also wore a triaxial accelerometer (GENEActiv, Activinsights Ltd, Kimbolton, UK) throughout the study to record step count, non-wear, sleep, sedentary time, and physical activity/exercise. **Results:** HYD had no reported side effects, reduced systolic blood pressure (9% at its highest reduction from 142 mmHg in PLA1 to 130 mmHg in HYD2), increased walking distance by 12% (from 309 m in PLA1 to 345 m in HYD2), and increased antioxidant capacity (by 19% (in HYD1) and 16% (in HYD2)), no other measures were affected. **Conclusion:** HYD seemed to increase total antioxidant capacity and walking distance, although the participant's physical activity levels were similar in PLA and HYD. There was lower protocol compliance to HYD inhalation in the later stages of the study but this still elevated TAC.

5.2. INTRODUCTION

The imperative need to slow down the progression of Parkinson's disease (PD) is a paramount challenge. The pursuit of therapeutic interventions which help alleviate PD symptoms, or even slow down the progression of PD, are needed as there is currently no cure. Hydrogen (H₂) has demonstrated promising results in animal models of PD; however, there is limited research in people with Parkinson's (PwP). PD animal models offer an insight in how interventions can

impact disease, and even prevent PD from progressing, however, H₂ and PD research has relied on 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6 – tetrahydropyridine (MPTP) animal models of PD - which are neurotoxin-induced models – but both models lack Lewy Bodies formation and 6-OHDA lacks blood-brain penetration, therefore intervention studies in a PD animal model may yield different results to humans. A H₂ gas and PwP study (Yoritaka et al., 2021) found lack of compliance to study protocol, with participants self-assessment inhaling time (182.8 ± 48.8 h) reported higher than seen on the machine (162.5 ± 63.4 h). This study excluded five participants due to inhaling less than 50% of the total time, participants were required to use the machines 1 h, twice a day for 16 weeks, and compliance decreased with elderly participants and higher dose of Levodopa. While the study reported lack of compliance, there were no adverse effects reported. Reporting lack of compliance to the intervention through a reliable measure (log time on the machines) allows other researchers to interpret findings and to understand that compliance to this protocol may prove difficult in PwP. The first study in H₂ and PwP (Hirayama et al., 2018) reported full compliance but lacked information and detail on how this was determined. The study found that H₂ gas increased urinary 8—hydroxy-2'-deoxyguanine (8-OHdG) (an oxidative stress marker) but had no effect on olfactory function, non-motor symptoms or activities of daily living (ADL). However, a previous study reported that drinking H₂ rich water reduced 8-OHdG (Ishibashi et al., 2012) in people with Rheumatoid Arthritis (RA), a chronic inflammatory condition, in which elevated 8-OHdG levels have been reported, while different conditions, both share similarities of increased inflammation and oxidative stress.

Adherence to oral intake of medication is known to be sub-optimal in PwP (Malek & Grosset, 2015), which can in turn increase PD symptoms, affect quality of life (QoL), impact carers, and may lead to medic teams concluding that PD medication is not working optimally. Adherence and compliance are often interchanged within literature; however, adherence refers to a patient matching recommendations by a clinician (Barofsky, 1978) and compliance is the extent to which a patient complies with prescriber's recommendations (Haynes, 1979). A person can 'over comply' with recommendations, especially if they feel the substance they are ingesting is beneficial to them, which could in some cases lead to toxicity. H₂ has been reported to be safe even at high level concentrations (Fontanari et al., 2000), and if a person was to over comply with H₂ consumption, it should not cause toxicity.

H₂ has been shown to have an anti-hypertensive effect in an animal model (Sugai et al., 2020) and hypertension, hypotension, and supine hypertension are due to autonomic dysfunction,

which can be affected in PwP. It was previously thought that PwP were at low risk of cardiovascular diseases (Scigliano et al., 2006), however recent evidence has shown that cardiovascular dysfunction such as orthostatic hypotension and supine hypertension (Palma & Kaufmann, 2018) can be a part of clinical autonomic dysfunction in PwP which is due to norepinephrine neuronal dysfunction and can often precede motor symptoms of PD (Kaufmann et al., 2017). PD is affiliated with a reduction of catecholamine norepinephrine in the myocardium; however, hypertension may occur independently of PD, and hypertension risk factors include ageing, obesity, and sedentary behaviour (Leszczak et al., 2024), all associated with PD experience.

Atrial fibrillation (AF) is a heart arrhythmia, characterised by irregular and rapid heart rhythm. Ageing is the primary risk factor in AF, with hypertension and obesity also being acknowledged as predisposing risk factors. A large study in Korea (Han et al., 2021) which included 57,585 PwP found that PD was associated with a higher risk of AF, even after risk factors such as age, sex and socioeconomic status were adjusted for. Biological sex is especially important with regards to cardiovascular disease (CVD) as post-menopausal females have more CVD complications compared to males of the same age (Hodis and Mack 2022), however, this study only had 40% male participants, so it may not be reflective of the disease as there is approximately a 1.4:1 male to female ratio of PD rates (Parkinson's UK, 2025). Also, as age was adjusted for, the younger participants of the study may have been diagnosed with young-onset PD (YOPD), and rare gene variants have been identified in Korean patients diagnosed with YOPD (Youn 2019) which may help with generalisation to PwP despite geographical variation.

Accelerometry has been demonstrated to be a powerful indicator in predicting PD development (Schalkamp et al., 2023; Williamson et al., 2021) and no known research has used accelerometry to assess H₂ and the impact on sleep, physical activity, and exercise in a PD population. PwP often suffer with impaired sleep, marred with nocturia, which in turn causes excessive daytime sleepiness and fatigue. H₂ has been reported to alleviate sleep disturbances and fatigue in a small multiple sclerosis (MS)/chronic fatigue syndrome cohort (Hirano et al., 2024). However, the potential underlying mechanism was not elucidated in this study, and while MS is a different disease entity and requires very different treatment to PD, both are neurodegenerative and manifest motor symptoms. Limitations of the study included no placebo was used, and participants inhaled H₂ gas for lengthy periods of time during a day (3-6 hours per day). Increasing sedentary behaviour is not ideal given benefits of physical activity and exercise, on

both neurological and physiological health, therefore there needs to be careful and considerate balance of inhaling H₂ to not elevate sedentary behaviour. A small number of participants were included in the study (n=4) and all four participants completed self- assessments. With a reduction in fatigue, this would affect physical activity, and objective measures of PA would be beneficial to investigate health enhancing interventions. An ABAB design is single-subject experimental design (SSED) and is one of the most direct and strongest “treatment effect demonstration” strategies (Byiers et al., 2012). Randomised controlled trials (RCTs) are often reported as ‘gold standard’ in study design, however, an ABAB design can demonstrate real-world effectiveness of an intervention, especially if there are resource limitations. Therefore, results from an ABAB design may be fed into a larger scale study. However, if there are no measurement changes after an intervention, due to the small number of participants, it may be hard to ascertain the reason why there was no effect.

This study incorporated an ABAB design to ascertain if H₂ gas is beneficial in a person with Parkinson’s. Various physiological and neurological measures were taken to determine if H₂ impacts non-motor and/or motor symptoms of PD, and total antioxidant capacity was measured in plasma.

5.3. METHODS

5.3.1. Ethical approval

The study was granted ethical approval by the University of Kent’s School of Sport and Exercise Sciences (SSES) Research Ethics Advisory Group, SSES REAG Number: 21_20_23, and was prospectively registered with the BMC ISRCTN registry, ISRCTN number: 46799043.

Permission to use the International Parkinson and Movement Disorder Society (MDS) Non-Motor Rating Scale was granted by the MDS Scientific Programs Co-Ordinator.

5.3.2. Participants

Figure 5.1 shows the CONSORT flow diagram of study recruitment

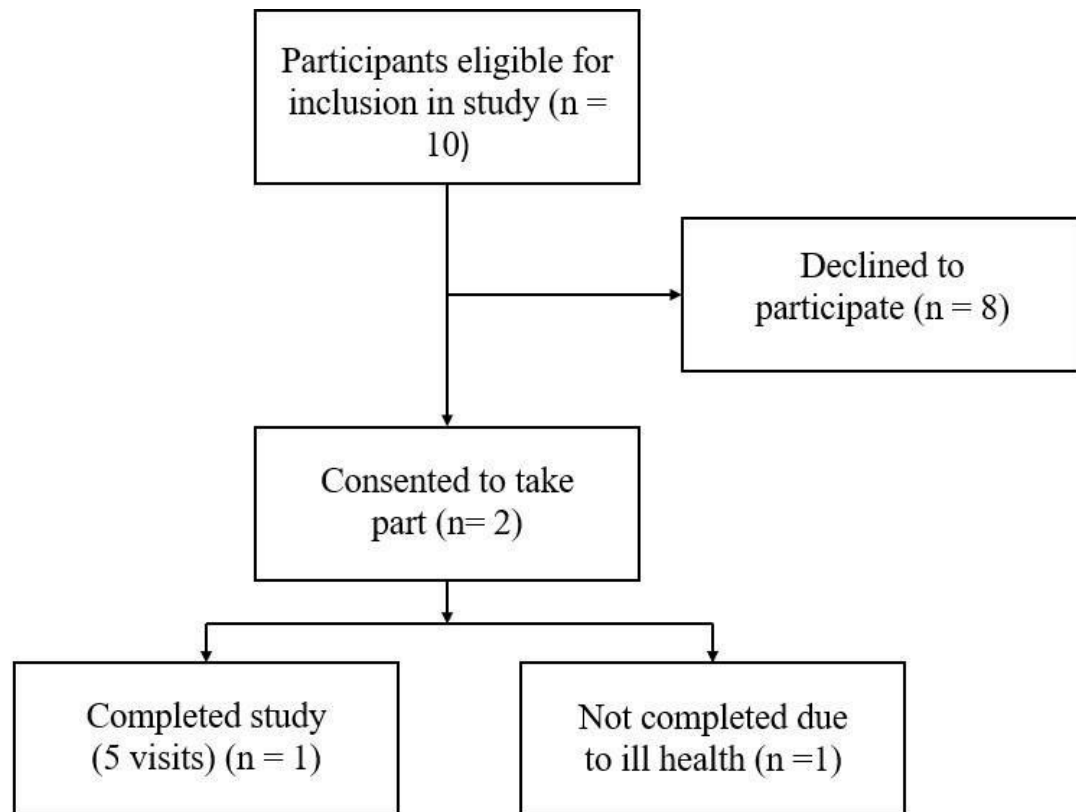


Figure 5.1 CONSORT flow diagram of case study recruitment. 10 participants were contacted. 8 participants declined to take part due to the study length. 2 participants consented to take part. 1 participant completed the study; 1 participant did not complete the study due to ill health.

Inclusion criteria

All participants had a diagnosis of PD by a Neurologist or Geriatrician and participants could be male or female. Participants had to be able to attend the School of Sport and Exercise Sciences (SSES) Laboratory for a period of 1.5 h. Participant's medication was not changed by the researcher. All participants had been stable for the past two months and be in stage H&Y stage 1-3.

Exclusion criteria

Participants whose Parkinson's was not controlled. Participants in stage 4 or 5 of the H&Y scale. Participants who could not consent for themselves.

Participant consent

Participants gave written informed consent, completed a medical history questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q) before participation in the study.

Recruitment

Recruitment was achieved by contacting participants from a previous study on the acute effects of H₂ (Chapter 4). Of the ten participants approached, two participants consented to take part (See Figure 5.1 for CONSORT flow diagram). Of these two participants, one participant rescheduled a total of five visits. Due to the unreliability of the participant (who had family and personal health issues), it was decided to withdraw the participant from the study. This participant had not yet started the study.

5.3.3. Experimental design

An ABAB design was implemented in the study which allowed the researcher to evaluate the effectiveness of an intervention by comparing the participant's measurements across different phases with and without intervention. The participant was not aware which machine was a placebo machine or a 'true' machine. The repeated measurements across multiple phases help increase the reliability of findings and provide a clearer understanding of the intervention's impact.

- A (baseline) = baseline measurements were taken without any intervention (in this study, this was after a placebo intervention).
- B (intervention) = measurements were taken after the intervention had been introduced. This phase assessed the effects of the intervention.

- A (withdrawal) = the intervention was withdrawn, and measurements were taken again. This phase helped determine whether any changes observed during the intervention phase are due to the intervention itself rather than other factors.
- B (reintroduction) = the intervention was reintroduced, and measurements were taken again. This phase provided further evidence of the intervention's effects by demonstrating whether changes occurred again when the intervention was reinstated.

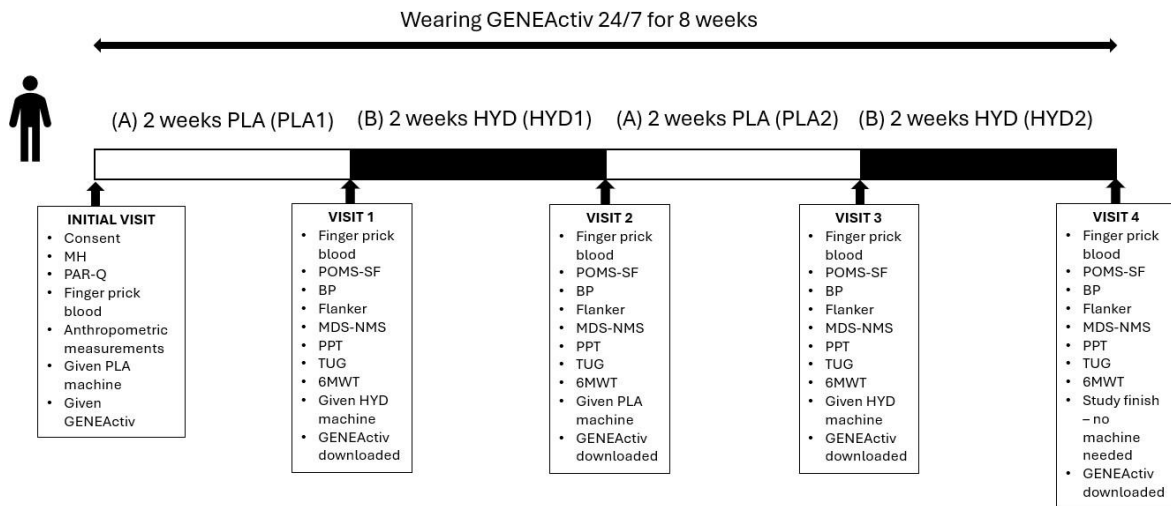


Figure 5.2 Study schema. PLA = placebo, HYD = hydrogen. POMS-SF = Profile of Mood States- Short Form. BP = blood pressure. Flanker = Flanker Compatibility Task. MDS-NMS = Movement Disorder Society – Non-motor symptoms. PPT = Purdue Pegboard Test. TUG = Timed Up and Go Test. 6MWT = 6 min walk test.

5.3.4. Gas machine

The participant inhaled either PLA or HYD gas (Osmio Solutions Ltd, Cliffe, Kent, UK) for one hour each day for a total of eight weeks. This was split into 30 min in the morning and 30 min in the evening, during ‘normal’ sitting time:

- A = PLA1 for 1 hour (30 min x 2 daily) for 2 weeks. (Week 0 -2)
- B = HYD1 for 1 hour (30 min x 2 daily) for 2 weeks. (Week 2 – 4)
- A = PLA2 for 1 hour (30 min x 2 daily) for 2 weeks. (Week 4 -6)
- B = HYD2 for 1 hour (30 min x 2 daily) for 2 weeks. (Week 6-8)

Humidifiers on the machine were covered so no difference in appearance was apparent, as the HYD machine had ‘condensation’ in the humidifier and the PLA machine did not.

Please see General Methods (Chapter 2.5) for further information on the machines including gas concentration and water content.

Nasal cannula

Gas was inhaled through a 210 cm nasal cannula (Osmio Solutions Ltd, Cliffe, Kent, UK), and new nasal cannulas were given to the participant for each day, and then disposed in clinical waste. Proper nasal cannula placement was demonstrated to the participant pre commencement of study.

Please see General Methods (Chapter 2.5.3) for further information on nasal cannulas.

5.3.5. Measures

The participant had already completed one study in H₂, so therefore no familiarisation visit was required. The participant attended the SSES Research Laboratory on five occasions for a total of 1.5 h each visit. The participant's partner also attended, however, was not in the Lab during testing. The initial visit comprised gaining informed consent, medical history and PAR-Q completion, along with anthropometric measurements (height and weight) and blood samples. The participant was given a placebo (PLA) machine, along with 14 nasal cannulas (one nasal cannula per day). The participant was advised to contact the researcher as soon as possible if he had any issues with the machine.

Measures were taken in the same order at each visit; Blood samples, POMS-SF, BP, Flanker Compatibility Task, Purdue Pegboard Test (PPT), MDS-NMS, TUG, 6MWT.

Anthropometric measurements

Height and weight were measured, and BMI calculated at the initial visit. Please see General Methods (Chapter 2.3) for further information.

Total antioxidant capacity

Plasma antioxidant capacity was determined by a chemiluminescence assay kit (No CV available from manufacturer) (ABEL Antioxidant Test Kit for Hydroxyl Radicals with Pholasin, Knight Scientific Ltd, Plymouth, UK) in accordance with manufacturer instructions.

Profile of Mood States – Short Form (POMS-SF-SF) (Grove, 1992)

Please see General Methods (Chapter 2.7) for further information.

Blood pressure

Systolic and diastolic blood pressure were measured. Please see General Methods (Chapter 2.4 for further information on this).

Flanker Compatibility Task (Presentation, Neurobehavioural Systems, Inc, California, USA)

Please see General Methods (Chapter 2.8) for further information.

Purdue Pegboard Test (PPT) (Tiffin & Asher, 1948)

Please see General Methods (Chapter 2.9) for further information.

Movement Disorder Society – Non-Motor Rating Scale

Please see General Methods (Chapter 2.10) for further information.

Timed up and Go Test (TUG)

Please see General Methods (Chapter 2.11.1) for further information.

6 min walk test (6MWT)

Please see General Methods (Chapter 2.11.2) for further information.

Accelerometer (GENEActiv)

The participant was instructed to wear the accelerometer around his waist 24 hours a day, seven days a week for a total of eight weeks except when the participant attended the lab and data was downloaded from the accelerometer.

Weeks 0 – 6 were captured with the accelerometer, however weeks 6-8 were found to not have recorded any data when downloaded.

Please see General Methods (Chapter 2.12) for further information.

5.4. RESULTS

Participant characteristics are presented in Table 5.1

Table 5.1. Participant characteristics

<i>Variable</i>	
Age (years)	78
Sex	Male
Occupation	Retired
Current location	East Kent, UK
Ethnicity	White British
Height (cm)	194
Body Mass (kg)	117
BMI (kg/m²)	31
Age of disease onset (years)	75
Disease duration (years)	3
Levodopa equivalent daily dose (mg)	850
Other prescribed medications	Perindopril 4mg daily Atorvastatin 20 mg daily Bisoprolol fumarate 2.5mg daily

Participant attendance

The participant attended all five visits with his partner who drove him to the testing site.

Recorded machine inhaling time is presented in Table 5.2

Table 5.2. Recorded machine inhaling time

<i>Machine</i>				
<i>Variable</i>	<i>A (PLA)</i>	<i>B (HYD)</i>	<i>A (PLA)</i>	<i>B (HYD)</i>
Machine time log	Unknown	14 hours	Unknown	5 hours
% of time compliance	Unknown	100%	Unknown	36%

Both machines had a time log on the back of the machine, which recorded time in hours. The placebo time log did not work and did not move past 8 hours (which was recorded before the participant was given the machine). Each machine should have recorded a total of 14 hours of use (due to the participant being asked to inhale gas for 30 min, twice a day). During HYD1, the machine was switched on for 14 hours, which was required from the participant. In the reintroduction of the HYD machine (HYD2), the machine was only switched on for 5 hours, therefore only 36% of the total required time. During Week 0 – 2 (PLA1) the participant reported that he was inhaling gas at 10:00 and 19:00 every day with no side effects. However, during this study period he was travelling to France by ferry and was not able to take the machine with him. As a result, the participant missed four days (eight sessions) of inhalation of placebo gas. The participant sent videos of the machine switched on to make sure it was correctly bubbling each time a new machine was taken home. Communication from the participant was excellent during the study.

5.4.1. TOTAL ANTIOXIDANT CAPACITY

Total antioxidant capacity is presented in Figure 5.3

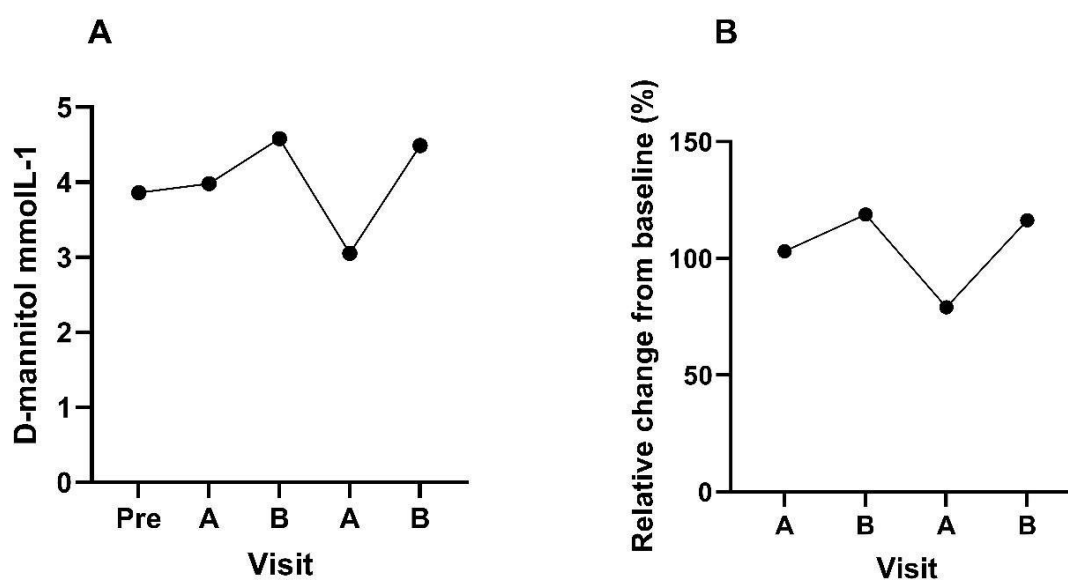


Figure 5.3 Effects of hydrogen and placebo gas inhalation intervention on total antioxidant capacity. A = Antioxidant capacity expressed as D-mannitol equivalent units. B = Percentage of relative change from baseline. Antioxidant capacity increased from baseline (by 19% after HYD1 and 16% after HYD2).

5.4.2. PROFILE OF MOOD STATES – SHORT FORM

Results of the POMS-SF are presented in Table 5.3

Table 5.3 Profile of Mood States- Short Form

<i>POMS-SF Subscale</i>	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
<i>Tension (Ten)</i>	0	0	0	0
<i>Anger (Ang)</i>	0	0	0	0
<i>Fatigue (Fat)</i>	0	0	0	0
<i>Depression (Dep)</i>	0	0	0	0
<i>Esteem-related affect (ERA)</i>	11	8	10	18
<i>Vigour (Vig)</i>	10	5	6	7
<i>Confusion (Con)</i>	0	0	0	0
<i>Total Mood Disturbance (TMD)</i>	79	87	84	75

POMS-SF items are scored 0-4 (0 = not at all, 1 = a little, 2 = moderate, 3 = quite a lot, 4 = extremely). There are seven subscales. Two items are reversed scores: ashamed and embarrassed (both part of the ERA subscale). Total Mood Disturbance is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: $TMD = [TEN+DEP+ANG+FAT+CON] - [VIG+ERA]$.

5.4.3. BLOOD PRESSURE

Blood pressure measures are presented in Figure 5.4

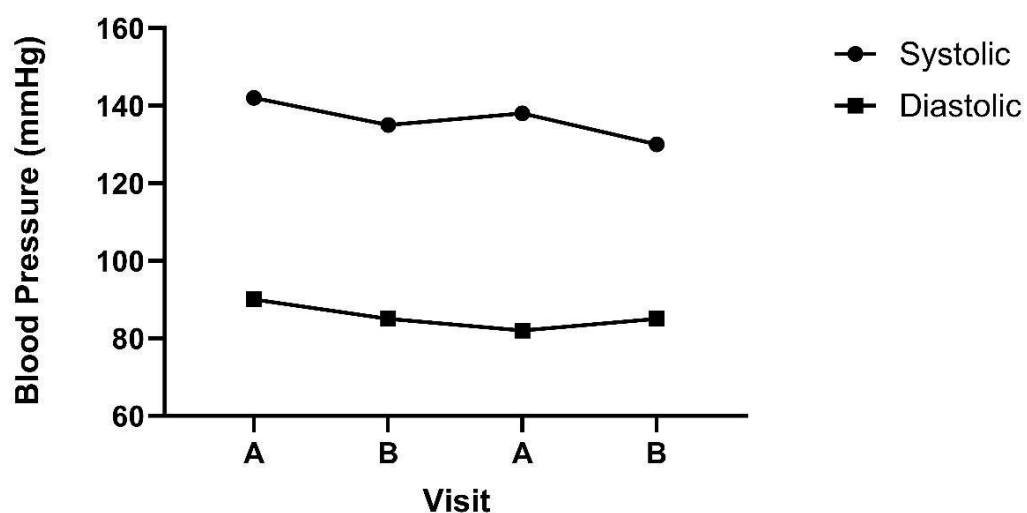


Figure 5.4 Systolic (SBP) and diastolic blood pressure (DBP) measured after a HYD and PLA 2-week inhaling intervention. Blood pressure measured in millimetres of mercury (mmHg). There was no change in Levodopa, perindopril or bisoprolol over the course of the study which may have impacted SBP.

5.4.4. FLANKER COMPATIBILITY TASK

Results of the Flanker Compatibility Task are presented in Figure 5.5

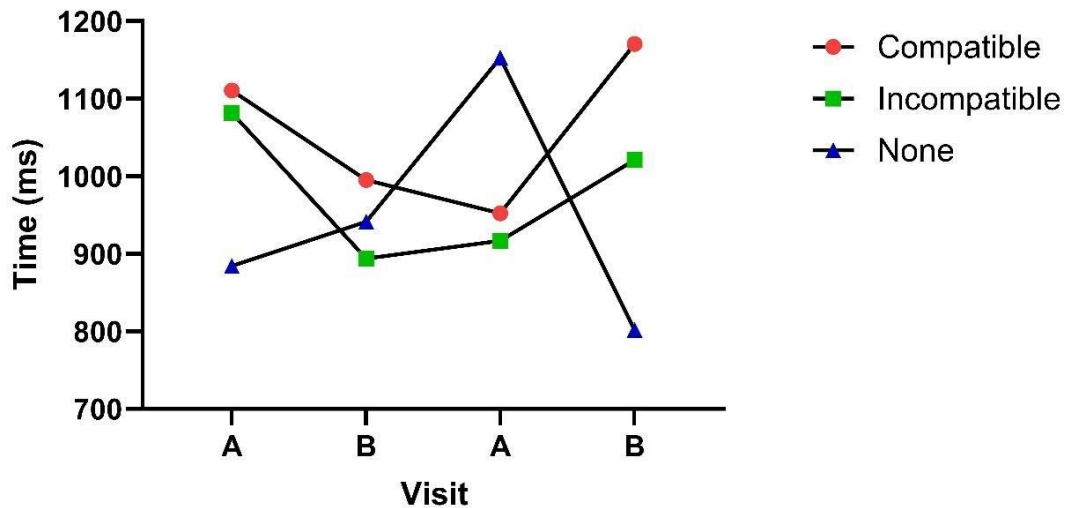


Figure 5.5 Flanker Compatibility Task Reaction Time. Measurements are to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli) at each visit. Data shown in milliseconds.

Table 5.4 Flanker Compatibility Task Accuracy

Results for the Flanker Compatibility Task Accuracy are presented in Table 5.4

Accuracy Subscale	A	B	A	B
Compatible	0.938	1	1	1
Incompatible	0.938	0.938	0.938	1
None	0.938	1	0.938	1
Mean of 3 subscales	0.938	0.98	0.96	1

Measurements are to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli) at each visit. Maximum score of 1.

5.4.5. MOVEMENT DISORDER SOCIETY NON – MOTOR RATING SCALE

Results of the MDS-NMS are presented in Table 5.5

Table 5.5 MDS-NMS scores

<i>MDS Subscale</i>	<i>A</i>	<i>B</i>	<i>A</i>	<i>B</i>
<i>Depression</i>	0	0	0	0
<i>Anxiety</i>	0	0	0	0
<i>Apathy</i>	0	0	0	0
<i>Psychosis</i>	0	0	0	0
<i>Impulse control</i>	0	0	0	0
<i>Cognition</i>	0	0	0	0
<i>Orthostatic Hypotension</i>	0	9	0	0
<i>Urinary (urgency, frequency, nocturia)</i>	6	6	9	6
<i>Sexual (drive, arousal)</i>	0	0	0	0
<i>Gastro-intestinal (drooling, swallowing, nausea, constipation)</i>	1	9	9	3
<i>Sleep</i>	0	0	0	4
<i>Pain</i>	0	0	0	0
<i>Other (weight loss, smell, tired, sweating)</i>	2	0	4	0
<i>Total Score</i>	9	24	22	13

Item total is calculated by frequency multiplied by severity. (Maximum frequency score is 4, maximum severity score is 4). Subscale total is the sum of all Item totals for each subscale. MDS – NMS Total Score is the sum of totals for all Subscales.

5.4.6. PURDUE PEGBOARD TEST

Results of the Purdue Pegboard Test are presented in Figure 5.6

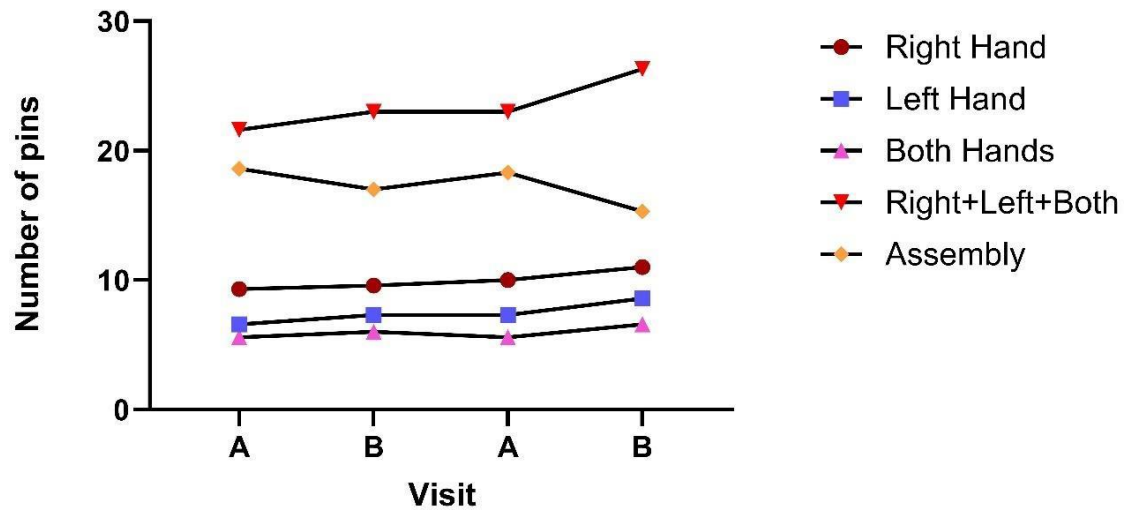


Figure 5.6 Purdue Pegboard Test results. Right, Left and Both Hands were completed over 30 seconds. Assembly was completed over 60 seconds. Each task was completed three times, and the mean measure of pins was taken (as per standard protocol).

5.4.7. FUNCTIONAL MOVEMENT

Results of Functional Movement Tests are presented in Figure 5.7

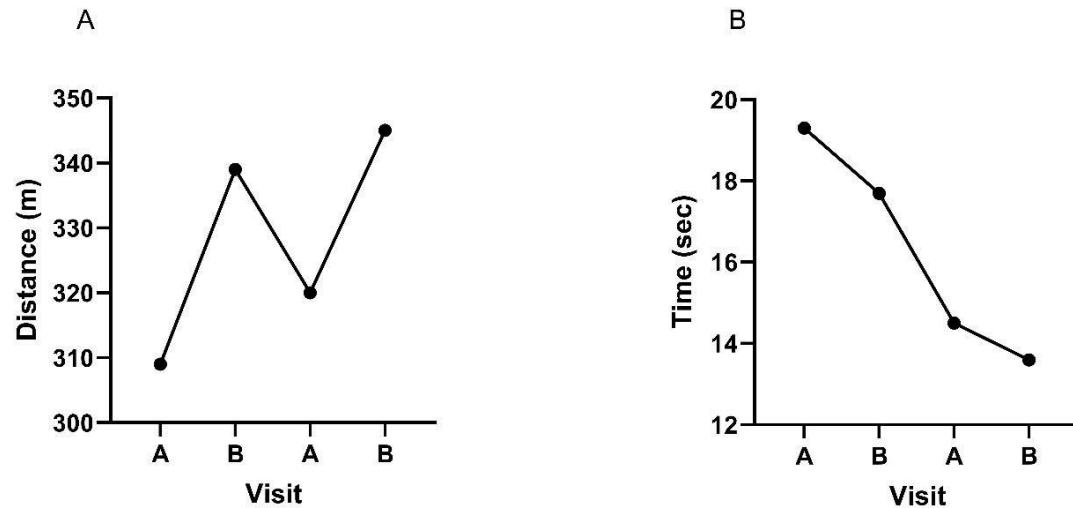


Figure 5.7 Functional movement measures. A = distance completed in a 6MWT in meters. B = Timed Up and Go Test in seconds. All tests were carried out with the participant using a walking stick. The participant walked further in HYD1 (339 m) and HYD 2 (345 m) than PLA1 (309 m) and PLA2 (320 m). The TUG completion reduced despite intervention (PLA1 = 19.3 sec, HYD1 = 17.7 sec, PLA2 = 14.51 sec, HYD 2 = 13.6 sec, a total of 30% over the course of the study).

5.4.8. ACCELEROMETRY - GENEACTIV

To note - Data unavailable for Week 6-8 (B - HYD2). The participant reported that he wore the accelerometer but when the data was downloaded, the accelerometer had not captured any data.

Accelerometry data for physical activity is presented in Figure 5.8

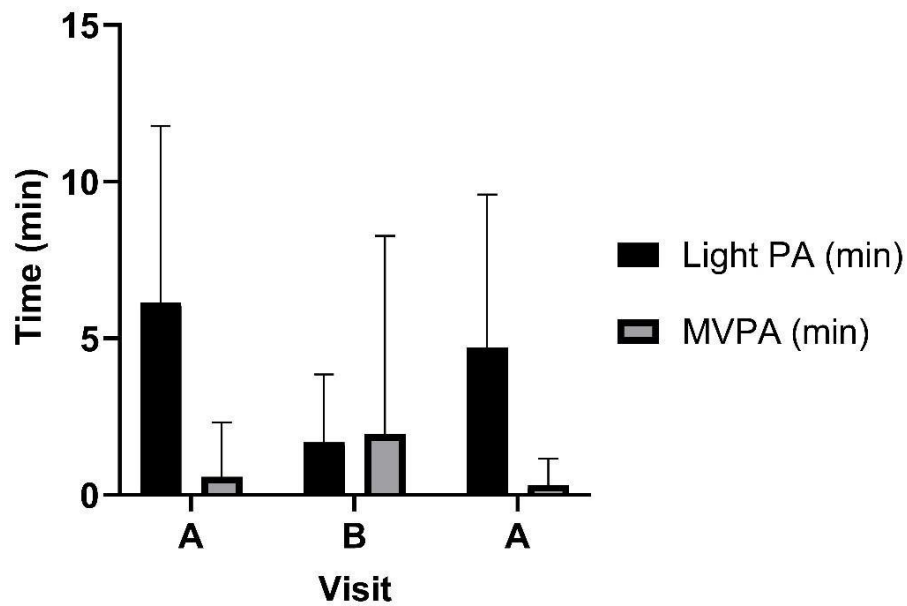


Figure 5.8 GENEActiv accelerometry physical activity data over the course of each intervention. Data presented as daily mean $\pm$ standard deviation. This demonstrated the participant is largely inactive, with virtually no time spent in moderate to vigorous physical activity (MVPA).

Accelerometry output for non-wear, step count, sedentary time and sleep are presented in Figure 5.9 over page

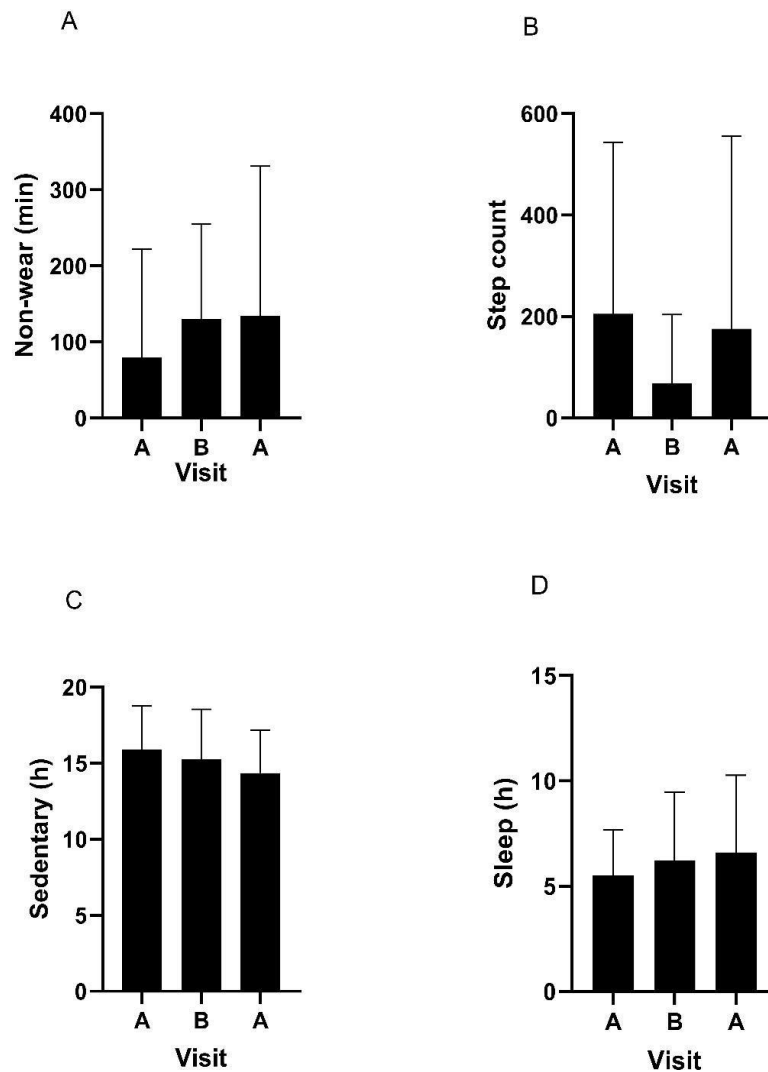


Figure 5.9 GENEActiv output for each variable. A = Non-wear. B = Step count. C = Sedentary time. D = Sleep. Data presented as daily mean $\pm$ standard deviation. Non-wear is less in PLA1 (80 min) than HYD1 (130 min) and PLA2 (135 min), suggesting better compliance at the start of the study, Step count is similar in PLA1 (205) and PLA 2 (176), with less steps taken in HYD1 (69); however, these variations are negligible, and potentially just ‘noise’ picked up from the accelerometer. Sedentary behaviour is similar throughout the study (PLA1 = 15.9 h, HYD1 = 15.27 h, PLA2 = 14.36 h). Sleep mean is also similar throughout the study (PLA1 = 5.5 h, HYD 1 = 6.2 h, PLA 2 = 6.6 h).

Daily measures for steps, non-wear, sleep, sedentary time and physical activity are presented in Table 5.6

Table 5.6 Daily measures for week 0 – 2 (A - PLA1)

<i>Day number</i>	<i>Steps</i>	<i>Non-wear (min)</i>	<i>Sleep (h)</i>	<i>Sedentary (h)</i>	<i>Light (min)</i>	<i>MVPA (min)</i>
1	0	0	8.28	15.65	4.65	0
2	0	0	7.52	16.31	7.78	1.53
3	0	0	10	14.12	0.86	0
4	31	77	3.85	20.19	0	0
5	741	450	5.83	12.66	11.76	6.5
6	0	125	5.80	14.18	1.56	0
7	509	133	4.49	15.21	14.05	0
8	0	0	5.87	17.93	13.78	0
9	822	0	5.62	18.28	8.9	0
10	0	0	6.25	17.67	0	0
11	771	0	5.65	18.24	13.78	0
12	0	332	3.68	20.18	0	0
13	0	1.1	1.87	16.62	1.15	0
14	2	0	2.57	4.73	7.65	0
Mean	205	79.86	5.5	15.9	6.14	0.57
SD	338.37	142.15	2.18	2.89	5.63	1.75
Highest daily value	822	450	10	20.19	14.05	6.5
Lowest daily value	0	0	1.87	4.73	0	0

Table 5.6 shows daily step count, non-wear, sleep, time spent sedentary, time spent in light PA and MVPA, with mean and standard deviation calculated for each measure. Highest and lowest values for each measure are also reported. Eight out of 12 days, the participant seems to not walk at all, with the participant walking less than 50 steps per day in two days. There is little non-wear time on these days, suggesting that the participant has extreme levels of inactivity. Sleep varies greatly, with day 13 measuring 1.87 h of sleep, mean sleep time is 5.5 h. The participant spent large amounts of time being sedentary.

Daily measures for steps, non-wear, sleep, sedentary time and physical activity are presented in Table 5.7

Table 5.7 Daily measures for week 2– 4 (B - HYD1)

<i>Day number</i>	<i>Steps</i>	<i>Non-wear (min)</i>	<i>Sleep (h)</i>	<i>Sedentary (h)</i>	<i>Light (min)</i>	<i>MVPA (min)</i>
15	435	0	3.22	11.4	5.7	3.66
16	4	0	5.80	18.04	0	0
17	8	0	7.23	18.25	0	0
18	2	26.35	13.79	13.83	0	0
19	309	3	2.73	16.17	4.17	0
20	0	169.26	11.72	11.57	4.6	0
21	2	141.42	3.49	16.42	0	0
22	103	249.66	8.37	10.67	1.23	0
23	0	88.17	6.25	17.14	1.43	0
24	0	279.43	4.33	14.83	0	0
25	83	213.9	4.78	16.77	0	0
26	0	119.88	2.86	17.7	0	0
27	2	121.77	5.57	16.62	1.43	23.65
28	15	410.2	6.76	14.42	4.9	0
Mean	68.78	130.21	6.20	15.27	1.68	1.95
SD	134.82	125.07	3.27	2.56	2.17	6.32
Highest daily value	435	410.2	13.79	18.25	5.7	23.65
Lowest daily value	0	0	2.73	10.67	0	0

Table 5.7 shows daily step count, non-wear, sleep, time spent sedentary, time spent in light PA and MVPA, with mean and standard deviation calculated for each measure. Highest and lowest values for each measure are also reported. Again, the participant is largely inactive, with a mean step count of 68 steps per day, and hardly any time spent in MVPA.

Daily measures for steps, non-wear, sleep, sedentary time and physical activity are presented in Table 5.8

Table 5.8 Daily measures for week 4– 6 (A - PLA2)

<i>Day number</i>	<i>Steps</i>	<i>Non-wear (min)</i>	<i>Sleep (h)</i>	<i>Sedentary (h)</i>	<i>Light (min)</i>	<i>MVPA (min)</i>
29	61	242.6	5.29	10.02	2.05	0
30	0	151.01	7.13	11.29	4.4	0
31	3	733.02	1.12	9.8	0	0
32	0	0.03	9.73	14.8	5.37	0
33	0	87.57	7.16	15.24	0	0
34	0	15.45	13.14	10.51	3.72	0
35	978	0	8.99	15.83	5.43	0
36	1142	167.69	9.84	15.37	11.78	2.65
37	196	41.55	2.66	15.70	4	0
38	0	3.77	5.49	18.21	4.08	0
39	82	1.08	7.99	15.95	8.28	1.92
40	0	202.73	4.54	15.65	0	0
41	0	0	10.03	14.5	16.93	0
42	0	239.3	0.26	18.18	0	0
Mean	175.85	134.7	6.6	14.36	4.71	0.32
SD	379.93	196.33	3.67	2.82	4.88	0.84
Highest daily value	1142	733.02	13.14	18.21	16.93	2.65
Lowest daily value	0	0	0.26	9.8	0	0

Table 5.8 shows daily step count, non-wear, sleep, time spent sedentary, time spent in light PA and MVPA, with mean and standard deviation calculated for each measure. Highest and lowest values for each measure are also reported. Again, there are large amounts of inactivity reported, with a mean step count of 176 steps per day. Hardly any time is spent in MVPA, with the participant achieving 12 days of no MVPA.

On one visit, the participant's partner said (while the participant had gone to the bathroom):

“He always underplays his [PD] symptoms, he makes out that he's doing better than what he is, even to the Neurologist. He sits in his chair all day.”

This comment agrees with the accelerometer data reported above.

Mean time (over 14 days) spent in non-wear, sleep, sedentary, light physical activity (PA), and moderate to vigorous PA (MVPA) in minutes is presented in Figure 5.10 (over page)

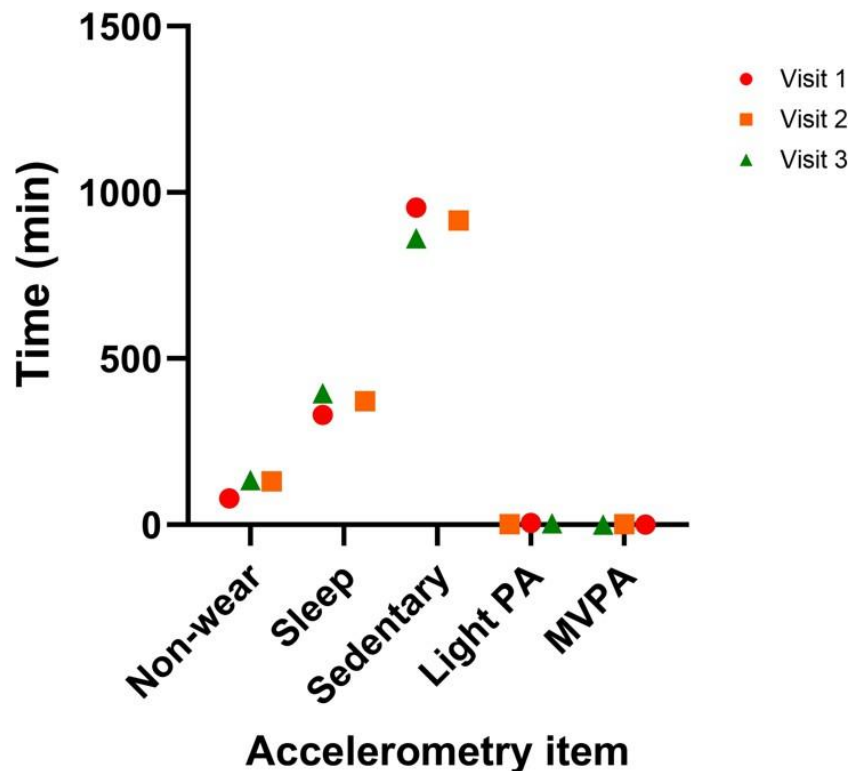


Figure 5.10 Mean time (over 14 days) spent in non-wear, sleep, sedentary, light physical activity (PA), and moderate to vigorous PA (MVPA) in minutes. Visit 1 = PLA1, Visit 2 = HYD2, Visit 3 = PLA2. Non-wear, sleep, sedentary behaviour, light PA and MVPA demonstrate similar periods of time during PLA1 (Visit 1), HYD1 (Visit 2) and PLA2 (Visit 3).

5.5. DISCUSSION

Poor study protocol adherence

In the last 2-week session (weeks 6-8) the participant only used the machine for 36% of the required time, these results are similar to Hong et al. (2021) who found that 25% of participants did not use the machine for 50% of the total time. Dopamine plays a crucial role in regulating motivation processes, and motivation is strongly linked to behaviour and approximately 40% of PwP suffer with motivation disorders (den Brok et al., 2015). However, the participant did not report any feelings of apathy in the MDS-NMS. There are many validated models which help to increase adherence, particularly with a focus on medications/interventions. The

Information – Motivation – Behaviour Skills model (IMB) (Fisher & Fisher, 1992) was recently demonstrated to improve self-efficacy and non-motor symptoms in PwP (Park et al., 2022), and future intervention studies could use the IMB model or similar to help increase adherence to study protocol, particularly in those people who may lack motivation such as PwP. Studies which have demonstrated significant findings in H₂ and PD animal models lack the inevitable variability of human studies, and compliance to protocol would not be an issue for animal studies.

Total Antioxidant Capacity

Inhaling hydrogen gas (HYD) increased total antioxidant capacity (TAC) in one participant. After the first hydrogen intervention (HYD1), TAC increased by 19% from baseline and after HYD2 showed a 16% increase. There was 100% compliance with study protocol in HYD1 and this reduced to 36% in HYD2. Despite the reduced inhaling time in HYD2, there was only 3% difference in the improvement in TAC from baseline, suggesting that HYD may improve TAC at either less inhalation time per day, or when missed days inhaling. While Hirayama et al. (2019) found an increase in 8-OHdG (an indirect marker of endogenous oxidative damage to DNA) after a H₂ gas inhalation, it was found that both H₂ (16%) and placebo (5%) gas increased 8-OHdG after four weeks. Time of urine sampling was not reported in the paper, and it has been demonstrated that 8-OHdG levels fluctuate during the day (Kanabrocki et al., 2002), therefore if participants gave samples at different times of day, this may not be a true indicator of 8-OHdG levels. The researchers used an enzyme-linked immunosorbent assay (ELISA) kit to quantify 8-OHdG levels, however, chromatographic assays have showed higher agreement across urine from different subjects (Barregard et al., 2013) and have been demonstrated to be due to the heterogeneity of analysis techniques, this makes it difficult to compare study results. While TAC increased with H₂, it is difficult to determine the mechanism of action of how this occurred due to other markers of inflammation and oxidative stress not being analysed, which would have given a fuller picture of the mechanism of action due to the complex interaction of oxidative stress and inflammation and cascade of effects. A marker of oxidative stress may have been beneficial to measure as well, as antioxidant capacity is not sufficient enough to make references about oxidative stress, however, the results from this study are preliminary. PwP have increased rates of oxidative stress and if H₂ did lower these rates, this would potentially slow down the

progression of the disease but in order to optimise H₂ benefits, dose effect needs to be elucidated.

Mood

Five POMS-SF subscales scored zero all through the study: tension, anger, fatigue, depression, and confusion. A higher ERA score at the last visit compared to previous could potentially be due to the participant completing the study. POMS-SF ERA subscale includes positive items: ‘proud’, ‘competent’, ‘confident’, ‘satisfied’, and two negative items ‘embarrassed’, ‘ashamed’ which are reversed-scored prior to being combined with the other items, there are no specific ways of detecting and measuring shame in PD (Fleury et al., 2020). Esteem is self-perception and abilities and limitations, PwP may experience shame and embarrassment from motor-symptoms and non-motor symptoms (Angulo et al., 2019) which may be why the MDS-NMS and POMS-SF follow the same pattern, again demonstrating that the POMS-SF could be used instead of the MDS-NMS. The participant’s vigour score was highest at the beginning of the study. Vigour refers to generalised motivating effects which comes from dopaminergic drive (Beierholm et al., 2013) and dopaminergic medication has been shown to boost motivation by contingent rewards (rewards based on performance) but declines by expected reward (reward irrespective of performance) (Grogan et al., 2020). The participant attended the lab on the same day of the week at the same time (Thursdays at 10 am) to avoid medication ‘on/off’ phases, so therefore the change in vigour results should not be due to dopaminergic medication. Vigour on the POMS-SF includes the words: ‘lively’, ‘active’, ‘energetic’, ‘full of pep’ and ‘vigorous’. The researcher had to clarify the term ‘full of pep’ (as this scale was created in Australia, this has potential to not translate well to a non-Australian population, despite being English speaking). The researcher described ‘full of pep’ as having energy and being in high spirits.

Blood pressure (BP)

Systolic blood pressure (SBP) measured lower with HYD than with PLA. As a 1-hour daily H₂ protocol has demonstrated anti-hypertensive effects in animal models (Sugai et al., 2020), with a potential mechanism of action that H₂ scavenges ROS (as demonstrated by Ohsawa et al., 2007) in the rostral ventrolateral medulla (RVLM) (which is critical in the regulation of blood pressure), as there is elevated ROS (in the form of hydroxyl radicals) in the RVLM (Kishi et

al., 2004) and impaired catecholaminergic neurons in the RVLM occur in early stage PD model (Zhang et al., 2015). The participant adhered to the study protocol of 14 hours inhaling over 2 weeks in HYD1, however, the participant did not adhere to study protocol in HYD2 (only 5 hours in total were recorded on the machine). If SBP was affected by H₂, it may be this occurs at shorter durations of inhaling than a total of one hour per day, potentially making this easier to comply with if a shorter duration is beneficial. Diastolic blood pressure (DBP) was similar during HYD and PLA, however, it may be HYD stabilised DBP. As BP in PwP is often affected by lowering blood pressure (Palma & Kaufmann, 2018), HYD may offer a stabilising effect, which would benefit those people who suffer with orthostatic hypotension.

Cognition

Reaction time (RT) was not impacted by HYD. In the previous chapter (Chapter 4), RT Compatible reduced from baseline to 2 h irrespective of intervention (HYD or PLA), and it was hypothesised that cognitive training over a 2 h period may increase RT in PwP, however, the current study has not shown a pattern of decrease in RT. The participant's 6MWT was greater with a HYD intervention than a PLA intervention, and previous research has shown that a cognitive training programme reduces freezing of gait (Walton et al., 2018) (which may benefit PwP who experience freezing of gait) with improved Flanker task performance predicting improved mobility tests performance (Gothe et al., 2014), however the current study has not demonstrated this. The participant had 100% accuracy in all stimuli in HYD2, with a potential learning effect from previously completing the Flanker. The participant scored 100% for 'compatible' in HYD1, PLA2, and HYD2.

Non-Motor symptoms

The participant reported orthostatic hypotension (OH) after HYD1. The participant was diagnosed with AF and was prescribed beta-blockers (Bisoprolol fumarate) and an ACE inhibitor (Perindopril) prior to the study commencement. Perindopril is associated with lower incidences of hypotension compared to enalapril and captopril (Haïat et al., 1999). It can be complex managing comorbidities due to side effects of prescription drugs, or drugs which may interact with each other. Despite the participant reporting OH after the HYD1 intervention, his

6MWT was higher, and TUG was quicker than at PLA1, and usually OH impairs walking time (Mol et al., 2018).

Total MDS-NMS scores were 9, 24, 22 and 13, which have a similar pattern to POMS-SF total mood disturbance (TMD) scores of 79/87/84/75 (where lower scores are considered more positive mood than higher scores which indicate a more negative mood). The MDS-NMS scoring for frequency/duration (percentages denote days per week or hours per waking day) are: 0 (never), 1 (rarely, 10% or less of time), 2 (sometimes, 11-25% of time), 3 (frequently, 26-50% of the time), 4 (majority of the time, equal to or more than 51% of the time). Severity scoring is measured at 0 (not present), 1 (no distress), 2 (mild, minor distress), 3 (moderate, considerable distress) and 4 (severe, major distress). Maximum scores vary according to different subscales due to the number of questions asked e.g. depression subscale asks five questions, and the orthostatic hypotension subscale asks 2 questions. Therefore, the POMS-SF may be a substitute questionnaire instead of the MDS-NMS which requires permission for use by the MDS Scientific Programs Co-ordinator at the International Parkinson and Movement Disorder Society (MDS). The MDS-NMS is expensive to use, and permissions are granted on a single-use, per language, and on a per study, per protocol, per language basis if being used for research purposes. Government or Non-Profit Funded Use costs \$1000 USD, if requested by Industry, the cost is \$20,000 USD, greatly increasing study costs. The POMS-SF is free to use and would hugely reduce study costs if attempting to capture mood, and the POMS-SF has been used in previous research in PwP (Leentjens et al., 2006).

Purdue Pegboard Test

PPT (Tiffin & Asher, 1948) scores for right hand, left hand, and both hands increased from PLA1 to HYD2 demonstrating that potentially repeated PPT can increase manual dexterity in PwP and as approximately 90% of PwP exhibit impaired manual dexterity (Raggi et al., 2011), this may be a relatively inexpensive way of increasing manual dexterity and therefore increase the ability to carry out activities of daily living (ADL) such as being able to do up buttons, tie shoelaces etc which could therefore lead to increased self-esteem and better overall wellbeing. As each PPT was carried out two weeks apart, this shows that repeated PPT can have quite a large amount of time in between sessions but still demonstrate increased scores. Parkinson's groups may wish to incorporate tasks such as the PPT as it is easy to deliver and relatively

inexpensive, potentially making this similar to a game and therefore enjoyable to complete, especially as a potential link between the PPT and cognition has been demonstrated (Hinkle & Pontone, 2021). While domain-specific cognitive changes have yet to be elucidated, it may be worthwhile for PwP to undertake the PPT.

Functional movement

The participant was able to walk further after a HYD intervention than with a PLA intervention. At HYD1, the participant walked 339 m and 345 m at HYD2. At PLA1, the participant walked 309 m, and at PLA2 walked 320 m. Comparing the longest distance covered (HYD2) to the shortest distance covered (PLA1), there was a 10% increase in distance covered (36 m) which has been demonstrated to be clinically important across multiple patient groups (Bohannon and Crouch, 2017). Therefore, HYD may improve sub-maximal effort in a PD population. Future studies should incorporate this simple test to assess functional movement. The participant's BMI was 31, categorised as obese. The participant is also diagnosed with hypercholesterolemia, hypertension and AF, and these conditions are managed with prescription drugs. Obesity increases the risk of all three conditions. Obesity has been reported to rapidly increase motor progression with early PD and increase risk of functional dependency (Kim & Jun, 2020). The participant's Timed Up and Go test (TUG) reduced over the course of the study by 30%. The TUG has demonstrated reliability in PD populations, and a cut-score of 11.5 seconds was identified as the most specific and sensitive to discriminate those who did or did not fall in a large PD cohort (Nocera et al., 2013), and comparing the participant's time to completion (13.6 seconds) to those of Nocera et al (2014), it would be prudent to suggest that the participant is at increased risk of falling.

Accelerometer (GENEActiv)

The participant does not complete the minimum recommended physical activity guidelines set by the World Health Organization (World Health Organization, 2024) which is 150-300 min of moderate intensity physical activity (PA) or 75-150 min of vigorous intensity PA per week for people aged 65 years and over (which should also include functional balance and strength training 3 days a week to help reduce falls). The GENEActiv accelerometer did not collect data in the HYD2 phase, which has impacted in data gathering. The accelerometer was worn around the participant's waist, as this has been demonstrated to discriminate sedentary behaviour and

moderate to vigorous physical activity (MVPA) better than when worn on the non-dominant wrist (Duncan et al., 2020). However, wearing the accelerometer on the waist may be more uncomfortable (especially when lying in bed) than when worn on the wrist, and this may have affected adherence to wear time. It has also been shown that accelerometers on the waist may not be able to measure activities of daily living (such as washing up) as well as when worn on the wrist. Non-wear daily mean increased through the study by 70%.

The International Physical Activity Questionnaire (IPAQ) showed the participant walked daily at a light level (slightly breathless) with no duration given. The IPAQ relies on recall and recall behaviour declines with age (Barnett et al., 2016). The participant's reported current levels of PA did not match the accelerometer data.

Despite the researcher ensuring the GENEActiv was switched on at the beginning of HYD2 (green light appearing), the GENEActiv did not record data. There was no way of checking whether it was working as no lights appear on the watch face, while this provides no visible feedback to the participant (such as step count, sleep time etc), it can hinder data gathering. An IPAQ, even though retrospective, could have been used alongside the GENEActiv in case of failure, however this has been shown to not be reliant and requires recall memory.

Limitations

GENEActiv accelerometer did not capture the last two weeks of the study despite being switched on and the participant reporting to have worn it except when bathing, therefore only two weeks of HYD was captured, compared to four weeks of PLA, therefore limits the comparison of HYD and PLA. There was poor adherence to inhaling the study protocol, PLA machine did not capture inhaling time.

While a case study provided rich data, it does limit its application to the general population. There was hesitancy from other potential participants due to the daily prolonged period of inhaling gas (30 min, twice a day) and subsequent burden of time commitment.

Other markers of inflammation and oxidative stress were not measured, which would have given more in-depth elucidation of potential mechanisms of action of H₂.

5.6. CONCLUSION

HYD had no reported side effects, reduced systolic blood pressure, enabled the participant to walk further (and therefore walk more quickly) but this did not change the participant's habitual PA and exercise levels. However, this may be attributed to a learning effect from repeated completion of tests. Protocol compliance reduced through the study, with 100% compliance during HYD1 and 36% in HYD 2. Despite differences in time spent inhaling H₂, antioxidant capacity increased from baseline by approximately the same amount during HYD1 (19%) and HYD2 (16%), therefore changes in antioxidant capacity may be achieved by inhaling less gas than 1 h per day.

Chapter 6. Measuring H₂ content and dissipation rate in Hydrogen Rich Water

6.1 ABSTRACT

Introduction: Researchers report varied hydrogen (H₂) concentrations in study trials in H₂ gas inhalation and H₂ rich water and there are different methods of measuring H₂ concentrations. The purpose of this study was to measure hydrogen (H₂) concentration of hydrogen rich water (HRW) using an alternative to the supplier's methylene blue drops and to measure H₂ dissipation rate. **Methods:** Two different methods were utilised to measure H₂ concentration: a dissolved hydrogen (DH) meter and methylene blue drops. H₂ concentration was also measured every 10 min for 1 h, and every 30 min for 4 h using a DH meter. Water temperature and total dissolved solids were also measured. **Results:** Methylene blue drops measured similar H₂ concentration (1.6ppm) compared to a DH meter (1.4ppm and 1.42ppm), and H₂ dissipation measured 9% reduction from baseline after 1 h, and 19% after 4 h. **Conclusion:** A DH meter and methylene blue drops report similar H₂ concentration in HRW, and H₂ concentration reduced overtime but elevated H₂ levels remained after a 4 h period.

6.2 INTRODUCTION

The optimal dose of hydrogen (H₂) is yet to be elucidated. Dose response and mechanism of action have yet to be fully understood in hydrogen (H₂), and variations in reported H₂ concentrations can make studies difficult to compare, and many of these studies have been carried out in animals with small sample sizes. H₂ can be ingested using different methods: drinking hydrogen rich water (HRW) and breathing H₂ gas. Consumption of HRW may be the most convenient method due to being portable, and regular tap water can be used in H₂ bottles, however, the bottle in the study retails at £180, which is a cost barrier to purchasing a bottle. While the dose effect of H₂ remains unknown, the H₂ content in the current study (1.42 ppm) is similar to H₂ content reported in other studies in people with Parkinson's (PwP) (Yoritaka et al., 2013), but lower than other reported levels in PwP (Yoritaka et al., 2018, Hong et al., 2021). H₂ content can be measured by portable dissolved hydrogen (DH) meters, gas chromatography and methylene blue drops. While H₂ content may be reported in papers (and varies wildly), this may not have been measured by the researchers, which may cause overestimation of H₂ concentration.

Only two known papers (Yoritaka et al., 2013, Yoritaka et al., 2018) have researched HRW intake in people with Parkinson's (PwP) with opposing findings. These papers reported very different H₂ concentrations: 1.6 ppm (Yoritaka et al. 2013) and 10 ppm (Yoritaka et al., 2018),

and both drinks were made from participants dissolving H₂ powder into water. An issue with H₂ powder is that it contains magnesium (Mg), and H₂ content of water will be calculated using the Mg content. However, not all H₂ gas will dissolve into the water as some of this gas will dissipate into atmospheric air, therefore reported levels of H₂ concentration may be incorrect. Hong et al. (2019) reported a 2.5 ppm H₂ concentration (although this study was not just HRW - it also asked that participants use photobiomodulation). Hong et al. (2019) gave participants HRW in metal cans and both Yoritaka studies (2013 and 2018) asked participants to dissolve powder into water. (Tanaka et al., 2020) demonstrated metal containers preserved H₂ content more than plastic containers and after 12 hours, H₂ concentration was maintained in metal while plastic containers contained virtually no H₂. The bottle used in the current study had a proton exchange membrane, and it has been demonstrated that electrolytically produced H₂ water (0.79 ppm) reduced hydroxyl radicals ($\cdot$ OH) more than other types of H₂ water (Kato et al., 2015).

Comparison of studies are reported in Table 6.1 over page.

Table 6.1 Reported H₂ water content in PwP studies

<i>Authors and country of origin</i>	<i>H₂ company</i>	<i>H₂ level</i>	<i>Preparation of H₂ water</i>	<i>Frequency of water delivery to participant</i>	<i>Required consumption of daily volume of water</i>	<i>Material of container</i>	<i>Compliance monitoring</i>
Yoritaka et al., (2013) - Japan	Reported as Aquerable (Ecom International) +	1.6ppm	Participants dissolved H ₂ powder into a bottle	Not reported	1000mL	Not reported	Compliance not discussed
Yoritaka et al., (2018) - Japan	Reported as Ecom International (Hydrogen 7.0) ++	10ppm	Dissolved H ₂ powder	Sent weekly	1000mL (2 x 500mL) (to be consumed within 3 hours)	Plastic	Compliance not discussed
Hong et al., (2021) – Taiwan +++	I-MIZU PLATINUM, Japan	2.5ppm	Cans of water (dissolved with H ₂)	Collected every 2 to 5 days from researcher	200mL	Metal can	Compliance not monitored due to home consumption of water

+Aquerablue spelled incorrectly in the paper as it should read Aquela Blue.

++ Paper reported H₂ content as 10 ppm, however, manufacturer's website reported 7 ppm (Aquela, 2024).

+++ Study investigated concomitant use of H₂ water and photobiomodulation (PBM) in Parkinson's disease (PD). All 18 participants received daily PBM and H₂, there was no control group.

The current study measured H₂ content of HRW using two methods: a dissolved H₂ (DH) meter (Amtast USA Inc., Florida, USA) and Methylene Blue drops (H₂ Sciences Inc, Nevada, USA) which contained Methylthioninium chloride (commonly referred to methylene blue). The supplier provided and recommended Methylene blue drops to confirm H₂ concentration, however, the current study needed an alternative to this measurement technique to check H₂ levels and quantify H₂ dissipation rate.

6.3 METHODS

H₂ bottle

The H₂ water bottle (Osmio Genesis, Osmio Water Ltd, Kent, UK) was a 240ml capacity, plastic bottle (which can withstand high pressure) with a proton-exchange membrane (PEM) that uses electrolysis to produce hydrogen rich water. An electrolysis chamber contains anode and cathode electrodes which are separated by a PEM (a thin, semi-permeable membrane). The anode promotes the production of oxygen (O₂) and hydrogen ions (H⁺) in water, while the cathode produces hydroxide ions (OH⁻) therefore creating hydrogen saturated water.

Water

Lab water (Milli-Q Direct Water Purification System, Merck Life Sciences Ltd, Watford, UK) was used in the bottle.

Total dissolved solids (TDS) and temperature

TDS and temperature were measured using a handheld TDS/temperature meter (Osmio Water Ltd, Kent, UK).

Comparative analysis of measurements techniques

Measures were first taken to compare two measurement techniques: a dissolved H₂ (DH) meter (Amtast USA Inc., Florida, USA) and methylene blue drops (H₂ Sciences Inc, Nevada, USA) which contained Methylthioninium chloride. This was replicated five times with the same bottle. The DH meter is a more convenient method of measuring H₂ compared to methylene blue drops as this allowed the researchers to carry out repeated measurements in the water (e.g. when measuring dissipation rate).

Measurement of H₂ dissipation

Measures were taken every ten min for one hour, and every 30 min for four hours (using the same water) using a DH meter to record H₂ concentration.

Methylene blue drops

Individual drops of methylene blue drops (H₂ Sciences Inc, Nevada, USA) were initially weighed (A&D BM 252, A&D Instruments Ltd, Abingdon, UK) as the researcher wanted to ensure accuracy as drops weighed different amounts when decanted from the bottle i.e. one drop weighed 0.031g and a further drop weighed 0.028g. The drops contained Methylthioninium chloride and when added to H₂ water, drops turned clear until titration end point was reached and drops turned blue (see Figure 6.1. for chemical structure).

6 ml of water was decanted into a 15 ml polypropylene tube (Fisherbrand, Fisher Scientific UK Ltd, UK). 30µL of H₂ methylene blue was pipetted (10-100µL Finnipette, Fisher Scientific UK Ltd, Loughborough, UK) into the tube containing water until the water remained blue. This was completed within 2 min.

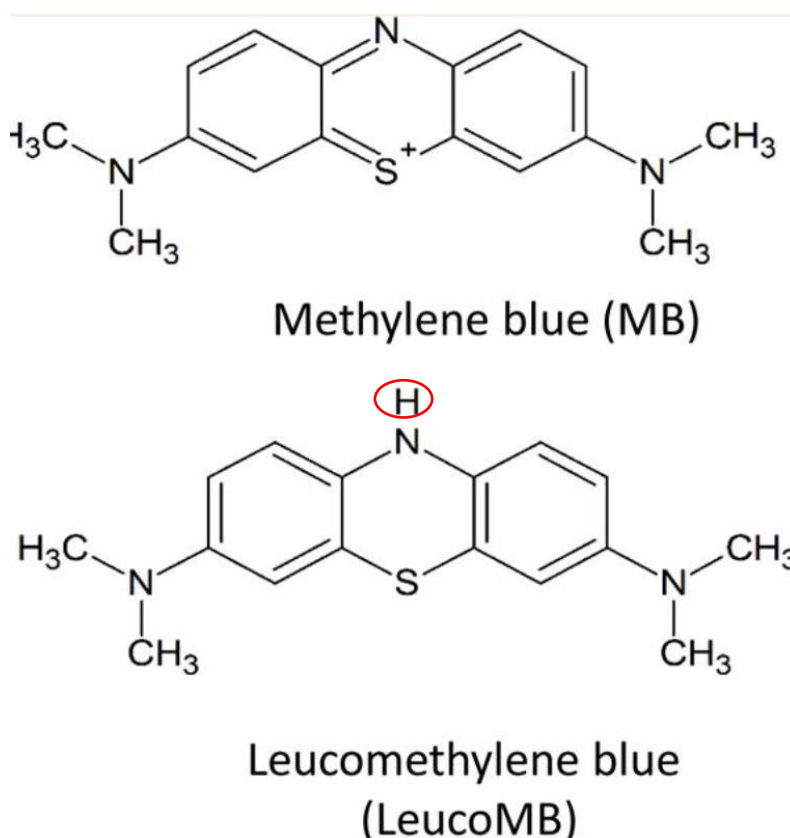


Figure 6.1 Methylene blue molecule changes after its reaction with H₂, highlighted in red. In its oxidised form, methylene blue appears blue, and then becomes clear during its reaction with H₂. Image adapted from (Tucker et al., 2018).

Statistical analysis

Assumptions of normal distribution of data were checked using the Shapiro-Wilk test. A one-way repeated measures was used to assess differences in the means of H₂ content over 1 h and 4 h. Comparison of H₂ concentration measured using a DH meter and methylene blue drops was not normally distributed, therefore a Wilcoxon signed-rank test was performed. Statistical significance level was set to $P < 0.05$ and analysis was performed with IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA)

A Bland and Altman (Altman & Bland, 1983) agreement test was used to describe the limits of agreement between methods of measurement of H₂ concentration (GraphPad Prism version 10.2.3 for Windows, GraphPad Software, Boston, USA). The difference between the measurements of H₂ concentration for the DH meter and methylene blue drops were calculated and plotted against the mean. Limits of agreement were defined as the mean bias plus or minus 1.96 times it's standard deviation.

6.4 RESULTS

6.4.1. Total dissolved solids and temperature of water

TDS and Temperature of MilliQ water and tap water results are presented in Table 6.2

Table 6.2. Total dissolved solids (TDS) and temperature of water

<i>Type of water</i>	<i>TDS (ppm)</i>	<i>Temperature (°C)</i>
MilliQ water	8	18.5
Tap water	229	18.5

6.4.2. Comparison of H₂ concentration measured using a DH meter and methylene blue drops

Results of the Wilcoxon signed-rank test revealed the mean values for H₂ did differ significantly between method of measurement ($Z = -2.121$, $p = 0.034$).

Results of H₂ concentration measured by Methylene blue drops and DH meter are presented in Table 6.3

Table 6.3. Comparison of H₂ concentration measured using two different methods

<i>Measure</i>	<i>H₂ conc. (ppm) (DH meter)</i>	<i>Number of Methylene drops</i>	<i>H₂ conc. from methylene drops (ppm)</i>	<i>Total volume of drops (μL)</i>
1	1.42	16	1.6	480
2	1.42	16	1.6	480
3	1.4	16	1.6	480
4	1.42	16	1.6	480
5	1.42	16	1.6	480

The same bottle was measured five times at full charge for H₂ content. Measurements were carried out using a DH meter (Amtast USA Inc., Florida, USA) and methylene blue drops (H₂ Sciences Inc, Nevada, USA). DH meter measurements were multiplied by 2 as the meter measures hydrogen molecules, rather than molecular weight.

Bland and Altman plot for DH meter and methylene blue drops are presented in Figure 6.2

Bland and Altman analysis showed high levels of agreement between methods for determining H₂ content, mean difference = -0.1840 with 95% limits of agreement -0.2015 and -0.1665.

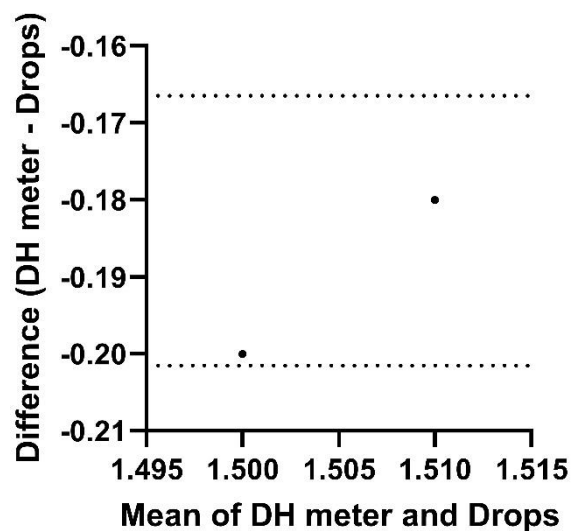


Figure 6.2 The relationship between DH meter and methylene blue drops derived measures of H₂ content presented as a Bland and Altman plot.

6.4.3. H₂ concentration over a 1 h period measured by DH meter

H₂ concentration and reduction from baseline are presented in Table 6.4

Table 6.4. H₂ concentration over a 1 h period

<i>Time (min)</i>	<i>H₂ conc. (ppm)</i>	<i>% reduction from baseline</i>
0	1.56	
10	1.56	0
20	1.52	2.6
30	1.52	2.6
40	1.48	5.1
50	1.48	5.1
60	1.42	9

H₂ concentration was measured at 10 min intervals using a DH meter to determine dissipation rate over a period of one hour.

Results of the one-way repeated measures ANOVA revealed ppm values did not differ significantly over time, no F value or p-value was reported possibly due to the small sample size.

6.4.4. H₂ concentration over a 4 h period measured by DH meter

H₂ concentration and reduction from baseline over a four-hour period are presented in Table 6.5

Table 6.5. H₂ concentration over a 4 h period

<i>Time (h)</i>	<i>H₂ conc. (ppm)</i>	<i>% reduction from baseline</i>
0	1.56	
0.5	1.52	2.6
1	1.42	9
1.5	1.38	11.5
2	1.36	12.8
2.5	1.34	14.1
3	1.32	15.4
3.5	1.3	16.7
4	1.26	19.2

H₂ concentration was measured at 30 min intervals using a DH meter to determine dissipation rate over a period of four hours.

Results of the one-way repeated measures ANOVA revealed ppm values did not differ significantly over time, no F value or p-value was reported possibly due to the small sample size.

H₂ concentration over time are presented in Figure 6.3

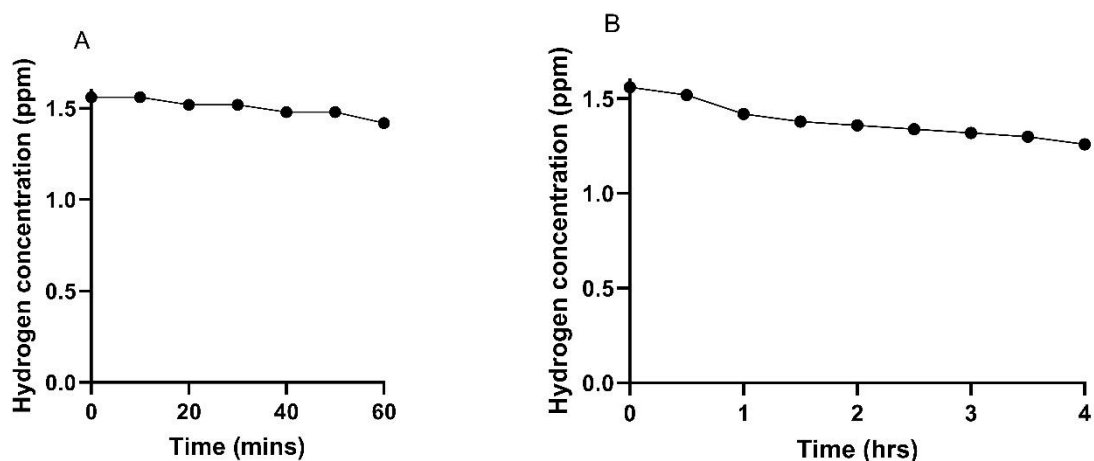


Figure 6.3. Graphical representation of H₂ (ppm) concentration. A = H₂ concentration (ppm) over 1 h. B = H₂ concentration (ppm) over 4 hrs measured by a DH meter (Amtast USA Inc., Florida, USA).

6.5 DISCUSSION

There are similar measurements by methylene blue drops and a DH meter (see Table 6.3), and there is agreement between the two methods based on a Bland and Altman plot (B&A), however the B&A plot does not reflect whether the limits of difference are acceptable or not as it only defines intervals of agreement (Giavarina, 2015). According to the manufacturer's website (H₂ Blue Test, 2017), 1 drop = 0.1 ppm, therefore 16 drops = 1.6 ppm, whereas the DH meter recorded 1.42 ppm or 1.4 ppm. Methylene blue drops are widely available, easy to use, inexpensive and have been demonstrated to measure H₂ as effectively as more complex and expensive methods (Seo et al., 2012), however, cannot be used to measure H₂ dissipation rate. There was a significant difference of H₂ content when measured over a 1 h period (9% reduction from baseline) and a 4 h period (19% reduction from baseline) with the lid kept on in between measurement. These results contrast with (Tanaka et al., 2020) who demonstrated that 50% of H₂ dissipated over four hours in a plastic bottle, and they also found that metal containers preserved H₂ content more than plastic with H₂ concentration maintained even after 12 hours. The same paper concluded as water total dissolved solids increased, H₂ concentration

decreased. Water measuring 83 ppm TDS contained 0.9 ppm H₂, while water containing 304 ppm TDS contained around 0.85 ppm of H₂. The water in the current study measured 8 ppm TDS and the highest H₂ concentration measured was 1.56 ppm. HRW is convenient, portable and easy to administer, and can be drunk in place of 'regular' water, allowing continuous dosing.

The current study assessed how long it took for H₂ to dissipate and whether levels would still be significantly elevated even if the participants took a while to consume the water. Although it was observed there was a decrease over time, this was slower compared to previous reports, and this suggests that if participants did not drink the water immediately, they would likely still receive a considerable H₂ dose (i.e. if consumed within 1 h, H₂ content was on average over 90% what it was immediately after generating.)

Limitations

Other methods of H₂ concentration analysis such as gas chromatography were not utilised in the current study. Validation of other methods of H₂ concentration would be beneficial due to the different measurement techniques reported in papers.

6.6 CONCLUSION

This study confirms that elevated concentrations of H₂ are maintained in HRW, even after four hours, with a drop of 19%. This demonstrated that if participants did not drink HRW immediately after production, then H₂ concentration would still be maintained by 90% after one hour, and approx. 80% after four hours.

CHAPTER 7. THE CHRONIC EFFECTS OF DRINKING HYDROGEN RICH WATER IN PEOPLE WITH PARKINSON'S

7.1. ABSTRACT

Introduction: Hydrogen rich water (HRW) has some neuroprotective effects in animal models of Parkinson's disease (PD), suggesting potential benefits for people with Parkinson's (PwP) but research has reported opposing findings. This study aimed to examine whether HRW influenced motor symptoms, non-motor symptoms and biomarkers of inflammation, neuroinflammation and antioxidant capacity in PwP. **Methods:** A randomised, single blind, placebo controlled, cross-over design was utilised in 12 PwP (male = 8, female = 4, age = 68 ± 8 years, age disease onset = 64 ± 10 years, height = 171 ± 0.08 cm, weight = 73.83 ± 12.63 kg, BMI = 25.2, H&Y stage = 1.67 ± 0.78). Participants were randomised to whether they first consumed hydrogen water (HYD) or placebo water (PLA: composition as tap water). Participants were asked to consume (ad libitum) 1 litre of water (HYD or PLA) per day for 4 weeks, with a week wash out period, followed by another 4-weeks of drinking 1 litre of water (HYD or PLA) for 4-weeks. Measures were taken at baseline, as well as at 1-week, and 4-weeks after commencement of each arm. Participants were asked to wear an accelerometer for the whole of the study, except the washout period. Blood samples were collected for measurement of: Brain Derived Neurotrophic Factor, Heat shock protein 70, Alpha-synuclein, Interleukin-6, 4-Hydroxynonenal, Tumor Necrosis Factor-alpha, C-reactive protein and Total Antioxidant Capacity. Profile of Mood States – Short Form (POMS-SF; to assess mood assessment), blood pressure, Flanker Compatibility Task (to assess cognitive function), the Purdue Pegboard Test (PPT; to assess fingertip dexterity and gross movement of hand, fingers, and arm), MDS-NMS (to assess non-motor symptoms), and functional movement (TUG and 6MWT) were recorded at baseline, after 1-week and 4-weeks of intervention. **Results:** Drinking HRW increased IL-6 (with outlier included) by 18% from baseline to 4 weeks and antioxidant capacity increased by 7% from baseline to 1 week, but then reduced from baseline to 4 weeks by 6%. MDS-NMS Urinary scores reduced from baseline to 1 week by 25% and from baseline to 4 weeks by 14%. Accuracy improved slightly from baseline to 4 weeks by 2% for both PLA and HYD. MDS-NMS scores for depression reduced from baseline to week 1 (HYD = 42%, PLA = 40%), anxiety reduced from baseline to week 1 (HYD = 40%, PLA = 42%) and apathy reduced from baseline to week 1 (HYD = 49%, PLA = 77%), these scores then increased from week 1 to week 4 (depression HYD = 50%, PLA = 27%; anxiety HYD = 19%, PLA = 34%; apathy HYD = 27%, PLA = 74%). MDS Total score reduced from baseline to week 1 (HYD = 34%, PLA = 28%), and then increased slightly from week 1 to week 4 (HYD = 10%, PLA =

4%). **Conclusion:** A 4-week HRW intervention did not improve motor and non-motor symptoms of PD, and HYD increased levels of IL-6, antioxidant capacity increased after 1 week of HYD but then reduced, suggesting adaptation occurred to the continued presence of HRW.

7.2. INTRODUCTION

Therapeutic interventions which are easy to administer and adhere to are needed to help reduce the impact of Parkinson's disease (PD) on people's quality of life (QoL) and activities of daily living (ADL). There is an imperative need to slow down progression of PD as there is no cure and exponential increases in diagnoses in part due to ageing and increased awareness of PD. To date there are only two known peer-reviewed papers (Yoritaka et al., 2013; Yoritaka et al., 2018) which have explored hydrogen rich water (HRW) in people with Parkinson's (PwP) and reported opposing findings. The mechanism of action of hydrogen (H_2) is still under investigation, however, seminal work by Ohsawa et al. (2007) demonstrated that H_2 selectively reduced hydroxyl radicals ($\cdot OH$), (the most reactive of free radicals) in cultured cells and decreased levels of oxidized guanine (8-OH-G) and 4-Hydroxynonenal (4-HNE). PD is a multifactorial, complex disease (Dorszewska et al., 2021), has no specific biomarkers (which can help identify PD before people become symptomatic, indicates normal biological processes and can measure responses to therapeutic interventions) (Biomarkers Definitions Working Group, 2001), and an abundance of complicated interactions between genetic factors and environmental factors have been observed in PD. There is no conclusive theory on how PD occurs, but it is thought that oxidative stress and inflammation may be important contributing factors of PD. As H_2 reduces $\cdot OH$ and has more broad antioxidant capacities (Ohsawa et al., 2007), this could potentially help in reducing the progression of PD via antioxidant/oxidative stress reducing mechanisms.

HRW is portable, easy to administer and can be consumed in place of tap water (unlike H_2 gas inhalation, which at present requires a person to be sedentary as the machine is mains powered). However, unlike other antioxidant and anti-inflammatory supplements (such as Vitamin C, Omega-3, N-acetyl cysteine etc) which can be consumed in tablet form, route of optimal administration, dose, and timing of H_2 has yet to be established and HRW studies have reported different H_2 saturations. H_2 is the smallest gas molecule; it diffuses quickly throughout the body and importantly can pass through the blood-brain barrier, potentially important to people with neurodegeneration. A recent study (Zanini et al., 2021) explored the effects of HRW on biomarkers of oxidative stress and inflammation, and functional assessments in 40 older adults

(20 males, 20 females, aged 70 years or over). Only ‘healthy’ people were included in this study, however, as ageing is a risk factor for PD, results can be tentatively extrapolated to PwP. HRW was reported to contain 15 ppm of H₂, which compared to previous HRW and PD studies is much higher (1.6 ppm (Yoritaka et al., 2013) and 10 ppm (Yoritaka et al., 2018)). The 15 ppm of HRW was produced by dissolving tablets (which contained magnesium (Mg)) into 250 mL of tap water, and researchers assumed H₂ content based on Mg content of the tablets, however, the tablets may not dissolve equally well, and H₂ dissipates quickly so H₂ concentration may not be accurate. Changes seen in the study could be due to Mg content rather than H₂ as Mg is required for many biochemical reactions such as protein synthesis, ATP synthesis, autonomic nerve functions, and interestingly contributes to glutathione (an antioxidant) synthesis. PwP often exhibit Mg deficiency (which causes oxidative damage in PwP) (Forte et al., 2005) and Mg has been demonstrated to inhibit α -syn aggregation (Golts et al., 2002) . If Mg status had not been measured (thereby not identifying Mg deficiency), any benefits observed may be due to the addition of Mg from the water. Telomere length increased in the HRW group and reduced in the control group from pre- to post-measurement. However, Chen & Zhan (2021) found telomere length was not associated with higher risk of PD in a Genome Wide Association Study of 30,000 PwP. There was a significant effect of condition in the Chair Stand Performance (CSP). No difference in oxidative stress markers or markers of inflammation were found between the two groups; C-reactive protein (an acute phase inflammatory reactant) HRW mean was 3.2 mg/L pre intervention and 3.3 mg/L post intervention, therefore it is possible that HRW potentially slightly increases acute inflammation.

Positive results from animal models of PD and HRW have yet to translate well in PwP studies, as opposing findings have been found, potentially due to a PD animal not representing the whole picture of PD due to the heterogeneity of the disease. There have been a myriad of published papers on the effects of H₂ and diseases, and there are two known peer-reviewed papers on HRW and PwP (Yoritaka et al., 2013; Yoritaka et al., 2018). These papers demonstrate opposing findings (significant improvement to MDS-UPDRS scores in six out of nine participants in the H₂ group after 48 weeks of drinking HRW, and no significant differences to MDS-UPDRS, H&Y stage or Parkinson’s Disease Questionnaire-39, no biological fluids were taken to assess for any biomarkers) with different H₂ saturation and required people to drink water over a long period of time (48 weeks and 72 weeks respectively). This study attempts to elucidate the mechanism by which HRW may be impacting PwP through

analysing blood samples for inflammatory markers and markers of total antioxidant capacity, and to assess if HRW improves QoL and ADL.

7.3. METHODS

7.3.1. Ethical approval

This study was granted ethical approval by the University of Kent's School of Sport and Exercise Sciences (SSES) Research Ethics Advisory Group, SSES REAG Number: 23_20_23, and was prospectively registered with the BMC ISRCTN registry, ISRCTN number: 17961308.

Permission to use the International Parkinson and Movement Disorder Society (MDS) Non-Motor Symptom Rating Scale was granted by the MDS Scientific Programs Co-ordinator.

7.3.2. Participants

Inclusion criteria

All participants had a diagnosis of PD by a Neurologist or Geriatrician. All participants were at stage 1-3 on the Hoehn and Yahr (H&Y) scale and could be male or female. Participants had to be able to attend the School of Sport and Exercise Sciences (SSES) Lab for a total of six visits, for approximately 1.5 hours each visit and commit to a 9-week study. Participant's medication was not changed by the researcher. All participants had been stable for the past two months.

Exclusion criteria

Participants whose Parkinson's was not controlled, participants in stage 4 and 5 of the H&Y scale. Participants who could not consent for themselves.

Participant consent

Participants gave written informed consent, completed a medical history questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q) before participation in the study.

Recruitment

Recruitment was carried out through Parkinson's UK (PUK) 'Take part in research', in which PUK send out invitations to research to its members. Posters were placed in public spaces such as community notice boards including in the Parkinson's Centre for Integrated Therapy in Canterbury, UK. Parkinson's groups were contacted, and the researcher went to one group based in Deal, Kent, UK. Initially 25 people in total from all avenues of recruitment were interested in the study but due to participants not wanting to drink one litre of water per day, or not wanting to attend the SSES Lab on six occasions, 12 participants were recruited.

7.3.3. Experimental design

The study was a placebo-controlled, randomised, single-blind, cross-over design.

All participants were already prescribed Levodopa treatment (please see results section for Levodopa equivalent daily doses).

Participants were required to attend the School of Sport and Exercise Science (SSES) lab on six occasions over the nine-week testing period for approximately 1.5 hours each visit. Participants attended at baseline, after one week, then after four weeks from baseline. There was a one-week washout period, followed by baseline assessments, then another visit after one week and then after four weeks from baseline on the other condition. Participants were not required to change their habitual diet/physical activity levels or medication. If there was any medication change during the trial, this was noted (one participant increased their Mirtazapine dosage, and one participant started taking medication for urinary urgency/frequency).

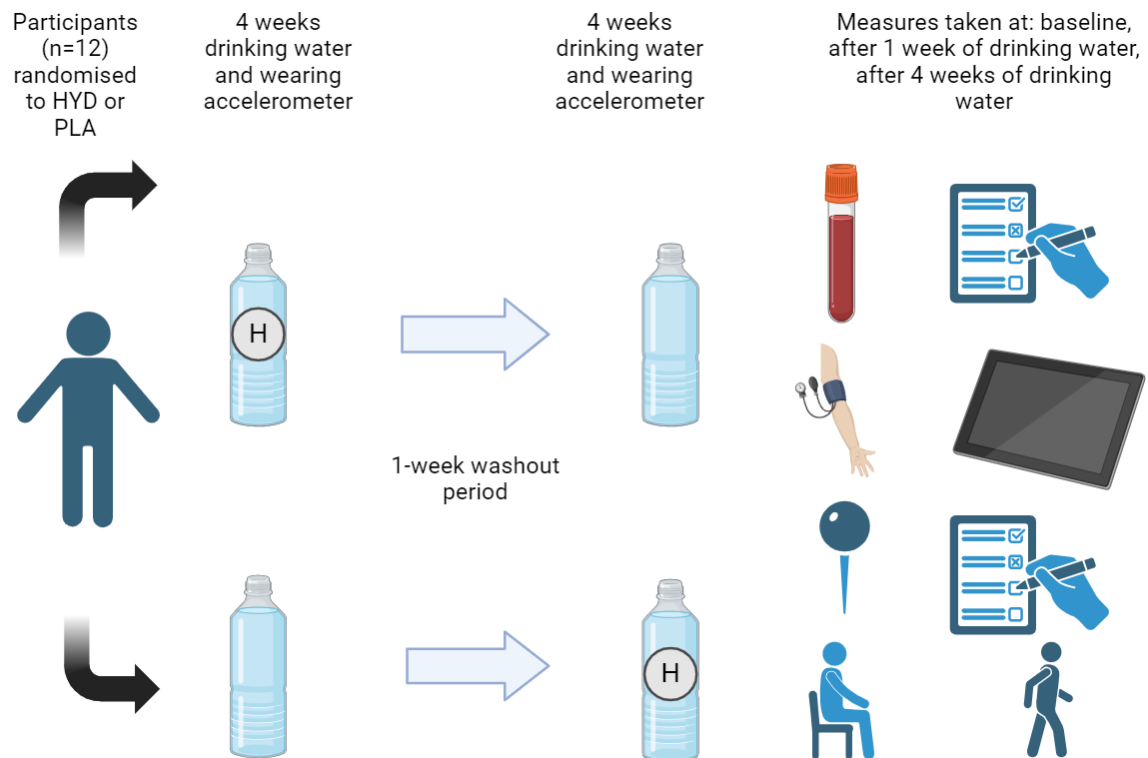


Figure 7.1. Study schema. 12 participants were randomised into receiving hydrogen (HYD) or placebo (PLA) water first. Participants were given an activity tracker to wear continuously (except when bathing/swimming) for four weeks, with one week of not drinking study water/wearing activity tracker. Blood samples were collected for the measurement of: Brain Derived Neurotrophic Factor, Heat shock protein 70, Alpha-synuclein, Interleukin-6, 4-Hydroxynonenal, Tumor Necrosis Factor-alpha, C-reactive protein and Total Antioxidant Capacity. Other measures were Profile of Mood States-Short Form, Blood Pressure, Flanker Compatibility Task, Purdue Pegboard Test, Movement Disorder Society – Non-motor Rating Scale, Timed Up and Go Test, Six Min Walk Test.

7.3.4. Drinking water

Participants were asked to drink one litre of study water per day (ad libitum) (in replacement of their normal drinking water, but other fluid consumption was not restricted including additional ‘normal’ water and hot drinks).

Hydrogen rich water

A 240 mL hydrogen water bottle was used in the study (Osmio Solutions Ltd, Cliffe, Kent, UK). Participants were asked to drink four bottles (220 mL) and one bottle of 120 mL, with markings on the bottle where the participant should drink to. The membrane could not be allowed to dry out (therefore participants were instructed to leave some water in), hence why the bottle only contained 220 mL (and not 240 mL). Participants were given a charging cable and advised to charge the bottle every other day (or when needed). Two participants had issues with the water bottle not generating as many bubbles as usual, and as a result their water bottles were changed as soon as possible after being notified they were not working properly (this was within 48 hr).

Control condition

A 600 mL water bottle (Brita Water Filter Systems Ltd, Oxfordshire, UK) was used as a placebo water bottle. Participants were instructed to drink one full bottle (600 mL), and then 400 mL per day. 400 mL was marked on the bottle. A ‘placebo’ water bottle could not be manufactured by Osmio Solutions Ltd. There was an element of deception in the study as participants were told that the research team were interested in the potential effects of different H₂ water types and were not aware that the placebo water did not contain any additional H₂. This was to protect against any ‘expectancy’ effects and participants were debriefed after all participants had completed the study.

Water requirements of the bottles

Tap water in Canterbury, UK contained around 225 parts per million (ppm) TDS (Hommix Water TDS-3 and Temperature Meter, Enfield, UK) (see Chapter 6 for further water tests and comparisons). The manufacturer claimed that distilled water or reverse osmosis water gave the best H₂ saturation. To make the water consumption more convenient for participants, it was decided to allow them to use tap water in the H₂ water bottle. Some participants visited the Lab using public transport, so it seemed unfeasible to ask them to carry large amounts of bottled water with them.

7.3.5. Measures

Participants attended the SSES Research Laboratory on six occasions.

Measures were taken in the same order at each visit; Blood samples, Profile of Mood States – Short Form (POMS-SF), Blood Pressure (BP), Flanker Compatibility Task (Flanker), Purdue Pegboard Test (PPT), Movement Disorder Society - Non-Motor Rating Scale (MDS-NMS), Timed Up and Go Test (TUG), Six-minute Walk test (6MWT).

Anthropometric measurements

Height and weight were measured (and BMI calculated) at the initial visit. Please see General Methods (Chapter 2.3) for further information on how this was carried out.

Blood samples

Blood samples at all six time points were taken (some participants were not included in this due to being unable to locate veins). Please see General Methods (Chapter 2.6) for further information on how this was carried out.

Blood analysis

Total Brain-Derived Neurotrophic Factor (BDNF)

Free and Trk-bound BDNF (Total BDNF) (Manufacturer's reported Intra-assay CV = 2.4% - 3.2%) were determined by enzyme-linked immunosorbent assay (ELISA) (Catalogue number: DBNT00, R&D Systems, Bio-Techne Ltd, Abingdon, UK) was determined in platelet poor plasma (PPP) in accordance with manufacturer instructions. To collect PPP, venous blood (collected in heparin vacutainers) (BD Vacutainer™) see (General Methods Chapter 2.6.1 for further explanation on venous blood collection) was centrifuged at 1500 relative centrifugal force (RCF) at 4°C for 10 min (Eppendorf UK Ltd, Stevenage, UK) immediately after collection. Clear plasma was pipetted into 1.5mL aliquots. This aliquot was then further centrifuged at 10,000 g for ten minutes at 4°C (AccuSpin Micro 17R, Fisher Scientific UK Ltd, Loughborough, UK). The top 0.5 mL of plasma was pipetted into a new aliquot and stored at –80°C until analysis.

Heat Shock Protein 70 (HSP70)

Plasma HSP70 levels were determined by ELISA (Manufacturer's reported Intra-assay CV = 1.8% - 13.2%)(Catalogue number: BMS2087, Invitrogen, Thermo Fisher Scientific, Loughborough, UK) in accordance with manufacturer instructions.

Human alpha-synuclein (α -syn)

Plasma α -syn levels were determined by ELISA (Manufacturer's reported Intra-assay CV = <10%)(Catalogue number: ELH-SNCA, RayBiotech, Georgia, USA) in accordance with manufacturer instructions.

Interleukin-6 (IL-6)

Plasma IL-6 levels were determined by ELISA (Manufacturer's reported Intra-assay CV = 3-5.8%)(Catalogue number: Q6000B, R&D Systems, Bio-Techne Ltd, Abingdon, UK) in accordance with manufacturer instructions.

4-Hydroxynonenal (4-HNE)

Plasma 4-HNE levels were determined by ELISA (Manufacturer's reported Intra-assay CV = <8%)(Catalogue number: EKF60360, Biomatik Corporation, Ontario, Canada) in accordance with manufacturer instructions.

Tumour Necrosis Factor alpha (TNF- α)

Plasma TNF- α levels were determined by ELISA (Manufacturer's CV = 2.8% - 8.2%)(Catalogue number: QTA00C, R&D Systems, Bio-Techne Ltd, Abingdon, UK) in accordance with manufacturer instructions.

C-Reactive Protein (CRP)

Plasma CRP levels were determined by ELISA (Manufacturer's reported CV = 3.6% - 3.7%) (Catalogue number: DCRP00B, R&D Systems, Bio-Techne Ltd, Abingdon, UK) in accordance with manufacturer instructions.

Total antioxidant capacity

Plasma antioxidant capacity was determined by a chemiluminescence assay kit (No CV available from manufacturer) (ABEL Antioxidant Test Kit for Hydroxyl Radicals with Pholasin, Knight Scientific Ltd, Plymouth, UK) in accordance with manufacturer instructions.

Profile of Mood States – Short Form (POMS-SF) (Grove, 1992)

Please see General Methods (Chapter 2.7) for further information on this.

Blood pressure

Systolic and diastolic blood pressure were measured. Please see General Methods (Chapter 2.4) for further information on this.

Flanker Compatibility Task (Presentation, Neurobehavioural Systems, Inc, California, USA)

Please see General Methods (Chapter 2.8) for further information on this.

Purdue Pegboard Test (PPT) (Tiffin & Asher, 1948)

Please see General Methods (Chapter 2.9) for further information on this.

Movement Disorder Society – Non-Motor Rating Scale

Please see General Methods (Chapter 2.10) for further information on this.

Timed up and Go Test (TUG)

Please see General Methods (Chapter 2.11.1) for further information on this.

6 min walk test (6MWT)

Please see General Methods (Chapter 2.11.2) for further information on this.

Accelerometer (GENEActiv)

Participants were instructed to wear the activity tracker around their waist 24 hours a day, seven days a week for a total of four weeks, then the activity tracker was returned to the researcher for one week, and then commencement of wearing the tracker started again after one week 'washout period'. Please see General Methods (Chapter 2.12) for further information on this.

Sample analysis

Please see General Methods (Chapter 2.6.3) for further information on this.

Statistical analysis

For repeated measures, assumptions of normal distribution of data were checked using the Shapiro-Wilk test (and Z scores for skewness and kurtosis for confirmation). Data that was not normally distributed were transformed prior to analysis to normalise the data distribution. The following transformations were attempted, and the method that provided the best normalisation (based on Z-scores) was used to transform these data for analysis: log, square root, cube root, power (x cubed), inverse and arcsine. Outliers were assessed by boxplot. Non extreme outliers were identified as not greater than three times the interquartile range from a quartile, while extreme outliers were identified as greater than three times the interquartile range from a quartile (Tukey, 1977). If outliers were identified by box plot, these were both included and excluded in the data analysis. Variables of IL-6 (with outlier included), CRP, systolic and diastolic blood pressure and Flanker Reaction Time (None) needed to be transformed before analysis.

The following data were analysed using a two-way analysis of variance (ANOVA): blood pressure, flanker compatibility task, functional movement, the International Parkinson and Movement Disorder Society Non-motor rating scale, Profile of Mood States (short form), Purdue Pegboard Test, plasma samples. Where the assumption of sphericity was violated (determined by Mauchly's test), the Greenhouse-Geiser adjustment was applied to the degrees of freedom.

Dependent (paired) samples t-test was used to assess differences in the means of placebo and hydrogen rich water trials of GENEActiv accelerometer if data was normally distributed (for sleep and sedentary time). If data was not normally distributed, a Wilcoxon signed-rank test was performed (for step count, light activity, moderate to vigorous physical activity and non-wear time).

Statistical significance level was set to $P < 0.05$ and analysis was performed with IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA)

7.4. RESULTS

Participant characteristics are presented in Table 7.1

Table 7.1. Participant characteristics.

<i>Variable</i>	<i>Mean ± SD</i>	<i>Minimum</i>	<i>Maximum</i>
Age (years)	68 ± 8	51	78
Sex	M (n = 8) F (n=4)		
Height (m)	1.71 ± 0.08	154	180
Weight (kg)	73.83 ± 12.63	51	99.3
BMI (kg/m ²)	25.06 ± 2.71	21.5	30.5
Age of Disease Onset Age (years)	64 ± 9.88	46	76
Disease Duration (years)	4 ± 3.89	0.5	14
Hoehn and Yahr stage	1.67 ± 0.78	1	3
Levodopa equivalent daily dose (mg)	486 ± 199	400	600
Employment Status	Retired = 9 Full-time employment = 1 Part-time employment= 2		
Medical conditions	Enlarged prostate, m = 1 Overactive Bladder, m =1 Atrial Fibrillation, m =1 f = 1 Hypercholesterolemia, m = 2 f = 1 Hypertension, m = 1 f = 2 Microvascular angina, m = 1 Depression, m = 2 Vitamin D3 deficiency, m = 1 Deep Vein Thrombosis, m = 1 Constipation, m = 1, f = 1		

Data presented as mean ± standard deviation.

7.4.1.1. BLOOD BIOMARKERS

Results for blood biomarkers are presented in Table 7.2

Table 7.2. Blood biomarkers at baseline, week 1 and week 4 of condition (PLA and HRW).

2-way ANOVA

<i>Biomarker</i>	<i>N</i>	<i>Condition</i>	<i>Baseline</i>	<i>Week 1</i>	<i>Week 4</i>	<i>Condition</i>	<i>Time</i>	<i>Interaction</i>
BDNF (pg/mL) (values expressed as $\times 10^{-3}$)	7	PLA	1.60 $\pm$ 0.79	2.07 $\pm$ 1.62	1.49 $\pm$ 0.79	P = 0.624 ES = 0.043	P = 0.184 ES = 0.246	P = 0.932 ES = 0.012
		HYD	1.84 $\pm$ 1.77	1.64 $\pm$ 0.67	1.35 $\pm$ 0.59			
Hsp70 (ng/mL)	7	PLA	2.19 $\pm$ 1.47	2.46 $\pm$ 1.25	2.95 $\pm$ 2.21	P = 0.563 ES = 0.059	P = 0.918 ES = 0.014	P = 0.530 ES = 1
		HYD	3.22 $\pm$ 2.01	2.99 $\pm$ 1.69	2.39 $\pm$ 1.05			
A-syn (ng/mL)	5	PLA	2.60 $\pm$ 1.09	2.71 $\pm$ 1.52	3.01 $\pm$ 1.699	P = 0.911 ES = 0.004	P = 0.266 ES = 0.282	P = 0.985 ES = 0.004
		HYD	2.61 $\pm$ 1.67	2.85 $\pm$ 0.86	3.06 $\pm$ 0.93			
IL-6 (pg/mL)+	7	PLA	2.33 $\pm$ 1.22	1.68 $\pm$ 0.52	1.53 $\pm$ 0.46	P = 0.362 ES = 0.139	P = 0.555 ES = 0.093	P = 0.045* ES = 0.403
		HYD	5.20 $\pm$ 9.40	5.21 $\pm$ 9.06	5.19 $\pm$ 7.85			
IL-6 (pg/mL)++	6	PLA	1.97 $\pm$ 0.84	1.75 $\pm$ 0.54	1.48 $\pm$ 0.48	P = 0.928 ES = 0.002	P = 0.918 ES = 0.017	P = 0.049* ES = 0.454
		HYD	1.66 $\pm$ 0.89	1.80 $\pm$ 0.94	2.33 $\pm$ 2.30			
4-HNE (pg/mL) (values expressed as $\times 10^{-3}$)	6	PLA	1.57 $\pm$ 0.32	1.49 $\pm$ 0.20	1.52 $\pm$ 0.30	P = 0.652 ES = 0.044	P = 0.593 ES = 0.099	P = 0.851 ES = 0.032
		HYD	1.75 $\pm$ 0.47	1.54 $\pm$ 0.6	1.64 $\pm$ 0.36			
TNF-α (pg/mL)	7	PLA	7.80 $\pm$ 1.65	7.63 $\pm$ 1.97	7.99 $\pm$ 2.67	P = 0.530 ES = 0.069	P = 0.831 ES = 0.030	P = 0.957 ES = 0.007
		HYD	7.93 $\pm$ 0.96	7.95 $\pm$ 0.811	8.44 $\pm$ 1.76			
CRP (ng/mL) (values expressed as $\times 10^{-3}$)	11	PLA	2.20 $\pm$ 3.15	3.07 $\pm$ 3.52	2.86 $\pm$ 4.32	P = 0.724 ES = 0.013	P = 0.538 ES = 0.060	P = 0.504 ES = 0.066
		HYD	2.96 $\pm$ 3.54	2.53 $\pm$ 3.92	1.83 $\pm$ 3.11			
Antioxidant Capacity (mmol/-L)	11	PLA	7.68 $\pm$ 2.99	7.1 $\pm$ 3.44	7.35 $\pm$ 2.69	P = 0.049** ES = 0.403	P = 0.765 ES = 0.033	P = 0.608 ES = 0.060
		HYD	9.22 $\pm$ 3.76	9.57 $\pm$ 5.73	8.28 $\pm$ 3.65			

BDNF = Brain Derived Neurotrophic Factor, Hsp70 = Heat shock protein 70, A-syn = alpha-synuclein, IL-6 = Interleukin-6, 4-HNE = 4-Hydroxynonenal, TNF- α = Tumour Necrosis Factor – alpha, CRP = C-reactive protein, Antioxidant Capacity as measured in D-mannitol equivalent units. Data presented as mean $\pm$ standard deviation. *Significant condition x time interaction. **Significant effect of condition. + = IL-6 with outlier included, ++ = IL-6 outlier excluded.

Total Brain-derived neurotrophic factor (BDNF) in platelet poor plasma

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's BDNF levels ($F(1,6)$, 0.266, $p = 0.624$, $\eta^2 = 0.043$). There was no significant main effect of time ($F(2,12)$, 1.955, $p = 0.184$, $\eta^2 = 0.246$), or condition x time interaction ($F(2,12)$, 0.071, $p = 0.932$, $\eta^2 = 0.012$).

Heat Shock Protein 70 (HSP70) in Plasma

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's HSP70 levels ($F(1,6)$, 0.375, $p = 0.563$, $\eta^2 = 0.059$). There was no significant main effect of time ($F(2,12)$, 0.086, $p = 0.918$, $\eta^2 = 0.014$), or condition x time interaction ($F(2,12)$, 0.670, $p = 0.530$, $\eta^2 = 1$).

Alpha-synuclein (α -syn) in Plasma

Two participants were removed from the data due to negative numbers. One outlier was identified by box plot, but this was not considered extreme (not greater than three times the interquartile range from a quartile), therefore it was included in the results.

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's α -syn levels ($F(1,4)$, 0.014, $p = 0.911$, $\eta^2 = 0.004$). There was no significant main effect of time ($F(1, 4)$, 1.568, $p = 0.266$, $\eta^2 = 0.282$), or condition x time interaction ($F(1,4)$, 0.015, $p = 0.985$, $\eta^2 = 0.004$).

Interleukin-6 (IL-6) in Plasma

Two outliers in the data were identified by box plot. One outlier was included in the data analysis as it was not considered extreme (not greater than three times the interquartile range from a quartile), while one outlier was removed as it was considered extreme (greater than 3 times the interquartile range from a quartile). Two sets of results are reported for IL-6, the first set of results with the outlier included in the data analysis. The second report excludes the extreme outlier.

IL-6 with outlier included in data analysis

Log transformed data results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's IL-6 levels ($F(1,6)$, 0.971, $p = 0.362$, $\eta^2 = 0.139$), or time ($F(2,12)$, 0.618, $p = 0.555$, $\eta^2 = 0.093$). There was an interaction between condition and time ($F(2,12)$, 4.042, $p = 0.045$, $\eta^2 = 0.403$). Post hoc analysis did not show any significant differences between time points.

IL-6 with outlier removed in data analysis

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's IL-6 levels ($F(1,6)$, 0.009, $p = 0.928$, $\eta^2 = 0.002$), or time ($F(2,12)$, 0.087, $p = 0.918$, $\eta^2 = 0.017$). There was an interaction between condition and time ($F(2,12)$, 4.151, $p = 0.049$, $\eta^2 = 0.454$).

IL-6 results are presented in Figure 7.2

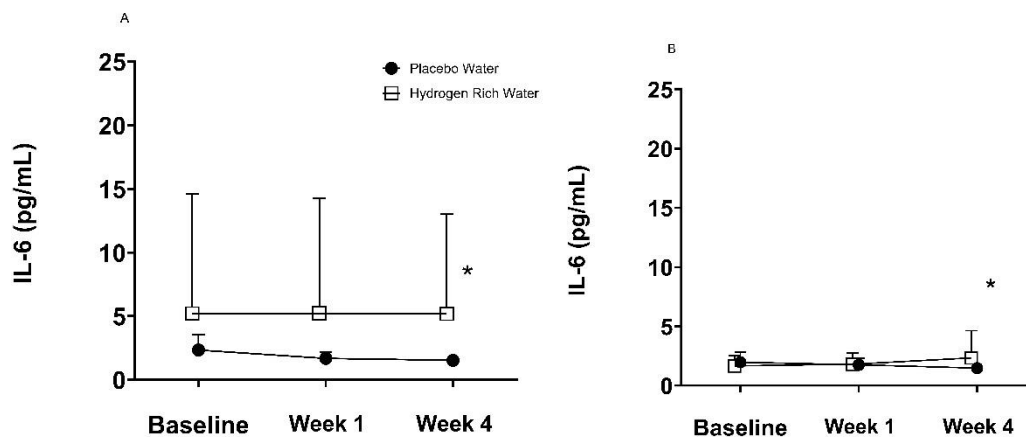


Figure 7.2. IL-6 results for placebo water and hydrogen rich water, measured at baseline, week 1, and week 4. A = Extreme outlier included in data set. B Extreme outlier removed from data set. Data presented as mean $\pm$ standard deviation. *Significant time x condition interaction.

Tumour Necrosis Factor alpha (TNF- α) in Plasma

One outlier in the data was identified by box plot and was not considered extreme (not greater than three times the interquartile range from a quartile) and was included in the data analysis.

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's TNF- α levels ($F(1,6)$, 0.445, $p = 0.530$, $\eta^2 = 0.069$). There was no significant main effect of time ($F(2,12)$, 0.188, $p = 0.831$, $\eta^2 = 0.030$), or condition x time interaction ($F(2,12)$, 0.044, $p = 0.957$, $\eta^2 = 0.007$).

4-Hydroxynonenal (4-HNE) in Plasma

One participant was removed due to incomplete data. Two outliers were identified by box plot but were not considered extreme (not greater than three times the interquartile range from a quartile), therefore were included in the results.

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's 4-HNE levels ($F(1,5)$, 0.230, $p = 0.652$, $\eta^2 = 0.044$). There was no significant main effect of time ($F(2,10)$, 0.551, $p = 0.593$, $\eta^2 = 0.099$), or condition x time interaction ($F(2,10)$, 0.164, $p = 0.851$, $\eta^2 = 0.032$).

C-Reactive Protein (CRP) in Plasma

One outlier was identified by box plot but was not considered extreme (not greater than three times the interquartile range from a quartile), therefore it was included in the results.

Log transformed data results of the two-way repeated measures ANOVA revealed that there was no significant main effect of condition on participant's CRP levels ($F(1,10)$, 0.132, $p = 0.724$, $\eta^2 = 0.013$). There was no significant main effect of time ($F(2,20)$, 0.639, $p = 0.538$, $\eta^2 = 0.060$), or condition x time interaction ($F(2,20)$, 0.709, $p = 0.504$, $\eta^2 = 0.066$).

Total antioxidant capacity in Plasma as measured in D-mannitol equivalent units

Five outliers were identified by box plot, and all were considered extreme (greater than three times the interquartile range from a quartile). Removing these extreme outliers would have left two sets of data to analyse, so they were included in the analysis.

A significant main effect of condition on participant's total antioxidant capacity was observed ($F(1,8) = 5.407$, $p = 0.049$, $\eta^2 = 0.403$). No significant main effect of time was observed, (F

2,16) = 0.273, $p = 0.765$, $\eta^2 = 0.033$), or condition x time interaction ($F(2,16) = 0.513$, $p = 0.608$, $\eta^2 = 0.060$).

7.4.1.2. BLOOD BIOMARKERS AS PERCENT CHANGE FROM BASELINE

Blood biomarker changes are presented in Table 7.3

Table 7.3. Blood biomarkers as percentage change from baseline of condition (PLA and HRW)

2-way ANOVA effects

<i>Biomarker</i>	<i>N</i>	<i>Condition</i>	<i>Baseline</i>	<i>Week 1 % change</i>	<i>Week 4 % change</i>	<i>Condition</i>	<i>Time</i>	<i>Interaction</i>
<i>BDNF</i>	7	PLA	100	147.38	96.94	P = 0.699	P = 0.104	P = 0.806
		HYD	100	120.91	93.76	ES = 0.027	ES = 0.366	ES = 0.035
<i>Hsp70</i>	7	PLA	100	121.04	131.08	P = 0.578	P = 0.463	P = 0.847
		HYD	100	99.89	117.58	ES = 0.055	ES = 0.120	ES = 0.027
<i>A-syn</i>	5	PLA	100	99.49	118.99	P = 0.501	P = 0.182	P = 0.642
		HYD	100	133.59	137.20	ES = 0.120	ES = 0.347	ES = 0.105
<i>IL-6 +</i>	7	PLA	100	86.03	77.16	P = 0.035*	P = 0.933	P = 0.050
		HYD	100	107.09	117.69	ES = 0.550	ES = 0.012	ES = 0.393
<i>IL-6 ++</i>	6	PLA	100	95.58	83.29	P = 0.196	P = 0.967	P = 0.266
		HYD	100	108.77	114.40	ES = 0.261	ES = 0.006	ES = 0.201
<i>4-HNE</i>	6	PLA	100	98.24	99.22	P = 0.850	P = 0.803	P = 0.898
		HYD	100	91.64	100.08	ES = 0.008	ES = 0.043	ES = 0.021
<i>TNF-α</i>	7	PLA	100	100.68	109.97	P = 0.972	P = 0.687	P = 0.978
		HYD	100	101.63	107.96	ES = 0.000	ES = 0.061	ES = 0.004
<i>CRP</i>	11	PLA	100	164.27	170.07	P = 0.150	P = 0.498	P = 0.471
		HYD	100	92.83	101.78	ES = 0.196	ES = 0.067	ES = 0.073
<i>Total Antioxidant Capacity</i>	11	PLA	100	95.79	98.90	P = 0.820	P = 0.883	P = 0.593
		HYD	100	107.31	93.52	ES = 0.007	ES = 0.015	ES = 0.063

BDNF = Brain Derived Neurotrophic Factor, Hsp70 = Heat shock protein 70, A – syn = alpha-synuclein, IL-6 = Interleukin-6, 4-HNE = 4-Hydroxynonenal, TNF-α - Tumour Necrosis Factor – alpha, CRP = C-reactive protein, Antioxidant Capacity as measured in D-mannitol equivalent units. + = IL-6 with outlier included, ++ = IL-6 outlier excluded. *Significant effect of condition.

Total Brain-derived neurotrophic factor (BDNF) in platelet poor plasma

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's BDNF levels ($F(1,6)$, 0.165, $p = 0.699$, $\eta^2=0.027$). There was no significant main effect of time ($F(1,138)$, 6.828, 3.458, $p = 0.104$, $\eta^2=0.366$), or condition x time interaction ($F(2,12)$, 0.219, $p = 0.806$, $\eta^2=0.035$).

Heat Shock Protein 70 (HSP70)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's HSP70 levels ($F(1,6)$, 0.346, $p = 0.578$, $\eta^2=0.055$). There was no significant main effect of time ($F(2,12)$, 0.821, $p = 0.463$, $\eta^2=0.120$), or condition x time interaction ($F(1.148, 6.888)$, 0.169, $p = 0.847$, $\eta^2=0.027$).

Alpha-synuclein (α -syn)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's α -syn levels ($F(1,4)$, 0.546, $p = 0.501$, $\eta^2=0.120$). There was no significant main effect of time ($F(1, 4)$, 2.121, $p = 0.182$, $\eta^2=0.347$), or condition x time interaction ($F(1,4)$, 0.469, $p = 0.642$, $\eta^2=0.105$).

Interleukin-6 (IL-6) including outlier

Results of the two-way repeated measures ANOVA revealed a significant main effect of condition on participant's IL-6 levels ($F(1,6)$, 7.322, $p = 0.035$, $\eta^2=0.550$). There was no significant main effect of time ($F(2,12)$, 0.070, $p = 0.933$, $\eta^2=0.012$), or condition x time interaction ($F(2,12)$, 3.878, $p = 0.050$, $\eta^2=0.393$).

Interleukin-6 (IL-6) excluding outlier

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's IL-6 levels ($F(1,6)$, 2.120, $p = 0.196$, $\eta^2=0.261$). There was no significant main effect of time ($F(2,12)$, 0.034, $p = 0.967$, $\eta^2=0.006$), or condition x time interaction ($F(1.114, 6.687)$, 1.510, $p = 0.266$, $\eta^2=0.201$).

IL-6 changes are presented in Figure 7.3

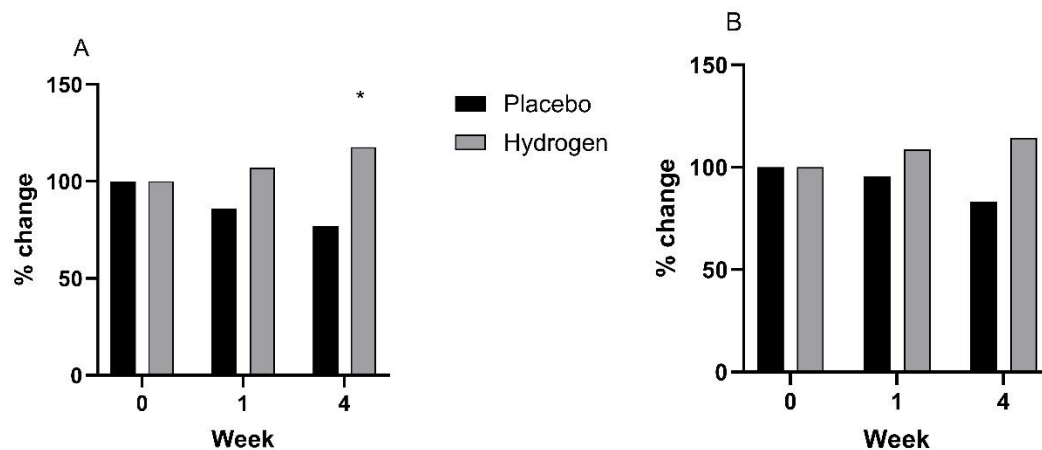


Figure 7.3. IL-6 percent change from baseline results for placebo water and hydrogen rich water, measured at baseline (0), week 1 (1), and week 4 (4). A = Extreme outlier included in data set. B = Extreme outlier removed from data set. *Significant main effect of condition.

4-Hydroxynonenal (4-HNE)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's 4-HNE levels ($F(1,5)$, 0.040, $p = 0.850$, $\eta^2 = 0.008$). There was no significant main effect of time ($F(2,10)$, 0.224, $p = 0.803$, $\eta^2 = 0.043$), or condition x time interaction ($F(2,10)$, 0.109, $p = 0.898$, $\eta^2 = 0.021$).

Tumour Necrosis Factor alpha (TNF- α)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's TNF- α levels ($F(1,6)$, 0.001, $p = 0.972$, $\eta^2 = 0.000$). There was no significant main effect of time ($F(2,12)$, 0.387, $p = 0.687$, $\eta^2 = 0.061$), or condition x time interaction ($F(2,12)$, 0.022, $p = 0.978$, $\eta^2 = 0.004$).

C-Reactive Protein (CRP) in Plasma

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's CRP levels ($F(1,10)$, 2.432, $p = 0.150$, $\eta^2 = 0.196$). There was no

significant main effect of time ($F(2,20)$, 0.721, $p = 0.498$, $\eta^2=0.067$), or condition x time interaction ($F(2,20)$, 0.783, $p = 0.471$, $\eta^2=0.073$).

Total antioxidant capacity in Plasma as measured in D-mannitol equivalent units

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participant's total antioxidant levels ($F(1,8)$, 0.055, $p = 0.820$, $\eta^2=0.007$). There was no significant main effect of time ($F(2,12)$, 0.125, $p = 0.883$, $\eta^2=0.015$), or condition x time interaction ($F(2,12)$, 0.540, $p = 0.593$, $\eta^2=0.063$).

7.4.2. PROFILE OF MOOD STATES (POMS) (SHORT-FORM)

Profile of Mood States short form results are presented in Table 7.4

Table 7.4. Profile of Mood States short form. (n = 12).

2-way RM ANOVA main effects

<i>Subscale</i>	<i>Condition</i>	<i>Baseline</i>	<i>Week 1</i>	<i>Week 4</i>	<i>Condition</i>	<i>Time</i>	<i>Interaction</i>
<i>Tension</i>	PLA	4.25 ± 5.75	4.75 ± 5.46	4.58 ± 5.64	P = 0.910	P = 0.826	P = 0.539
	HYD	4.58 ± 6.85	4.16 ± 4.64	5 ± 5.325	ES = 0.001	ES = 0.017	ES = 0.055
<i>Anger</i>	PLA	1.58 ± 3.75	2.16 ± 4.40	1.41 ± 2.81	P = 1	P = 0.637	P = 0.96
	HYD	1.75 ± 2.52	1.0 ± 1.53	2.41 ± 3.55	ES = 0.000	ES = 0.040	ES = 0.192
<i>Fatigue</i>	PLA	4.58 ± 5.64	4.5 ± 5.56	4.16 ± 4.01	P = 0.826	P = 0.841	P = 0.360
	HYD	4.33 ± 4.86	4.0 ± 5.16	5.25 ± 5.17	ES = 0.005	ES = 0.016	ES = 0.081
<i>Depression</i>	PLA	3.50 ± 6.48	2.91 ± 5.26	3.75 ± 5.78	P = 0.342	P = 0.254	P = 0.148
	HYD	3.50 ± 5.86	2.83 ± 4.01	5.33 ± 6.86	ES = 0.082	ES = 0.117	ES = 0.160
<i>ERA</i>	PLA	12.50 ± 5.01	13.3 ± 5.804	12.5 ± 5.86	P = 0.583	P = 0.297	P = 0.351
	HYD	12.50 ± 5.86	12.91 ± 5.68	12.91 ± 5.40	ES = 0.028	ES = 0.104	ES = 0.091
<i>Vigour</i>	PLA	8.08 ± 4.52	8.91 ± 5.26	7.91 ± 5.88	P = 0.415	P = 0.463	P = 0.716
	HYD	9.41 ± 5.66	9.0 ± 5.29	8.41 ± 4.44	ES = 0.061	ES = 0.068	ES = 0.030
<i>Confusion</i>	PLA	3.0 ± 4.22	3.08 ± 4.27	2.5 ± 2.19	P = 0.368	P = 0.588	P = 0.848
	HYD	2.41 ± 2.50	2.50 ± 2.71	2.41 ± 2.99	ES = 0.074	ES = 0.047	ES = 0.015
<i>TMD</i>	PLA	97.16 ± 29.53	94.58 ± 30.44	94.08 ± 24.99	P = 0.620	P = 0.597	P = 0.392
	HYD	94.58 ± 26.17	93.58 ± 20.75	100.16 ± 30.57	ES = 0.023	ES = 0.034	ES = 0.082

ERA = esteem related affect. TMD = Total mood which is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: TMD = [TEN+DEP+ANG+FAT+CON] – [VIG+ERA]. POMS-SF items are scored 0-4 (0 = not at all, 1 = a little, 2 = moderate, 3 = quite a lot, 4 = extremely.) There are seven subscales. Two items

are reversed scores: ashamed and embarrassed (both part of the ERA subscale). Data presented as mean $\pm$ standard deviation.

POMS Tension

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS Tension subscale ($F(1,11) = 0.013$, $p = 0.910$, $\eta^2 = 0.001$). There was no significant main effect of time ($F(2,22) = 0.193$, $p = 0.826$, $\eta^2 = 0.017$), or condition x time interaction ($F(2,22) = 0.635$, $p = 0.539$, $\eta^2 = 0.055$).

POMS Anger

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS Anger subscale ($F(1,11) = 0.000$, $p = 1.000$, $\eta^2 = 0.000$). There was no significant main effect of time ($F(2,22) = 0.460$, $p = 0.637$, $\eta^2 = 0.040$), or condition x time interaction ($F(2,22) = 2.613$, $p = 0.096$, $\eta^2 = 0.192$).

POMS Fatigue

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS Fatigue subscale ($F(1,11) = 0.050$, $p = 0.826$, $\eta^2 = 0.005$). There was no significant main effect of time ($F(2,22) = 0.175$, $p = 0.841$, $\eta^2 = 0.016$), or condition x time interaction ($F(1.203,13.231) = 0.970$, $p = 0.360$, $\eta^2 = 0.081$).

POMS Depression

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS depression subscale ($F(1,11) = 0.987$, $p = 0.342$, $\eta^2 = 0.082$). There was no significant main effect of time ($F(2,22) = 1.460$, $p = 0.254$, $\eta^2 = 0.117$), or condition x time interaction ($F(2,22) = 2.088$, $p = 0.148$, $\eta^2 = 0.160$).

POMS Esteem-related Affect (ERA)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS ERA subscale ($F(1,11)$, 0.320, $p = 0.583$, $\eta^2 = 0.028$). There was no significant main effect of time ($F(2,22)$, 1.283, $p = 0.297$, $\eta^2 = 0.104$), or condition x time interaction ($F(2,22)$, 1.097, $p = 0.351$, $\eta^2 = 0.091$).

POMS Vigour

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS Vigour subscale ($F(1,11)$, 0.716, $p = 0.415$, $\eta^2 = 0.061$). There was no significant main effect of time ($F(2,22)$, 0.797, $p = 0.463$, $\eta^2 = 0.068$), or condition x time interaction ($F(2,22)$, 0.340, $p = 0.716$, $\eta^2 = 0.030$).

POMS Confusion

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS Confusion subscale ($F(1,11)$, 0.880, $p = 0.368$, $\eta^2 = 0.074$). There was no significant main effect of time ($F(2,22)$, 0.544, $p = 0.588$, $\eta^2 = 0.047$), or condition x time interaction ($F(2,22)$, 0.166, $p = 0.848$, $\eta^2 = 0.015$).

POMS TMD

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the POMS TMD ($F(1,11)$, 0.261, $p = 0.620$, $\eta^2 = 0.023$). There was no significant main effect of time ($F(1,310)$, 14.406, 0.391, $p = 0.597$, $\eta^2 = 0.034$), or condition x time interaction ($F(2,22)$, 0.978, $p = 0.392$, $\eta^2 = 0.082$).

7.4.3. BLOOD PRESSURE

Blood pressure results are presented in Table 7.5

Table 7.5. Blood pressure measurements at baseline, week 1 and week 4 of trials (PLA and HRW). (n = 12).

2-way RM ANOVA main effects							
<i>Blood pressure</i>	<i>Condition</i>	<i>Baseline</i>	<i>Week 1</i>	<i>Week 4</i>	<i>Condition</i>	<i>Time</i>	<i>Interaction</i>
<i>Systolic (mm/Hg)</i>	PLA	120 (1)	118 (4)	118 (18)	P = 0.831 ES = 0.004	P = 0.212 ES = 0.132	P = 0.901 ES = 0.009
	HYD	118 (20)	116 (16)	118 (20)			
<i>Diastolic (mm/Hg)</i>	PLA	76 (11)	76 (13)	76 (15)	P = 0.964 ES = 0.000	P = 0.187 ES = 0.141	P = 0.177 ES = 0.146
	HYD	79 (14)	75 (14)	75 (15)			

Blood pressure measured in millimetres of mercury (mmHg). Data presented as median (IQR).

Systolic BP (mm/Hg)

Log transformed data results of a two-way repeated measures ANOVA revealed no significant main effect of condition on systolic blood pressure ($F(1,11) = 0.048$, $p = 0.831$, $\eta^2 = 0.004$). There was no significant main effect of time, ($F(2,22) = 1.668$, $p = 0.212$, $\eta^2 = 0.132$), or time x condition interaction ($F(2,22) = 0.105$, $p = 0.901$, $\eta^2 = 0.009$).

Diastolic BP (mm/Hg)

Log transformed data results of a two-way repeated measures ANOVA revealed no significant main effect of condition on diastolic blood pressure ($F(1,11) = 0.002$, $p = 0.964$, $\eta^2 = 0.000$). No significant main effects of time ($F(2,22) = 1.811$, $p = 0.187$, $\eta^2 = 0.141$), or condition x time interaction ($F(2,22) = 1.873$, $p = 0.177$, $\eta^2 = 0.146$) were observed.

7.4.4. FLANKER COMPATIBILITY TASK

7.4.4.1. Flanker – Accuracy

Flanker test accuracy measurements are presented in Table 7.6

Table 7.6. Flanker test accuracy measurements. (n= 12).

2-way RM ANOVA main effects

<i>Accuracy Subscale</i>	<i>Condition</i>	<i>Baseline</i>	<i>Week 1</i>	<i>Week 4</i>	<i>Condition</i>	<i>Time</i>	<i>Interaction</i>
<i>Compatible</i>	PLA	0.969±0.073	0.989±0.024	0.995±0.179	P = 0.819 ES = 0.005	P = 0.487 ES = 0.052	P = 0.205 ES = 0.134
	HYD	0.989±0.024	0.989±0.024	0.979±0.04			
<i>Incompatible</i>	PLA	0.9689±0.42	0.9740±0.727	0.9481±0.106	P = 0.561 ES = 0.032	P = 0.041* ES = 0.252	P = 0.810 ES = 0.011
	HYD	0.9584±0.076	0.9742±0.0319	0.9377±0.0753			
<i>None</i>	PLA	0.9767±0.463	0.9844±0.0283	0.987±0.021	P = 0.608 ES = 0.025	P = 0.532 ES = 0.056	P = 0.254 ES = 0.118
	HYD	0.997±0.008	0.9844±0.364	0.974±0.373			

Results show accuracy responses to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli), with a maximum score of 1. Data presented as mean ± standard deviation.

*Significant main effect of time.

Accuracy – Compatible

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' Accuracy - Compatible, ($F(1,11) = 0.055$, $p = 0.819$, $\eta^2 = 0.005$). There was no significant main effect of time ($F(1.242,13.663) = 0.599$, $p = 0.487$, $\eta^2 = 0.052$), or condition x time interaction ($F(2,22) = 1.707$, $p = 0.205$, $\eta^2 = 0.134$).

Accuracy – Incompatible

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' Accuracy - Incompatible, ($F(1,11) = 0.359, p = 0.561, \eta^2 = 0.032$).

There was a significant main effect of time ($F(2,22) = 3.714, p = 0.041, \eta^2 = 0.252$). However, post hoc analysis did not identify any differences between individual time points. There was no significant time x condition interaction ($F(1.346,14.801) = 0.117, p = 0.810, \eta^2 = 0.011$).

Accuracy – None

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' Accuracy - None, ($F(1,11) = 0.278, p = 0.608, \eta^2 = 0.025$). There was no significant main effect of time ($F(2,22) = 0.649, p = 0.532, \eta^2 = 0.056$), or condition x time interaction ($F(1.147,12.620) = 1.465, p = 0.254, \eta^2 = 0.118$).

7.4.4.2. Flanker – Reaction Time

Flanker reaction time results are presented in Table 7.7

Table 7.7. Flanker reaction time measurements. (n=12).

2-way RM ANOVA main effects

Reaction Time Subscale	Condition	Baseline	Week 1	Week 4	Condition	Time	Interaction
Compatible	PLA	707.56 (194.10)	686.50 (259.07)	686.03 (259.89)	P = 0.590 ES = 0.027	P = 0.563 ES = 0.039	P = 0.846 ES = 0.015
	HYD	666.15 (152.59)	651.93 (220.21)	644.34 (188.03)			
Incompatible	PLA	728.37 (183.56)	741.37 (210.56)	741.71 (181.89)	P = 0.416 ES = 0.061	P = 0.486 ES = 0.049	P = 0.410 ES = 0.078
	HYD	666.18 (158.26)	675.46 (115.10)	647 (137.53)			
None	PLA	649.92 (179.06)	646.21 (257.79)	622.81 (190.78)	P = 0.921 ES = 0.001	P = 0.135 ES = 0.185	P = 0.573 ES = 0.049
	HYD	659.17 (138.48)	618.26 (145.64)	629.67 (153.82)			

Results show reaction time measurements to different stimuli (compatible stimuli, incompatible stimuli, and no stimuli). Data is shown in milliseconds and presented as median (IQR).

Average Reaction Time – Compatible (ART -C)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' ART - C ($F(1,11) = 0.309$, $p = 0.590$, $\eta^2 = 0.027$). There was no significant main effect of time ($F(1.273,14.003) = 0.443$, $p = 0.563$, $\eta^2 = 0.039$), or condition x time interaction ($F(2,22) = 0.169$, $p = 0.846$, $\eta^2 = 0.015$).

Average Reaction Time – Incompatible (ART-IC)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' ART-IC ($F(1,11) = 0.716, p = 0.416, \eta^2 = 0.061$). There was no significant main effect of time ($F(1.126, 12.387) = 0.565, p = 0.486, \eta^2 = 0.049$), or condition x time interaction ($F(2,22) = 0.928, p = 0.410, \eta^2 = 0.078$).

Average Reaction Time – None (ART-None)

Log transformed results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' ART-None ($F(1,11) = 0.010, p = 0.921, \eta^2 = 0.001$). There was no significant main effect of time ($F(1.171, 12.882) = 2.502, p = 0.135, \eta^2 = 0.185$), or condition x time interaction ($F(2,22) = 0.572, p = 0.573, \eta^2 = 0.049$).

7.4.5. PURDUE PEGBOARD TEST

Purdue Pegboard Test results are presented in Table 7.8

Table 7.8. Gross movement of fingers, hand and arm; and fingertip dexterity measured. (n=12).

2-way RM ANOVA main effects							
<i>PPT</i>	<i>Condition</i>	<i>Baseline</i>	<i>Week 1</i>	<i>Week 4</i>	<i>Condition</i>	<i>Time</i>	<i>Interaction</i>
Right	PLA	10.8 ± 2.89	11.15 ± 3.21	11.01 ± 3.38	P = 0.778	P = 0.979	P = 0.467
	HYD	11.04 ± 2.78	10.76 ± 3.44	10.92 ± 3.17	ES = 0.008	ES = 0.002	ES = 0.067
Left	PLA	10.41 ± 4.03	10.34 ± 4.38	10.74 ± 4.14	P = 0.736	P = 0.496	P = 0.780
	HYD	10.35 ± 3.66	10.38 ± 3.56	10.44 ± 3.65	ES = 0.011	ES = 0.062	ES = 0.022
Both	PLA	7.74 ± 2.95	7.77 ± 3.24	7.88 ± 3.39	P = 0.614	P = 0.984	P = 0.668
	HYD	7.82 ± 2.87	7.71 ± 3.38	7.62 ± 2.98	ES = 0.024	ES = 0.001	ES = 0.036
Right + Left + Both	PLA	29.02 ± 9.64	29.30 ± 10.63	29.71 ± 10.74	P = 0.490	P = 0.564	P = 0.973
	HYD	28.46 ± 9.55	28.54 ± 10.67	28.96 ± 9.71	ES = 0.044	ES = 0.051	ES = 0.003
Assembly	PLA	24.04 ± 7.83	24.76 ± 10.57	24.42 ± 8.75	P = 0.785	P = 0.769	P = 0.799
	HYD	23.85 ± 8.91	23.99 ± 10.11	24.58 ± 10.02	ES = 0.007	ES = 0.024	ES = 0.020

Data shows number of pegs placed in holes using the Purdue Pegboard Test (PPT). Data presented as mean ± standard deviation.

PPT Right Hand

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the PPT Right Hand task ($F(1,11)$, 0.083, $p = 0.778$, $\eta^2 = 0.008$). There was no significant main effect of time ($F(2,22)$, 0.021, $p = 0.979$, $\eta^2 = 0.002$), or condition x time interaction ($F(2,22)$, 0.788, $p = 0.467$, $\eta^2 = 0.067$).

PPT Left Hand

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the PPT Left Hand task ($F(1,11)$, 0.120, $p = 0.736$, $\eta^2 = 0.011$). There was no significant main effect of time ($F(2,22)$, 0.723, $p = 0.496$, $\eta^2 = 0.062$), or condition x time interaction ($F(2,22)$, 0.251, $p = 0.780$, $\eta^2 = 0.022$).

PPT Both hands

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the PPT Both hands task ($F(1,11)$, 0.270, $p = 0.614$, $\eta^2 = 0.024$). There was no significant main effect of time ($F(2,22)$, 0.017, $p = 0.984$, $\eta^2 = 0.001$), or condition x time interaction ($F(2,22)$, 0.412, $p = 0.668$, $\eta^2 = 0.036$).

PPT R, Left, Both total (R+L+B)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the total of PPT R+L+B ($F(1,11)$, 0.511, $p = 0.490$, $\eta^2 = 0.044$). There was no significant main effect of time ($F(2,22)$, 0.589, $p = 0.564$, $\eta^2 = 0.051$), or condition x time interaction ($F(2,22)$, 0.028, $p = 0.973$, $\eta^2 = 0.003$).

PPT Assembly

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the PPT Assembly task ($F(1,11)$, 0.079, $p = 0.785$, $\eta^2 = 0.007$). There was no significant main effect of time ($F(2,22)$, 0.266, $p = 0.769$, $\eta^2 = 0.024$), or condition x time interaction ($F(2,22)$, 0.277, $p = 0.799$, $\eta^2 = 0.020$).

7.4.6. THE INTERNATIONAL PARKINSON AND MOVEMENT DISORDER SOCIETY – NON – MOTOR RATING SCALE

Movement Disorder Society – Non-motor Rating Scale results are presented in Table 7.9 (over page)

Table 7.9. MDS-NMS scores. (n = 12).

Subscale	Condition	Baseline	Week 1	Week 4	2-way ANOVA effects		
					Condition	Time	Interaction
Depression	PLA	14.08±15.62	8.41±10.08	10.66±12.69	P = 0.666 ES = 0.018	P = 0.014* ES = 0.324	P = 0.798 ES = 0.010
	HYD	12.75±13.63	7.41±7.42	11.08±14.52			
Anxiety	PLA	10.66±11.49	6.16±8.56	8.25±7.79	P = 0.901 ES = 0.001	P = 0.012* ES = 0.430	P = 0.896 ES = 0.010
	HYD	11.08±15.14	6.66±10.08	7.91±11.84			
Apathy	PLA	8.33±10.46	1.916±2.968	3.333±6.169	P = 0.675 ES = 0.017	P = 0.012* ES = 0.332	P = 0.522 ES = 0.057
	HYD	7.16±10.70	3.666±6.499	4.666±7.958			
Psychosis	PLA	1.16±2.58	1.08±2.74	0.50±1.73	P = 0.747 ES = 0.010	P = 0.602 ES = 0.035	P = 0.156 ES = 0.171
	HYD	1.0±3.46	0.75±2.59	1.41±4.60			
Impulse Control	PLA	1±3.46	0.75±2.59	0.25±0.86	P = 0.171 ES = 0.163	P = 0.423 ES = 0.062	P = 0.444 ES = 0.054
	HYD	0.66±2.30	0.0±0.0	0.75±2.59			
Cognition	PLA	8.16±8.89	6.5±5.60	6±5.78	P = 0.302 ES = 0.096	P = 0.475 ES = 0.055	P = 0.805 ES = 0.020
	HYD	5.5±5.26	4.66±6.45	4.83±8.8			
Orthostatic Hypotension	PLA	1.08±1.56	1.66±2.01	1.16±2.48	P = 0.217 ES = 0.135	P = 0.238 ES = 0.122	P = 0.097 ES = 0.191
	HYD	4.25±6.26	2.25±3.86	1.08±1.37			
Urinary 1	PLA	9.25±8.83	8.08±9.39	10.0±9.57	P = 0.029^ ES = 0.366	P = 0.137 ES = 0.165	P = 0.890 ES = 0.011
	HYD	6.75±9.22	5.08±6.34	6.66±6.85			
Urinary 2	PLA	9±9.22	7.36±9.5	9.55±9.9	P = 0.051 ES = 0.329	P = 0.125 ES = 0.188	P = 0.622 ES = 0.046
	HYD	7.36±9.42	5.55±6.44	6.36±7.1			
Sexual	PLA	0.0±0.0	0.0±0.0	0.0±0.0	P = 0.339 ES = 0.083	P = 0.384 ES = 0.083	P = 0.384 ES = 0.083
	HYD	0.66±2.30	0.0±0.0	0.0±0.0			
Gastro-intestinal	PLA	4.58±4.03	3.83±4.28	3.66±3.91	P = 0.574 ES = 0.030	P = 0.904 ES = 0.009	P = 0.110 ES = 0.110
	HYD	3.25±3.25	3.50±3.84	4.33±3.33			
Sleep	PLA	12.66±8.92	8.16±7.14	6.750±5.78	P = 0.558 ES = 0.032	P = 0.001* ES = 0.452	P = 0.800 ES = 0.020
	HYD	10.25±7.25	7.66±5.19	6.50±4.62			
Pain	PLA	5.66±5.01	7.08±5.68	4.00±4.86	P = 0.863 ES = 0.003	P = 0.043* ES = 0.248	P = 0.472 ES = 0.055
	HYD	4.66±6.55	6.16±5.74	5.16±4.17			
Other	PLA	4.33±4.75	2.500±3.580	4.333±5.087	P = 1.000 ES = 0.000	P = 0.073 ES = 0.211	P = 0.199 ES = 0.137
	HYD	6.00±7.61	3.250±6.454	1.916±2.968			
Total Score	PLA	80.50±49.69	57.91±29.17	60.41±36.81	P = 0.249 ES = 0.119	P = 0.001* ES = 0.449	P = 0.817 ES = 0.012
	HYD	74.50±46.32	49.08±28.88	54.16±39.36			

Item total is calculated by frequency multiplied by severity. Subscale total is the sum of all Item totals for that subscale. MDS – NMS Total Score is the sum of totals for all Subscales. Data presented as mean $\pm$ standard deviation. *Significant main effect of time for: Depression, Anxiety, Apathy, Sleep, Pain, and Total Score. ^Significant main effect of condition on Urinary. Urinary 1 = including participant who was prescribed urinary frequency medication. Urinary 2 = excluding participant who was prescribed urinary frequency medication.

MDS-NMS scores as percentage change from baseline are presented in Table 7.10

Table 7.10. Total MDS-NMS scores as percentage change from baseline.

<i>Condition</i>	<i>Baseline</i>	<i>Week 1 (% of baseline)</i>	<i>Week 4 (% of baseline)</i>
PLA	100	80	79
HYD	100	76	112

Scores reduce by 20% from baseline for PLA, and reduce by 24% from baseline after one week, and then increase by 12% from baseline for HYD.

Depression

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the depressions subscale/scoring from the MDS-NMS ($F(1,11) = 0.197$, $p = 0.666$, $\eta^2 = 0.018$). There was a significant main effect of time ($F(2,22) = 5.262$, $p = 0.014$, $\eta^2 = 0.324$). Post-hoc analysis showed a significant main effect between baseline and week 1 with values at baseline ($=13.41$) significantly higher ($p = 0.032$) than at week 1 ($=7.91$) but not between any other timepoints. There was no significant condition x time interaction ($F(1.24,13.641) = 0.110$, $p = 0.798$, $\eta^2 = 0.010$).

Anxiety

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the anxiety subscale/scoring from the MDS-NMS ($F(1,11) = 0.016$, $p = 0.901$, $\eta^2 = 0.001$). There was a significant main effect of time ($F(1.114, 12.252) = 8.310$, $p = 0.012$, $\eta^2 = 0.430$). Post-hoc analysis showed a significant main effect between baseline and week 1 with values at baseline ($=10.84$) significantly higher ($p = 0.034$) than week 1 (6.38), and significantly higher ($p = 0.028$) than week 4 (8.08).

There was no significant condition x time interaction ($F(2,22) = 0.111$, $p = 0.896$, $\eta^2 = 0.010$).

Apathy

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the apathy subscale/scoring from the MDS-NMS ($F(1,11) = 0.186$, $p = 0.675$, $\eta^2 = 0.017$). There was a significant main effect of time ($F(2,22) = 5.474$, $p = 0.012$, $\eta^2 = 0.332$), post-hoc analysis showed no difference between any time point. There was no significant condition x time interaction ($F(2,22) = 0.670$, $p = 0.522$, $\eta^2 = 0.057$).

Psychosis

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the psychosis subscale/scoring from the MDS-NMS ($F(1,11) = 0.110$, $p = 0.747$, $\eta^2 = 0.010$). There was no significant main effect of time ($F(1.372, 15.094) = 0.398$, $p = 0.602$, $\eta^2 = 0.035$), or condition x time interaction ($F(1.124, 12.364) = 2.268$, $p = 0.156$, $\eta^2 = 0.171$).

Impulse control

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the impulse control subscale/scoring from the MDS-NMS ($F(1,11) = 2.147$, $p = 0.171$, $\eta^2 = 0.163$). There was no significant main effect of time ($F(1.101, 12.109) = 0.729$, $p = 0.423$, $\eta^2 = 0.062$), or condition x time interaction ($F(1.004, 11.048) = 0.633$, $p = 0.444$, $\eta^2 = 0.054$).

Cognition

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the cognition subscale/scoring from the MDS-NMS ($F(1,11) = 1.171, p = 0.302, \eta^2 = 0.096$). There was no significant main effect of time ($F(1,11) = 0.646, p = 0.475, \eta^2 = 0.055$), or condition x time interaction ($F(2,22) = 0.219, p = 0.805, \eta^2 = 0.020$).

Orthostatic hypotension

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the orthostatic hypotension subscale/scoring from the MDS-NMS ($F(1,11) = 1.719, p = 0.217, \eta^2 = 0.135$). There was no significant main effect of time ($F(2,22) = 1.533, p = 0.238, \eta^2 = 0.122$). There was no significant condition x time interaction ($F(2,22) = 2.599, p = 0.097, \eta^2 = 0.191$).

Urinary MDS-NMS subscale (which includes urinary urgency, urinary frequency and nocturia) – including participant who was prescribed urinary frequency medication during the study.

Results of the two-way repeated measures ANOVA revealed a significant main effect of condition on the urinary subscale/scoring from the MDS-NMS ($F(1,11) = 6.343, p = 0.029, \eta^2 = 0.366$). There was no significant main effect of time ($F(2,22) = 2.176, p = 0.137, \eta^2 = 0.165$), or condition x time interaction ($F(2,22) = 0.117, p = 0.890, \eta^2 = 0.011$).

Urinary MDS-NMS subscale (which includes urinary urgency, urinary frequency and nocturia) – excluding participant who was prescribed urinary frequency medication during the study.

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the urinary subscale/scoring from the MDS-NMS ($F(1,10) = 4.911, p = 0.051, \eta^2 = 0.329$). There was no significant main effect of time ($F(2,20) = 2.308, p = 0.125, \eta^2 = 0.188$), or condition x time interaction ($F(2,20) = 0.486, p = 0.622, \eta^2 = 0.046$).

MDS Urinary results are presented in Figure 7.4

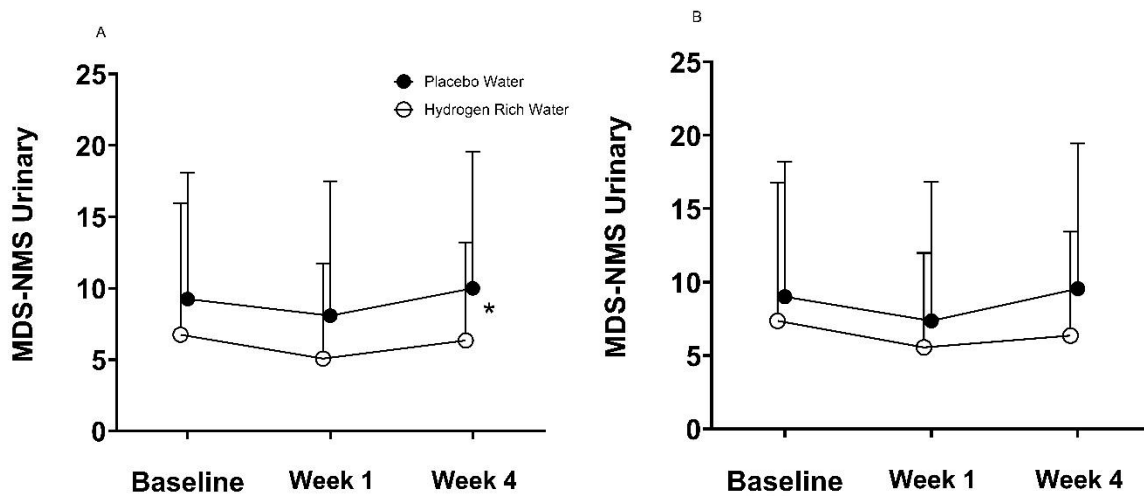


Figure 7.4. MDS-NMS Urinary scores for placebo water and hydrogen rich water, measured at baseline, week 1, and week 4. A = Including participant who was prescribed urinary frequency medication during the study. B = Excluding participant who was prescribed urinary frequency medication during the study. Data presented as mean $\pm$ standard deviation. *Significant main effect of condition.

Sexual subscale (which includes sexual drive and sexual arousal)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the sexual subscale/scoring from the MDS-NMS ($F(1,11) = 1.000$, $p = 0.339$, $\eta^2 = 0.083$). There was no significant main effect of time ($F(2,22) = 1.000$, $p = 0.384$, $\eta^2 = 0.083$), or condition x time interaction ($F(2,22) = 1.000$, $p = 0.384$, $\eta^2 = 0.083$).

Gastrointestinal (which includes drooling of saliva, difficulty in swallowing, nausea and constipation)

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the gastrointestinal subscale/scoring from the MDS-NMS ($F(1,11) = 0.335$, $p = 0.574$, $\eta^2 = 0.030$). There was no significant main effect of time ($F(2,22) = 0.101$, $p = 0.904$, $\eta^2 = 0.009$), or condition x time interaction ($F(2,22) = 1.356$, $p = 0.110$, $\eta^2 = 0.110$).

Sleep

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the sleep subscale/scoring from the MDS-NMS ($F(1,11) = 0.365$, $p = 0.558$, $\eta^2 = 0.032$). There was a significant main effect of time ($F(2,22) = 9.057$, $p = 0.001$, $\eta^2 = 0.452$), post hoc analysis showed no significant main effect between any time point. There was no significant condition x time interaction ($F(2,22) = 0.225$, $p = 0.800$, $\eta^2 = 0.020$).

Pain

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the pain subscale/scoring from the MDS-NMS ($F(1,11) = 0.031$, $p = 0.863$, $\eta^2 = 0.003$). There was a significant main effect of time ($F(2,22) = 3.629$, $p = 0.043$, $\eta^2 = 0.248$). Post-hoc analysis showed a significant main effect between week 1 and week 4, with values at week 1 (6.59) significantly higher ($p = 0.049$) than at week 4 (4.58). There was no significant condition x time interaction ($F(1.261, 13.874) = 0.640$, $p = 0.472$, $\eta^2 = 0.055$).

MDS Other

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the MDS other subscale/scoring from the MDS-NMS ($F(1,11) = 0.000$, $p = 1.000$, $\eta^2 = 0.000$). There was no significant main effect of time ($F(2,22) = 2.947$, $p = 0.073$, $\eta^2 = 0.211$), or condition x time interaction ($F(2,22) = 1.742$, $p = 0.199$, $\eta^2 = 0.137$).

MDS Total

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on the MDS-NMS total scoring ($F(1,11) = 1.483$, $p = 0.249$, $\eta^2 = 0.119$). There was a significant main effect of time ($F(2,22) = 8.947$, $p = 0.001$, $\eta^2 = 0.449$), post hoc analysis showed a significant main effect between baseline and week 1, with values at baseline (77.5) significantly higher ($p = 0.002$) than at week 4 (57.28). There was also a significant main effect between baseline and week 4, with values at baseline significantly higher ($p = 0.019$) than at week 4 (57.28). There was no significant condition x time interaction ($F(2,22) = 0.139$, $p = 0.817$, $\eta^2 = 0.012$).

7.4.7. FUNCTIONAL MOVEMENT

Functional movement results are presented in Figure 7.5

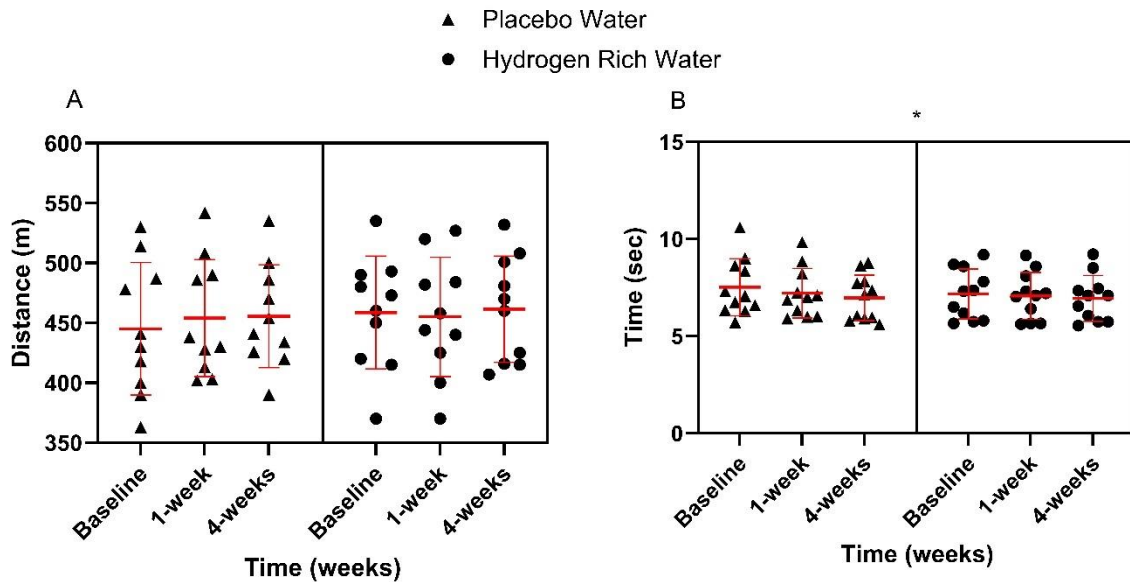


Figure 7.5. Functional movement (n = 10). A = 6MWT, B = TUG. To note: two participants did not complete all six time points in the 6MWT, and one participant did not complete all six time points in the TUG (these results were excluded from analysis). (N = 10). Data presented as mean $\pm$ standard deviation. *Significant main effect of time in the TUG.

Functional movement changes from baseline are presented in Table 7.11

Table 7.11. Functional movement as percentage change from baseline.

Test	Condition	Baseline	Week 1 (% of baseline)	Week 4 (% of baseline)
6MWT	PLA	100	102	103
	HYD	100	100	101
TUG	PLA	100	96	94
	HYD	100	99	97

6MWT PLA and HYD increase slightly over 4 weeks (PLA = 3% and HYD = 1%). TUG test reduces for both PLA (6%) and HYD (3%) over 4 weeks.

6MWT

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' 6MWT ($F(1,9) = 0.586$, $p = 0.464$, $\eta^2 = 0.061$). There was no significant main effect of time ($F(2,18) = 1.236$, $p = 0.314$, $\eta^2 = 0.121$), or condition x time interaction ($F(2,18) = 0.771$, $p = 0.477$, $\eta^2 = 0.079$).

TUG

Results of the two-way repeated measures ANOVA revealed no significant main effect of condition on participants' TUG ($F(1,10) = 0.650$, $p = 0.439$, $\eta^2 = 0.061$). There was a significant main effect of time ($F(2,20) = 4.105$, $p = 0.032$, $\eta^2 = 0.291$), but post hoc analysis did not identify any differences between individual time points. There was no significant condition x time interaction ($F(2,20) = 0.747$, $p = 0.486$, $\eta^2 = 0.070$).

7.4.8. ACCELEROMETRY - GENEACTIV

Steps

Results of the Wilcoxon signed-rank test revealed mean values for step count did not differ significantly between condition ($Z = -0.549$, $p = 0.583$).

Sleep

Results of the paired samples t-test revealed mean values for sleep did not differ significantly between condition ($t(11) = 0.710$, $p = 0.246$).

Sedentary time

Results of the paired samples t-test revealed mean values for sedentary time did not differ significantly between condition ($t(11) = -1.115$, $p = 0.144$).

Light activity

Results of the Wilcoxon signed-rank test revealed mean values for light activity did not differ significantly between condition ($Z = -1.255$, $p = 0.209$).

Moderate and Vigorous Physical Activity (MVPA)

Results of the Wilcoxon signed-rank test revealed mean values for MVPA did not differ significantly between condition ($Z = -1.177$, $p = 0.239$).

Non-wear time

Results of the Wilcoxon signed-rank test revealed mean values for non-wear time did not differ significantly between condition ($Z = .000$, $p = 1.000$).

GENEActiv non-wear time is presented in Figure 7.6

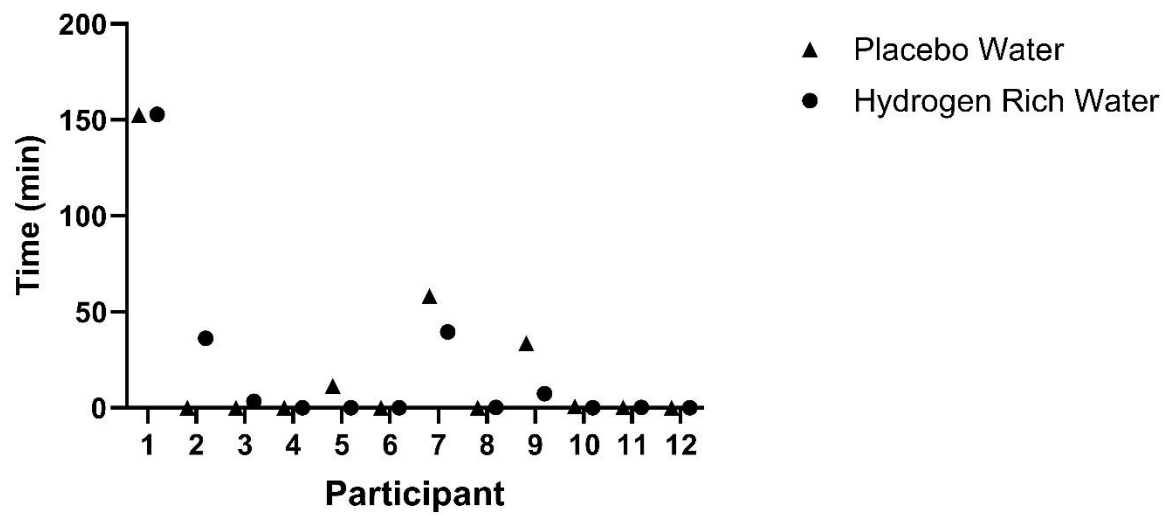


Figure 7.6. GENEActiv output for non-wear time for each variable. Four participants had some non-wear over each period. Represented as daily mean per condition.

GENEActiv output for each output is presented in Figure 7.7

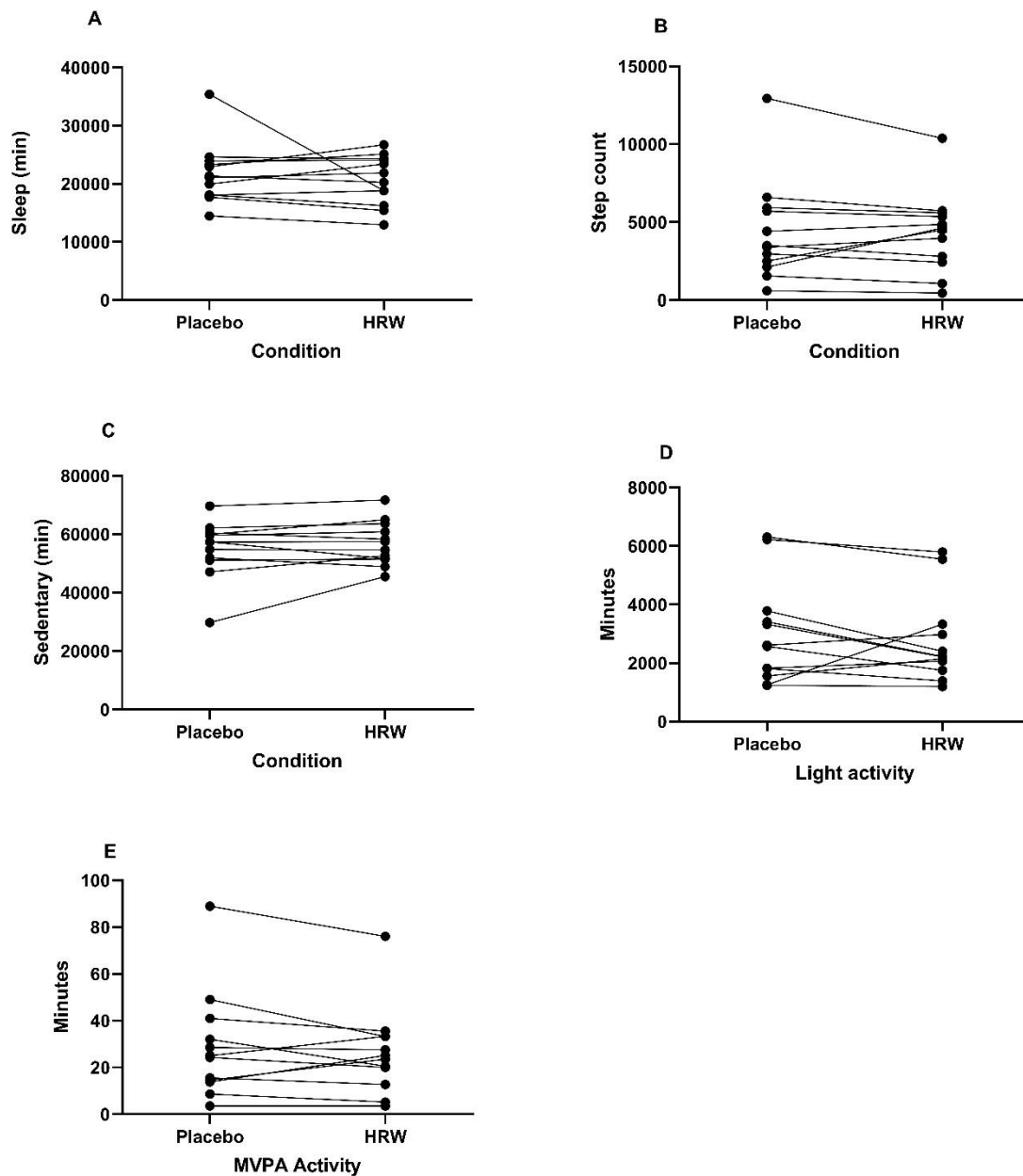


Figure 7.7. GENEActiv output for each variable. A = Sleep. B = Step count. C = Sedentary time. D = Light activity. E = Moderate and Vigorous Activity. Data presented as individual values.

7.5. DISCUSSION

The current study demonstrated drinking 1 litre (L) of hydrogen rich water (HRW) for 4 weeks did not have a significant effect on motor or non-motor symptoms of Parkinson's disease (PD), however Hydrogen (H₂) increased IL-6 levels over 4 weeks and antioxidant capacity over 1 week.

This is the first known study to examine HRW in people with Parkinson's (PwP) without the addition of Magnesium (Mg). Previous studies have dissolved powder into water, however, this contained Mg which is required for many biochemical reactions such as protein synthesis, adenosine triphosphate (ATP) synthesis, autonomic nerve functions, and contributes to glutathione (GSH) synthesis. Mg status was not measured in previous HRW studies. GSH redox imbalances are implicated in PD (Bjørklund et al., 2021), and Mg containing water may impact GSH redox imbalances in PwP. However, the bottle used in the study demonstrated lower H₂ content than reported in previous studies (Yoritaka et al., 2013, Yoritaka et al., 2018) (please see Chapter 7 for further information on H₂ content of water). PwP often exhibit Mg deficiency (which causes oxidative damage in PwP) (Forte et al., 2005) and Mg has been demonstrated to inhibit alpha - synuclein (α -syn) aggregation (Golts et al., 2002). If Mg status had not been measured in previous HRW studies (thereby not identifying Mg deficiency), any benefits observed may be due to the addition of Mg from the water.

Mechanism of action of H₂

No biomarker exists for PD, which ultimately proves difficult for clinicians and researchers, however, while this is still under investigation, other biomarkers implicated in PD (such as inflammatory markers and antioxidant capacity) may be of interest, which can help in understanding whether an intervention is beneficial for PwP. There is an abundance of literature to suggest that PwP have increased levels of cytokines (signalling proteins of the immune system) and oxidative stress and there is a complex interaction between biomarkers which regulate different biochemical pathways. Measuring different biomarkers in accessible peripheral blood can help elucidate mechanisms of action of therapeutic interventions and can help in identifying potential interventions that may help improve the quality of life for people with PwP. While cerebral spinal fluid (CSF) is the gold standard for measurement of biomarkers of neurodegenerative disease (Chan et al., 2019), this is invasive and requires a

highly trained medic, therefore peripheral fluid markers (such as blood) are more accessible in a research setting. However, not all blood samples in the current study were collected from participants due to difficulty in locating veins, therefore a small study sample size (n=12) was further reduced in some biomarker analysis (by up to 58% in some cases).

The mechanism of action of hydrogen (H₂) is still being researched, however, seminal work by Ohsawa et. al (2007) demonstrated that H₂ selectively reduced hydroxyl radicals ($\cdot$ OH), (the most reactive of free radicals) in cultured cells and decreased levels of oxidized guanine (8-OH-G) and 4-Hydroxynonenal (4-HNE). As H₂ reduces $\cdot$ OH and has more broad antioxidant capacities (Ohsawa et al., 2007), this could potentially help in reducing the progression of PD via antioxidant/oxidative stress reducing mechanisms.

A medium effect size was found for alpha-synuclein (α -syn) (with an increase from baseline to 4 weeks by 37% for H₂), C-reactive protein (CRP) (remained stable from baseline to 4 weeks for H₂) and total antioxidant capacity (TAC) (increased by 7% after one week of H₂ and then reduced from baseline to 4 weeks by 6%) and a large effect size with a significant effect was observed for Interleukin-6 (IL-6) (increased by 17% from baseline to 4 weeks with outlier included in results, with outlier excluded, IL-6 still increased from baseline to 4 weeks by 14%). However, small effect sizes were found for Brain derived neurotrophic factor (BDNF), Heat shock protein 70 (Hsp70), 4 – Hydroxynonenal (4-HNE), and Tumor-Necrosis Factor – alpha (TNF- α). These results must be interpreted with caution as small sample sizes have been analysed, and further studies assessing the impact of H₂ on PwP with larger sample sizes may observe different findings. Despite the small number of samples analysed for IL-6 (7 with outlier, 6 without outlier), a significant effect was found condition when analysing data as percentage change from baseline with outlier included. There is a complex interplay between antioxidant capacity, CRP, α -syn and IL-6 and PD is a multi-faceted, complex, heterogeneous disease, and all biomarkers were analysed in plasma samples in duplicate, which were collected using the same methods and thus allowed for reliable data collection and enabled valid comparisons between different time points.

IL-6 is mostly regarded as a pro-inflammatory cytokine; however, it also has anti-inflammatory properties as a myokine (produced by skeletal muscle in response to muscular contractions) and triggers acute phase response in host defence including CRP (Tanaka et al., 2014). Elevated IL-6 levels have been detected in PwP and IL-6 has been demonstrated to both correlate and non-correlate with PD severity (Green et al., 2019; Scalzo et al., 2010), and disease progression

usually takes place over months and years, the expectation that PD would progress over a short period of time (4 weeks) would be fairly unlikely. The current study analysed plasma samples and IL-6 levels have been demonstrated to measure higher in plasma than serum samples (Gong et al., 2019) and, potentially measuring plasma could have given higher IL-6 levels, it would have been useful (but more expensive and time consuming) to measure serum as well as plasma for IL-6 to understand if IL-6 levels did increase in serum as well.

However, with the increase of IL-6 found in the current study, this would suggest an increase in CRP levels (due to their complex interplay) but CRP levels were found to remain stable over 4 weeks. While an inflammatory marker, the relationship between IL-6 and oxidative stress is highly interdependent as IL-6 contributes to oxidative stress through influencing nitric oxide (NO) and superoxide levels (Didion, 2017), however, TNF- α also stimulates NO production but no significant effect was found for TNF- α levels. TNF- α is a pro-inflammatory cytokine and a mediator in neuroinflammation, which ultimately contributes to dopaminergic neuron death, and possibly leads to neurodegeneration (Hirsch & Hunot, 2009), and a link has long been established between BDNF levels and proinflammatory cytokines including TNF- α (Saha et al., 2006) in that, increased levels of proinflammatory cytokines will reduce levels of BDNF and vice versa. No significant effect was found for BDNF levels and decreased levels of BDNF may lead to increased expression of alpha-synuclein (α -syn) (Kang et al., 2017) (Katila et al., 2017) which is widely known to be related to PD pathogenesis (Rocha et al., 2017).

Total antioxidant capacity (TAC) increased after one week of H₂ and then decreased after 4 weeks of H₂, which could potentially be due to adaptation occurring to the continued presence of H₂ and participants becoming tolerant of H₂. Blood samples at more time points (e.g. daily) could pinpoint when TAC started to increase and decrease, however, methodological considerations of discomfort to the participant would make this unfeasible over a 4-week period. Hirayama et al., 2018 found that after four weeks H₂ gas inhalation, 8-hydroxy-2'-deoxyguanine (8-OHdG) increased which suggests that more DNA oxidative damage had occurred than pre-H₂ consumption. However, there is no data on when urine was collected and it has been shown that first morning urine is particularly reliable as it is largely unaffected by dietary, physical activity and environmental exposures (Zanolin et al., 2015), if urine was collected at different time points to the pre-measurement, this may have affected results. In the current study, all participants were asked to eat similar foods prior to attending the lab to help reduce any effects of any dietary antioxidants they may have consumed. Increased oxidative stress has been demonstrated to exacerbate aggregation of α -syn, thereby modulating

progression of PD (Scudamore & Ciossek, 2018) and α -syn levels increased over time in the current study with a medium effect size. Reported total α -syn plasma levels have varied widely in previous literature e.g. 1.56 pg/mL (Barbour et al., 2008) and 120 pg/mL (Foulds et al., 2011) and both studies used enzyme linked immunosorbent assays to measure α -syn, which is the same method used in the current study. There may be reported different levels due to residual erythrocytes or haemolysis occurring during collection or processing of samples which can contaminate α -syn measures. While CSF is the gold standard for α -syn sample collection, lumbar puncture is invasive, requires local analgesic and a highly trained medic is required to carry out the procedure, and is costly. If venous blood α -syn were more accurate, this could be a less expensive, more comfortable and convenient sample method. α -syn is ubiquitous throughout the body, and methods and biological fluids to determine α -syn are still yet to be determined (Chahine et al., 2023), however, saliva could offer a more stable and reproducible measure of α -syn due to avoiding haemolysis contamination (Kang et al., 2016), future studies in H₂ and PD could use saliva to determine α -syn levels.

Ohsawa et al., (2007) observed that H₂ decreased levels of 4-HNE and 8-hydroxyguanine (8-OH-G) after 24 h of a culture medium being induced with ROS, and when H₂ was removed from the medium, the protective effect disappeared. However, the current study found that H₂ did not affect levels of 4-HNE, but the study period that participants were drinking H₂ was greatly increased compared to Ohsawa et al., (2007), and not measured after one day. Mitochondrial oxidative stress in dopamine neurons increases the accumulation of 4-HNE, which is the most abundant and cytotoxic lipid peroxidation product, and increased levels of 4-HNE have been found in PD. 4-HNE is a marker of oxidative stress which is formed by omega-6 polyunsaturated fatty acid oxidation, 4-HNE stimulates inflammation, is highly toxic to mammalian cells, and can react with glutathione.

Elevated levels of Hsp70 protect against α -syn misfolding (Moloney et al., 2014; Danzer et al., 2011), therefore a therapeutic intervention that improves Hsp70 levels could benefit PwP, as protection against misfolding of α -syn would slow down the progression of PD. Heat shock proteins are induced in response to different physiological and environmental stresses such as cold, heat and energy depletion. No known research has explored H₂ and its effect on Hsp70 levels in PwP, however H₂ gas has been shown to reduce Hsp70 levels in the soleus muscle of sedentary and exercising rats compared to a control group (Nogueira et al., 2021).

Increased PLA Hsp70 levels may be due to participant's being more hydrated than usual due to the study protocol requirement of drinking one litre of water per day, which helps to prevent dehydration. H₂ has long been established to reduce oxidative stress (Ohsawa et al., 2007) which may be a result of increasing Hsp70, which thus increases detrimental mitochondrial ROS production.

HYD did not impact levels of 4-HNE, Mitochondrial oxidative stress in dopamine neurons increases the accumulation of 4-HNE, which is the most abundant and cytotoxic lipid peroxidation product, and increased levels of HNE have been found in PD. In the HYD phase, total antioxidant capacity increased at week 1 which could have decreased 4-HNE levels. 4-HNE is a marker of oxidative stress which is formed by omega-6 polyunsaturated fatty acid oxidation, 4-HNE stimulates inflammation, is highly toxic to mammalian cells, and can react with glutathione. High concentrations of 4-HNE inhibit NF- κ B (Wang et al., 2002), and H₂ has been demonstrated to potentially inhibit the NF- κ B pathway (Zhuang et al., 2013).

While some markers of inflammation and oxidative stress were affected by H₂, others were not. This could be due to specificity of biological fluids (such as in α -syn), or methods in measurement. TAC shows promise that it can be improved by H₂ after one week of consumption but not longer periods (e.g. 4 weeks), this could be the body is becoming accustomed to the continued presence of H₂. Further research is needed in PwP to help elucidate if TAC/oxidative stress is affected by H₂ in a similar way to which has been found in an animal model of PD (Fujita et al., 2009). The results from the current study need to be extrapolated tentatively due to the small sample size of the study, larger cohorts may offer further evidence of the mechanism of action of H₂.

Non-motor symptoms

Non-motor symptoms (NMS) of PD often precede motor symptoms of PD by many years (Berg et al., 2015) which often go undetected, and not diagnosed properly as PD. Non-motor symptoms of PD are varied across individuals and can drastically impair quality of life. NMS can be quantified using validated questionnaires such as the MDS-NMS (Chaudhuri, 2019) which is useful in both a clinical setting to assess the severity of PD, and a research setting. Some more common NMS include depression, anxiety and apathy, cognitive issues, constipation, loss of smell, psychosis, incontinence, orthostatic hypotension, sleep disorders, pain and sexual dysfunction.

HRW did not improve reaction time, accuracy, mood, or blood pressure after a 4-week intervention of drinking 1 L per day. While the Profile of Mood States Short -Form (POMS-SF) did not demonstrate any changes in mood, this may be more suited to an acute intervention study (Sistarelli et al., 2023) in demonstrating improved mood, however, this showed that participants arrived at the testing site in a similar mood and any changes observed in other non-motor or motor symptoms could potentially not be attributed to a different mood such as fatigue, feelings of anxiety etc.

The use of the Movement Disorder Society – Non – Motor Rating Scale (MDS-NMS) was used in the current study to quantify non-motor symptoms of PD. Other studies in H₂ and PwP have used the Movement Disorder Society – Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) and the use of the MDS-NMS in the current study does limit comparison slightly to these studies, however, part 1 of the MDS-UPDRS does measure non-motor aspects of experiences of daily living. A study assessing the association between scores of the MDS-UPDRS part 1 and the MDS-NMS demonstrated that patients with more severe non-motor symptoms did not score similarly in the MDS-UPDRS and MDS-NMS, but the association was stronger for mild to moderate NMS severity (Martinez-Martin et al., 2015), therefore it may be that lower scores on the MDS-NMS in the current study may be able to be compared to other HRW studies. The MDS-UPDRS remains the gold standard, however, it has been shown that floor effect can impact trials of disease modification as it fails to capture the complexity and heterogeneity of PD (Mari & Mestre, 2022). The MDS-UPDRS is lengthy and is usually carried out by a Neurologist. Non-motor symptoms are often under-appreciated in clinical practice, but non-motor symptoms can be as debilitating as motor-symptoms (D’Iorio et al., 2017), non-motor symptoms burden increase as PD advances (Ray Chaudhuri et al., 2019) and it is essential that non-motor symptoms are captured using a validated scale such as the MDS-NMS, which is quicker to administer than the MDS-UPDRS. A recent consensus paper for proposed outcome measures for disease-modifying trials in PD suggested that the MDS-NMS was a supplemental outcome measure, as well as the MDS-UPDRS (gait-axial score) (Gonzalez-Robles et al., 2023).

Participant’s blood pressure (BP) was not affected by HYD over four weeks; however, a large effect size was observed for diastolic BP, potentially a larger study cohort could elucidate a significant effect of H₂ on BP. BP for three participants who were taking medication for hypertension remained stable. These findings contrast with Sugai et al. (2020) who showed H₂ gas had an anti-hypertensive effect in animal models, however this was in a rat model of

hypertension and the mechanistic action of H₂ may differ in a PD population due to autonomic dysfunction in PwP which is due to norepinephrine neuronal dysfunction and can often precede motor symptoms of PD (Kaufmann et al., 2017). PD is affiliated with a reduction of catecholamine norepinephrine in the myocardium; however, hypertension may occur independently of PD, and hypertension risk factors include ageing, obesity, and sedentary behaviour. The same researcher measured BP using the same manual equipment to increase measurement reliability.

H₂ did not impact accuracy results, however, participants demonstrated decreased accuracy by a small percentage (2% for both PLA and H₂) in the Flanker Incompatible task but not incompatible or no tasks.

Reaction time (RT) was not affected by HRW, however, there was a medium effect size observed for reaction time – incompatible. No other H₂ studies and PwP have investigated RT, and RT has been implicated in postural instability and freezing of gait in PwP. However, in the current study, postural instability was not measured.

Motor-symptoms

H₂ did not improve motor symptoms of PD. While other research has demonstrated that H₂ can improve motor symptoms (Yoritaka et al., 2013) this was over a period of drinking the same amount of water (1 L) as the current study but over a period of almost one year. While the current study can tentatively extrapolate that changes may be observed in antioxidant capacity and inflammation, these biochemical changes may not be seen functionally for a while.

Manual dexterity

HYD did not significantly impact manual dexterity. Gonzalez-Robles et al., 2023 recent consensus paper suggested that the PPT can be supplemental to outcome measures for disease modifying trials in PD. While no gold standard for assessing manual dexterity in PwP exists (Proud et al., 2013), there is evidence that the PPT not only measures manual dexterity and visuomotor co-ordination (Desrosiers et al., 1995) but can measure cognition (Hinkle & Pontone, 2021), however, domain-specific cognitive changes have yet to be elucidated. The PPT may be more sensitive in tracking PD progression in prodromal and early clinical stages

compared to later stages of the disease (Zhou et al., 2023), however, most participants in the current study were in the earlier stages of disease, and the PPT did not elicit any changes in manual dexterity.

Functional movement

H₂ did not improve functional movement, however, there was a small improvement in the timed up and go test (TUG) completion time, irrespective of condition, over the course of the study. This demonstrates that repeated TUG can reduce time to completion, potentially offering a ‘training effect’ which is especially important to people who may have gait impairment and balance issues such as those with PD.

However, all participants took less than 11.5 sec to complete the TUG which has been demonstrated to be the most specific and sensitive time to discriminate participants at risk of falling in a large cohort of PwP (Nocera et al., 2013). This demonstrates that even though there was a wide range of ages (youngest participant was 51 years old, eldest participant was 78 years old) and disease duration (from 6 months to 14 years), all participants were at low risk of falling according to their TUG completion time. PwP who take longer than 11.5 sec to complete the TUG may be more suited to observing if H₂ impacts functional movement. As

Accelerometry

Step count, sedentary time, light activity, moderate to vigorous activity (MVPA) and non-wear time did not differ between conditions. Mean daily average of MVPA was 29 min for PLA and 26 min for HYD, therefore most participants are meeting the minimum recommended activity guidelines set by the World Health Organization (World Health Organization, 2024) of 150-300 min (which equates to approx. average of 21-42 min per day) of moderate physical activity (PA) per week for people aged 65 years and over. The accelerometer was worn around the participant’s waist, which has been demonstrated to discriminate sedentary behaviour and MVPA better than when worn on the non-dominant wrist in the general population (Duncan et al., 2020). However, this may not have accurately measured activities of daily living, and while most participants wore the belt continuously (apart from bathing), four participants had noticeable non-wear time (see figure 7.6.), which is a third of all participants. Participants were not made aware that the belt could distinguish between being worn and not being worn,

however, the researcher advised the participants to wear the belt all the time apart from bathing. If a larger study was carried out, site location of the belt could be changed from the waist to the wrist, while this may skew results, compliance to wear protocol may improve, however, this may prove problematic in a Parkinson's population if they have a tremor. This is especially important if the research is understanding acceleration and gait. As accelerometry can be expensive and requires the user to adhere to wearing it, the PPT could be an alternative to this as PPT scores have been correlated with impaired range and irregular patterns of acceleration (Wilkes et al., 2023). The PPT is cheap to administer and requires no compliance with protocol from the participant when away from the testing site.

Taken together, these results suggest that a week HRW does not elicit an improvement in motor and non-motor symptoms of PD but may influence antioxidant capacity and inflammation. The dose effect of HRW remains unknown, and while this study's protocol was 1 L of HRW per day (which is in line with previous research), this may not be acutely beneficial, however, participants cannot continuously dose with HRW. Perhaps non-motor and motor symptoms would improve over a longer period than 4 weeks as per previous research, but seemingly, antioxidant capacity increased after one week of HRW and then reduced from baseline to 4 weeks of drinking HRW. It would be beneficial to measure blood samples at different points such as every day for one week, however, this would be uncomfortable for the participant and would require daily testing site attendance. PD is an incredibly complex, multi-faceted disease and not everyone with PD will experience the same symptoms, however, it is known that PwP have increased levels of inflammation and oxidative stress compared to 'healthy' people, which are both known drivers of the disease. Therefore, if inflammation and oxidative stress can be reduced by a therapeutic intervention, it may slow down the progression of the disease.

Limitations

The study was a single-blind study. The manufacturer could not provide a PLA bottle, and therefore a larger water bottle was given to participants. One participant reported it was easier to drink from the HYD bottle as it was not as big, other participants preferred the larger bottle due to needing less refills.

Biomarkers in other biological fluids such as urine or saliva could have been taken. 8-OHdG could have been analysed using urine, to help further understand if H₂ did reduce oxidative stress, as although antioxidant capacity increased after one week, this is not sufficient to make inferences about oxidative stress levels. A-syn could have been measured through saliva as blood is more likely to be contaminated with residual erythrocytes or haemolysis occurring during collection or processing of samples.

Participants had to charge the HYD bottle. While most participants found this requirement easy, one participant struggled with this and was given a charger with a plug to charge the bottle (as the bottles were charged up using a USB). There was a small sample size (with only participants in H&Y stage 1 – 3 included) and not all blood samples were taken, which further reduced the sample size for some biomarker analysis. 25 people contacted the researcher and people became disengaged due to the nine-week intervention and six visits to the lab. One person asked the participant if it was possible if the testing could be carried out at his home but due to health and safety, this was not possible. Therefore approx. 50% of people who initially contacted the researcher then went on to take part in the study. The study could have benefited from being carried out over a longer period, as longer durations of HRW consumption have reported benefits, however, recruitment for a nine-week study in total was proving difficult.

It was difficult to know if the intervention was fully adhered to despite asking participants if they were able to follow the protocol of drinking 1 litre of study water per day.

7.6. CONCLUSION

HRW did not improve motor or non-motor symptoms in PwP, however, H₂ increased IL-6 over 4 weeks and improved antioxidant capacity after one week consumption. No other markers of inflammation or neuroinflammation were affected, although this was underpowered due to a small sample size. Dose effect of H₂ is yet to be established and the heterogeneity of PD further complicates this elucidation.

Chapter 8. General discussion

Summary of findings

The aim of this thesis was to investigate if hydrogen (H₂) could improve motor and non-motor symptoms in people with Parkinson's (PwP), and to investigate the potential mechanisms of action of H₂ through blood biomarker analysis. An acute inhalation period was first deployed to investigate these changes, and this is the first known research to measure changes acutely in PwP during H₂ inhalation (a pilot study preceded this to help with study design), this was then followed by a case study utilising an ABAB study design, and finally hydrogen rich water (HRW) was investigated in a chronic study. H₂ was first found to be a promising therapeutic in disease nearly 50 years ago (Dole et al., 1975) and since Ohsawa et al. (2007) first demonstrated that H₂ reduced oxidative stress, research momentum and curiosity has increased in the potential of H₂ to improve health outcomes. Promising findings from animal models of Parkinson's disease (PD) (Fujita et al., 2009; Fu et al., 2009) struggled to translate well in PwP which may be in part due to the heterogeneity of Parkinson's disease and the ability to strictly control environments in animal research. PD in rats only represent dopaminergic neuron loss, therefore cannot be used to investigate neurodegeneration mechanism or disease pathology progression. Thus, a novel intervention for PD may give varying results in PwP compared to animals.

Previous studies in the effects of H₂ in PwP (Yoritaka et al., 2013; Yoritaka et al., 2018; Hirayama et al., 2019; Yoritaka et al., 2021) have demonstrated different findings potentially due to different dose ranges, differences in methodologies (different dosages, different timings (e.g. 20 mins a day, one hour a day), different routes of administration (water or gas), different PD staging, and different analysis of different biomarkers in different biological fluids (serum, urine etc), which makes direct comparisons between different studies difficult. As there is no biomarker for PD and working on the premise that Ohsawa et al. (2007) established; that H₂ reduces oxidative stress, measuring indirect markers of oxidative stress or total antioxidant capacity could help bolster the theory that H₂ effects oxidative stress/antioxidant capacity. While there may be conflicting evidence if H₂ can help improve symptoms of PD, benefits of H₂ are that it does not interact with other drugs, has no known side effects, and does not disturb metabolic oxidation-reduction reactions or reactive oxygen species (ROS) mediated cell signalling.

Therefore, the overall aim of this thesis was to establish if H₂ could improve motor symptoms and non-motor symptoms of PD and to try and elucidate the underlying mechanisms of action of H₂ in PwP.

Benefits of a pilot study

A pilot study (Chapter 3) in ‘healthy’ controls was first carried out to understand if the study design of inhaling H₂ gas was feasible, and if the intervention was accepted by participants. This study was invaluable in shaping the study that followed this (Chapter 4) as the inhalation period was initially set at four hours, with five venepunctures. However, this length of time was found to be difficult and uncomfortable for participants (which resulted in reduction of inhalation period in Chapter 4 (2 hours with 3 venepunctures). Results from this study were consistent with findings of research in H₂ and healthy adults (Cole et al., 2021); H₂ was safe (as determined by grading adverse events according to the Common Terminology Criteria for Adverse Events) but did not improve mood, cognition, or manual dexterity. While this study allowed the researcher to refine clinical practices and investigate if timings of testing were feasible, younger people were recruited and it would have been beneficial to include older people in the study as they may have had different needs.

Non-motor symptoms

H₂ did not affect non-motor symptoms of PD. However, during the acute gas inhalation study (Chapter 4), participants demonstrated faster reaction times across all Flanker tasks over time, irrespective of condition. However, this is probably due to a practise effect through becoming familiarised to the test and these results were not reflected in the chronic water study (Chapter 7).

Non-motor symptoms massively impact PwP and are the ‘invisible’ symptoms of the disease and precede a PD diagnosis by years (Fullard et al., 2017). While staging of PD is reliant on motor symptoms, it is important that efforts are made to reduce non-motor symptoms of PD which can drastically impact quality of life. PwP will not experience the same non-motor symptoms, nor will these occur linearly, and symptoms will impact different people differently, therefore it is difficult to observe if any given intervention helps to alleviate any given symptom.

The MDS-NMS was used in Chapter 5 and Chapter 7 to assess changes in non-motor symptoms, although this did not show any changes to non-motor symptoms except for urinary (including frequency and urgency), however, this was attributed to one participant being prescribed urinary urgency medication during the study, and when this participant was removed from analysis, there was no significant effect of H₂. Previous H₂ research has implemented the MDS-UPDRS which measures non-motor symptoms and motor-symptoms. While the gold standard for measuring disease progression, the limitations of floor effect and its failure to capture the complexity and heterogeneity of PD (Mari & Mestre, 2022) can impact trials of disease modification.

Motor symptoms

Likewise with non-motor symptoms, not all motor symptoms will be experienced by everyone diagnosed with PD and females will tend to suffer with tremor more than males. People in the later stages of PD were not included in the studies, and these people would have increased disability, which would potentially give different results. Tremor was not impacted by an acute intervention, however, participants in Chapter 4 did not have severe tremor as measured by the MDS-UPDRS 3.15, studies which include people with a higher tremor amplitude may have different findings (and more females due to their PD being more tremor dominant). Functional movement, as assessed by the timed up and go test and six-minute walk test were not affected by H₂. Purdue Pegboard Test (PPT) measurements were not affected, however, there is no gold standard for measuring manual dexterity in PwP (Proud et al., 2013) and while the PPT has been demonstrated to be on the only mechanical test which differentiated PwP from controls (Růžička et al., 2016), this may be less sensitive in people in the earlier stages of the disease.

Mechanisms of action

Despite ongoing attempts at trying to elucidate the mechanism of action of H₂, it remains unknown exactly how H₂ impacts physiology. What further complicates this elucidation in PwP is the complex heterogeneity of PD and no specific PD biomarkers. Dose effect of H₂ also remains unknown and there are differences in methods of analysis from different biological fluids (urine, blood etc). The seminal work of Ohsawa et al. (2007) first deployed the notion

that H₂ reduced oxidative stress, and as a complex interplay exists between inflammation and oxidative stress, it would seem feasible to suggest that H₂ may have anti-inflammatory benefits.

H₂ increased TAC whether administered through gas inhalation or by drinking hydrogen rich water (HRW), however, TAC increased more with gas (56% after two hours inhalation - Chapter 4, 19% and 16% after two weeks inhalation – Chapter 5) than with HRW (7% after one week – Chapter 7). However, these results are preliminary in nature and must be interpreted with caution as sample sizes are small (including just one participant in Chapter 5) and measurements were taken at different times. There is a larger tissue saturation with H₂ gas (Liu et al., 2014) compared to HRW and gas inhalation has increased bioavailability compared to oral administration as it bypasses the digestive tract. There is only 40% uptake of H₂ from HRW (Shimouchi et al., 2012), therefore this may suggest that results from this study suggest that H₂ gas inhalation may result in increased TAC than with HRW. As participants were asked to consume 1 litre per day of HRW, an increase in this amount maybe could improve TAC more than just 1 litre. However, some participants reported struggling drinking 1 litre of water per day and said they found it a lot. Given that after two weeks inhalation, TAC increase is lower (19% and 16%) than after two hours of inhalation (56%), this may suggest an adaptive effect to daily use of H₂ gas inhalation, however, these were analysed in small samples. As TAC measurements were similar for the two H₂ inhalation periods (19% and 16%), but the participant did not adhere to the inhalation period in the second phase of H₂ (this was approx. a third of the total requested time), potentially concluding that shorter bouts of breathing, or intermittent days of breathing, may have similar effects to 30 min x 2 daily. While TAC increased with H₂, there was no significant effect of H₂ on measures of oxidative stress ((4-Hydroxynonenal (4-HNE)) and Heat shock protein 70 (Hsp70) in Chapter 7, oxidative stress markers were not measured for any other H₂ gas intervention which may have helped give a more detailed and in-depth picture of the mechanism of action on antioxidant capacity and oxidative stress. Markers of neuroinflammation and inflammation were also measured in Chapter 7, and a significant effect was found for Interleukin –6 (IL-6), in that H₂ increased IL-6 levels from baseline to after week 1 (7% with outlier included in analysis, 9% excluding outlier in analysis) and week 4 (18% with outlier included in analysis, 14% excluded in analysis). As there was no other significant effect of other markers, the underlying mechanism is difficult to elucidate, larger sample sizes may have offered more of an insight into these mechanisms.

H₂ concentration in gas and hydrogen rich water

Chapter 2 demonstrated that oxygen measured 32.3% in the H₂ gas machine, and thus it was assumed that H₂ concentration was 67.7%. Chapter 6 measured H₂ at 1.6 ppm (at its highest concentration) thereby allowing the researcher to demonstrate that participants were inhaling/drinking elevated levels of H₂, including measuring H₂ dissipation rate in HRW (there was a 19% reduction in H₂ over a four-hour period, and H₂ content of water measured similarly to previous reported H₂ levels. This was an important observation as the researcher wanted to confirm that there was increased H₂ levels in the water that participants would consume in Chapter 7.)

Limitations

Sample sizes of all studies were small, and blood analysis samples were even smaller (due to not being able to locate veins), furthermore, people in the later stages of PD were not included. TAC was measured in Chapters 4, 5 and 7 but markers of neuroinflammation, inflammation and oxidative stress were only measured in Chapter 7. Given that TAC improved more with a gas inhalation protocol than with HRW, this may have impacted these biomarkers differently. TAC was only measured at baseline and after 2 hours of gas inhalation in Chapter 4, more blood sample at increased timings would have been beneficial to plot the increase of TAC over time.

In previous H₂ and PwP research, the MDS-UPDRS was used, in this thesis, it was decided not to use whole parts of the MDS-UPDRS and instead use the MDS-NMS (or the MDS UPDRS 3.15), allowing direct comparison difficult to other studies.

Other methods used throughout the thesis such as the PPT and the Flanker effect task may not have been sensitive enough to detect changes in manual dexterity and cognition, especially in acute studies.

Future directions of research

As TAC has been shown to increase with H₂, the need to elucidate the best dose effect should be explored, however, the heterogeneity of PD does make this difficult. As there is no biomarker for PD, candidate markers of inflammation and oxidative stress should also be

explored alongside TAC. While TAC increased with HRW, H₂ gas inhalation seemed to improve TAC more, however, timings of these interventions were different and in small sample sizes so must be interpreted with caution.

Genetic testing was not within the scope of this thesis, however, as previous research in H₂ and PwP has been carried out in Japan (Yoritaka et al., 2013; Yoritaka et al., 2018; Hirayama et al., 2019; Yoritaka et al., 2021), and a LRRK2 gene mutation of Il22020Thr is found in Japanese populations (Kluss, Mamais and Cookson 2019), this may have affected results from their studies. Future studies in a UK population could carry out genetic testing.

As Sano, Shirakawa, *et al.* (2020) showed that H₂ concentrations and arterial blood saturations varied in different sized animals with the lowest weight animal having lower H₂ concentrations and arterial blood saturations than the other animals, this may suggest that concentration and saturation may be affected by body weight. Future studies could attempt to account for these differences but this would be reliant on the elucidation of dose effect.

PD research is difficult in that there is no biomarker for the disease and symptomology among PwP is different, however, it is known that PwP have increased oxidative stress and levels of inflammation. If H₂ can reduce oxidative stress and levels of inflammation, then this may help slow down the progression of PD, however, H₂ consumption through either H₂ gas inhalation or HRW requires compliance to study protocol, and compliance strategies could be employed in future studies.

Thesis conclusion

H₂ increased TAC in a small sample size, furthermore TAC increased more with H₂ gas inhalation than with HRW. However, these results are preliminary in nature and need to be interpreted with caution given the small sample sizes in the thesis studies and different timings of consumption. Adaptation may have occurred to the continued presence of H₂ (and thus participants became tolerant of H₂) given that an acute gas intervention increased TAC by more than 30% compared to a 2-week intervention, and a 7% increase after 4 weeks of drinking HRW. H₂ did not improve non-motor or motor symptoms of PD but tests used to measure may not be sensitive enough to identify any changes.

Sadly, there is no cure for Parkinson's yet, therefore it is important that therapeutic interventions are researched which improve quality of life for PwP. PD is a complex disease,

with a myriad of interactions between genetics and environmental factors which makes it difficult to elucidate beneficial interventions, particularly in small sample sizes.

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Appendix A



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School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent
Chipperfield Building
Kent
CT2 7PE

Ethics Reference:
25_20_21

Date: 21/12/21

Dear Kim,

Re: The effects of hydrogen gas on healthy participants – a Pilot study

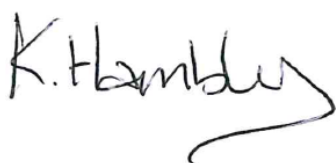
I am delighted to confirm that SSES REAG has approved your research study (REF No.) and you are now permitted to recruit participants and commence your research.

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,

A handwritten signature in black ink, appearing to read "K. Hambly", with a long, sweeping underline.

Karen Hambly

(Chair SSES REAG)

Appendix B



□

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent
Chipperfield Building
Kent
CT2 7PE

Ethics Reference:
26_20_21

Date: 20/12/21

Dear Kim,

Re: The acute effects of hydroxy gas on people with Parkinson's

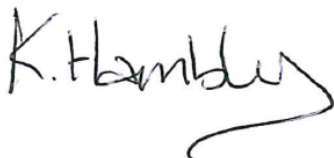
I am delighted to confirm that SSES REAG has approved your research study (REF No.) and you are now permitted to recruit participants and commence your research.

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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,

A handwritten signature in black ink, appearing to read "K. Hambly", with a long, sweeping flourish at the end.

Karen Hambly

(Chair SSES REAG)

Appendix C



□

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent
Chipperfield Building
Kent
CT2 7PE

Ethics Reference:
21_20_23

Date: 17/02/23

Dear Kim,

Re: The chronic effects of hydroxy gas inhalation on people with Parkinson's

I am delighted to confirm that SSES REAG has approved your research study 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,

A handwritten signature in black ink, appearing to read "Samiel Smith", with a stylized, flowing script.

Samiel Smith

(Chair SSES REAG)

Appendix D



□

School of Sport & Exercise Sciences
Research Ethics and Advisory Group (REAG)
University of Kent
Chipperfield Building
Kent
CT2 7PE

Ethics Reference:
23_20_23

Date: 02_02_23

Dear Kim,

Re: The chronic effects of hydrogen rich water on people with Parkinson's

I am delighted to confirm that SSES REAG has approved your research study (REF No.) 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,

Lex Mauger

Research Lead