



Kent Academic Repository

Fennell, Christopher, Mauger, Alexis R. and Hopker, James G. (2023) *Inter-day reliability of heart rate complexity and variability metrics in healthy highly active younger and older adults*. *European Journal of Applied Physiology*, 124 (5). pp. 1409-1424. ISSN 1439-6319.

Downloaded from

<https://kar.kent.ac.uk/103906/> The University of Kent's Academic Repository KAR

The version of record is available from

<https://doi.org/10.1007/s00421-023-05373-3>

This document version

Author's Accepted Manuscript

DOI for this version

Licence for this version

UNSPECIFIED

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in **Title of Journal**, Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our [Take Down policy](https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies) (available from <https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies>).

1 **Inter-day reliability of heart rate complexity and variability metrics in healthy highly**
2 **active younger and older adults.**

3
4 Original Investigation

5
6 Christopher R. J. Fennell, Alexis R. Mauger, James G. Hopker,

7
8
9 School of Sport and Exercise Sciences, University of Kent, Canterbury, Kent, England.

10
11
12 **Corresponding Author.** Mr Christopher Fennell; School of Sport and Exercise Sciences,
13 University of Kent, Chipperfield Building, Canterbury, Kent, CT2 7PE, UK. Email:
14 crjf3@kent.ac.uk

15
16
17 **Running head:** Inter-day reliability of HRV metrics

18
19 **ORCID ID**

20 Christopher RJ Fennell: 0000-0002-3797-6299

21 Alexis R Mauger: 0000-0001-6685-5800

22 James G Hopker: 0000-0002-4786-7037

23
24
25 Abstract word count: 249

26 Text-only word count: 5668

27 Number of figures and tables: Figures 3 & Tables 5

28 References: 59

47 **ABSTRACT**

48 **Purpose.** To investigate the inter-day reliability of time-domain, frequency-domain, and nonlinear HRV metrics
 49 in healthy highly active younger and older adults. The study also assessed the effect of age on the HRV metrics.
 50

51 **Methods.** Forty-four older adults (34M, 10F; 59 ± 5 years; $\dot{V}O_{2\text{peak}} = 40.9 \pm 7.6$ ml.kg⁻¹.min⁻¹) and twenty-two
 52 younger adults (16M, 6F; 22 ± 4 years; $\dot{V}O_{2\text{peak}} = 47.2 \pm 12.8$ ml.kg⁻¹.min⁻¹) attended the laboratory. Visit one
 53 assessed aerobic fitness through an exercise test. In visits two and three, participants completed a 30-minute supine
 54 RR interval measurement to derive the HRV metrics.
 55

56 **Results.** The younger group (YG) and older group (OG) demonstrated poor to good day-to-day relative and
 57 absolute reliability for all HRV metrics (OG, ICCs = 0.33 to 0.69 and between day CVs = 3.8 to 29.2%); YG,
 58 ICCs = 0.37 to 0.93 and between day CVs = 3.5 to 36.5%). There was a significant reduction in ApEn ($P < 0.001$),
 59 SampEn ($P = 0.031$), RMSSD ($P < 0.001$), SDNN ($P < 0.001$), LF power ($P < 0.001$) and HF power ($P < 0.001$),
 60 HRV metrics with ageing. There was no significant effect of age the complexity metrics DFA $\alpha 1$ ($P = 0.107$), $\alpha 2$
 61 ($P = 0.147$) and CI-8 ($P = 0.493$).
 62

63 **Conclusion.** HRV metrics are reproducible between days in both healthy highly active younger and older adults.
 64 There is a decline in linear and nonlinear HRV metrics with age, albeit there being no age-related change in the
 65 nonlinear metrics, DFA $\alpha 1$, $\alpha 2$ and CI-8.
 66

67 **KEYWORDS:** complexity; ageing; reproducibility; heart rate.
 68

69 **ABBREVIATIONS**

ANOVA	Analysis of variance
ANS	Autonomic nervous system
ApEn	Approximate entropy
CI-8	Complexity index under 8 scales
CV	Coefficient of variation
DFA	Detrended fluctuation analysis
HF	High frequency power
HRV	Heart rate variability
ICC2,1	Intraclass correlation coefficient
IET	Incremental exercise test
LF	Low frequency power
LOA	Limits of agreement
MDC	Minimal detectable change
MSE	Multiscale entropy
OG	Older group
RMSSD	Root mean square of successive differences between normal RR intervals
SampEn	Sample entropy
SDNN	Standard deviation of normal RR intervals
SD2	Standard deviation of points along the line of identity of the Poincare plot
SEM	Standard error of measurement
$\dot{V}O_{2\text{peak}}$	Peak oxygen uptake
$\dot{V}E/\dot{V}O_2$	Ventilatory equivalent of oxygen
$\dot{V}E/\dot{V}CO_2$	Ventilatory equivalent of carbon dioxide
YG	Younger group

71
 72
 73
 74
 75
 76
 77
 78

1. INTRODUCTION

Biological systems produce dynamic nonlinear outputs that are measurable across time, such as the variable fluctuations in the beat-to-beat (RRi) of the heart (Lipsitz & Goldberger 1992; Peng et al. 1995). The apparent “chaotic looking” behaviour of the fluctuations in an RR interval time series is accepted to contain meaningful structural richness; which can be assessed by using methods derived from nonlinear dynamics that can quantify the *complexity* (i.e., degree of self-similarity of fluctuations over multiple orders of temporal magnitude; Peng et al. 1995) and *entropy* (i.e., the regularity or randomness of the fluctuations; Richman & Moorman 2000) of the RR interval signal. While traditional linear time-domain methods provide a measure of variability between successive RR intervals, frequency-domain methods provide an estimation of the absolute or relative power of the RR interval signal (Shaffer & Ginsberg 2017).

Together the time-domain, frequency-domain, and nonlinear heart rate variability (HRV) metrics reflect the global functioning of the autonomic nervous system (ANS) through the interplay of sympathetic and parasympathetic activity at the sinus node (Task force 1996; Schwab et al. 2003). From a health-related and clinical perspective, a notable increase or decrease in heart rate complexity and variability away from an individual’s optimal range, may be indicative of an increased risk of sudden death, or adverse cardiac events such as arrhythmias, myocardial infarcts, postural hypotension, and congestive heart failure (Kleiger et al. 1987; Goldberger et al. 1988; Lipsitz 1989; La Rovere et al. 1998; Stein et al. 2005). Moreover, research has shown a higher HRV to be positively associated with working memory (Mosley et al. 2018), cognitive performance (Hansen et al. 2004), emotional regulation (Williams et al. 2015) and incidence of depression (de la Torre-Lugue et al. 2016).

Research utilising a wide variety of HRV metrics has shown that during wakeful rest, both heart rate complexity (Kaplan et al. 1991; Iyengar et al. 1996; Pikkujamsa et al. 1999; Beckers et al. 2006; Voss et al. 2015) and variability (Jensen-Urstad et al. 1997; Umetani et al. 1998; Goff et al. 2010; Hernandez-Vicente et al. 2020) progressively decrease from early adulthood through to older age in healthy individuals. The World Health Organisation projects the number of people in the world over 60 years of age to increase from 1 billion (as of 2020) to 1.4 billion by 2030 and 2.1 billion by 2050 (data from who.int). Given the potentially negative physiological and psychological implications associated with a decrease in heart rate complexity and variability, it is pertinent there is continued research into the utility of HRV in older adults.

Previous research has assessed the intra and inter-day reliability of a few specific time-domain, frequency-domain (Al Haddad et al. 2011; Cipryan & Litschmannova 2013; Uhlrig et al. 2020) and nonlinear HRV metrics (Maestri et al. 2007a). However, to the authors knowledge the inter-day reliability of the nonlinear HRV metrics has yet to be assessed in a homogenous group of healthy older adults. The current study therefore sought to extend upon the current literature investigating the reliability of HRV metrics, with the primary aim to provide new data on the day-to-day reliability of a range of HRV metrics in healthy active younger and older adults. The study also sought to assess the effect of age on HRV.

2. METHODS

2.1. Participants

Sixty-six healthy individuals (50 male; 16 female) were recruited to participate in the study. Participants were divided into two age groups, the younger group (YG) were aged 18 to 30 years ($N = 22$; 16M, 6F) and the older group (OG) were aged 50 to 70 years ($N = 44$; 34M, 10F).

All participants were regular exercisers, having performed above the World Health Organisation guidelines (i.e., 2.5 to 5 hours of moderate exercise per week; Bull et al. 2020) for ≥ 2 years. All participants were recruited to be closely matched for physical activity levels and exercise capacity. Participants were required to be non-obese, non-smokers, have no known or signs/symptoms of cardiovascular, neuromuscular, renal, or metabolic conditions and not be taking medications or dietary supplements that would affect cardiac function. The study was completed with full ethical approval of the University of Kent Research Ethics Committee (Proposal number: 21_2020_21), according to Declaration of Helsinki standards. All participants provided written informed consent prior to testing.

2.2. Experimental Design

Each participant completed three visits to the laboratory at the same time of day (± 1 hour) between the hours of 8am and 4pm (AM visits, YG $N = 8$ and OG $N = 21$; PM visits, YG $N = 14$ and OG $N = 23$). Visit one involved participant screening, laboratory familiarisation, and an incremental exercise test (IET) to determine aerobic fitness. At visits two and three, participants completed the 30-minute supine resting RR interval measurement to derive the HRV metrics.

138 Visits were conducted on non-concurrent days (with a minimum gap of 2 full days and maximum gap of 5 days
 139 between visits) and participants were instructed to refrain from any exercise in the day prior to testing and intense
 140 exercise in the two days prior. Participants were instructed to arrive euhydrated and in a post-prandial state, having
 141 eaten at least 4-hours prior to testing. Participants were told to not consume caffeine within 8-hours and alcohol
 142 within 24-hours of testing.

143

144 **2.3. Preliminary measurements and incremental exercise testing (visit one)**

145 At visit one prior to exercise testing all participants provided written informed consent, completed a health
 146 questionnaire and the long form international physical activity questionnaire (Craig et al. 2003). Resting blood
 147 pressure, participant height, body mass and body composition were then measured, after which the participants
 148 completed a cycling IET to determine markers of aerobic fitness.

149

150 The IET protocol was performed on an electro-magnetically braked ergometer (Excalibur Sport, Lode BV,
 151 Groningen, The Netherlands). Participants completed a 10-minute warm-up at 50 W, after which the required
 152 cycling power output increased by 25 W every minute (i.e., 1 W every 2.4 s) until they reached volitional
 153 exhaustion (operationally defined as a cadence of < 60 revolutions/min for > 5 s, despite strong verbal
 154 encouragement).

155

156 During the IET, respiratory gas exchange data were assessed using online breath-by-breath gas analysis
 157 (Metalyzer 3B; CORTEX Biophysik GmbH, Leipzig, Germany). Prior to all testing the gas analyser was
 158 calibrated according to the manufacturer recommendations using with ambient air and known concentrations of
 159 oxygen and carbon dioxide. The bidirectional turbine (flow meter) was calibrated with a 3-litre calibration syringe.

160

161 The participant's peak oxygen uptake ($\dot{V}O_{2peak}$) was assessed as the highest oxygen uptake that was attained during
 162 a 1-minute period in the test. Participants gas exchange threshold was determined as the breakpoint in carbon
 163 dioxide production and oxygen consumption (i.e., the point at which the carbon dioxide production begins to
 164 increase out of proportion to the oxygen consumption). This breakpoint also coincided with the increase in both
 165 ventilatory equivalent of oxygen ($\dot{V}E/\dot{V}O_2$) and end-tidal pressure of oxygen with no concomitant increase in
 166 ventilatory equivalent of carbon dioxide ($\dot{V}E/\dot{V}CO_2$; Beaver et al. 1986; Pallares et al. 2016). The respiratory
 167 compensation point was determined as an increase in both the $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ and a decrease in partial
 168 pressure of end-tidal carbon dioxide (Whipp et al. 1989; Lucia et al. 1999).

169

170 **2.4. Measurement of RR intervals (visits two and three)**

171 For collection of RR intervals participants were in a supine resting position, in a temperature-controlled room set
 172 at 20 C. The room was kept dark and quiet, and participants were instructed not to verbalise throughout the
 173 measurement and breathe freely at their normal resting rate. Before the 30-minute RR interval measurement
 174 commenced, an initial 20-minute supine rest period was carried out to ensure participants were at complete rest
 175 and their heart rates were stable.

176

177 To collect the RR intervals participants wore a Polar H10 heart rate monitor with a Pro Strap (Polar Electro Oy,
 178 Kempele, Finland), which has been shown to provide strong agreement and comparable RR interval signal quality
 179 to conventional ECG devices (Gilgen-Ammann et al. 2019; Schaffarczyk et al. 2022). The elastic electrodes of
 180 the Pro Strap were moistened, and the strap lengthened to fit around the participant's chest circumference as
 181 described by the manufacturer. The RR intervals were acquired at 1000 Hz via the Elite HRV application (Elite
 182 HRV, Asheville, NC, USA) on a mobile device positioned directly next to the participant. The RR intervals were
 183 then exported as a text file for processing and analysis offline in MATLAB.

184

185 **2.5. RR interval data pre-processing**

186 All RR interval time series were pre-processed to exclude artifacts and outliers. RR intervals less than 0.2 s and
 187 greater than 2.0 s were removed. Secondly, RR intervals that differed from the mean of the surrounding 40 RR
 188 intervals by more than 20% were excluded.

189

190 The number of RR interval artifacts and outliers from all RR interval time series on Day 1 were: YG, 19.6 ± 20.5
 191 RR intervals or $1.12 \pm 1.24\%$ (range 0.05 to 4.33%) of total RR intervals and OG, 7.5 ± 10.6 RR intervals or 0.46
 192 $\pm 0.64\%$ (range 0.00 to 2.65%) of total RR intervals and Day 2: YG, 16.3 ± 15.9 RR intervals or $0.94 \pm 0.94\%$
 193 (range 0.00 to 3.03%) of total RR intervals and OG, 6.7 ± 12.1 RR intervals or $0.42 \pm 0.76\%$ (range 0.00 to 4.10%)
 194 of total RR intervals.

195

196 **2.6. Heart rate complexity - nonlinear metric analysis**

197 2.6.1. Approximate and Sample entropy

198 Approximate entropy (ApEn; Pincus 1991) and sample entropy (SampEn; Richman & Moorman 2000) quantify
 199 the conditional probability that a template length of m and $m + 1$ data points is repeated during the time series
 200 within a tolerance of r (set at a % of the time series SD). SampEn differs from ApEn, as it avoids counting self-
 201 matches by taking the logarithm after averaging, thus reducing the inherent bias existing within the ApEn
 202 calculation.

203
 204 In the current study template length was set at $m = 2$ and tolerance $r = 0.2$ of the SD of the RR interval time series,
 205 for both ApEn and SampEn analysis (Kaplan et al. 1991). ApEn was calculated as shown by equation [1] and
 206 SampEn by equation [2], where N is the number of data points in the time series, m is the length of the template,
 207 A_i is the number of matches of the i th template of length $m + 1$ data points, and B_i is the number of matches of
 208 the i th template of length m data points:

$$210 \quad [1] \text{ApEn}(m, r, N) = \frac{1}{N - m} \sum_{i=1}^{N-m} \log \frac{A_i}{B_i}$$

$$211 \quad [2] \text{SampEn}(m, r, N) = -\log \left(\frac{\sum_{i=1}^{N-m} A_i}{\sum_{i=1}^{N-m} B_i} \right)$$

214 2.6.2. Detrended fluctuation analysis

215 The detrended fluctuation analysis (DFA) algorithm was used, as outlined by Peng et al. (1994), to measure the
 216 fractal scaling of the RR interval time series. The DFA algorithm allows for the detection of long-range
 217 correlations embedded in seemingly non-stationary physiological time series data. The RR interval time series is
 218 first integrated, using equation [3]:

$$221 \quad [3] y(k) = \sum_{j=1}^k (RR_j - \overline{RR}), \quad k = 1, \dots, N$$

222 The integrated time series are then divided into boxes of equal length, n . Within each box length n , a least squares
 223 line is fitted to the data, $y_n(k)$ denotes the trend in each box. The integrated time series $y(k)$ is then detrended by
 224 subtracting the local trend, $y_n(k)$, within each box. The root-mean-square fluctuation of the integrated and
 225 detrended time series is calculated by equation [4]:

$$228 \quad [4] F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^N [y(k) - y_n(k)]^2}$$

229 The DFA computation [4] is repeated across all box sizes to provide a relationship between $F(n)$, the average
 230 fluctuation as a function of box size, and the box size, n , the number of RR interval data points in a box. The slope
 231 of the double log plot, $\log F(n)$ vs $\log n$, determines the scaling exponent α . DFA α was calculated with box sizes
 232 ranging from 4 to ≤ 64 data points. DFA α_1 was calculated over box sizes of $4 \leq n \leq 16$ data points (i.e., scaling
 233 exponent calculated over short time scales) and DFA α_2 was calculated over box sizes of $16 \leq n \leq 64$ data points
 234 (i.e., scaling exponent calculated over long time scales), as used previously by Peng et al. (1995).

235 The DFA produces a scaling exponent α . An $\alpha = 0.5$ indicates that the value of one RR interval is completely
 236 uncorrelated from any previous values (i.e., unpredictable white noise; indicative of a very rough time series). An
 237 $\alpha = 1.5$ indicates Brown noise and a loss of long-range correlations (i.e., a smooth output with long term memory).
 238 While an α of 1.0 (i.e., 1/f or pink noise) is suggestive of a physiological output of high complexity, that is
 239 statistically self-similar with long range-correlations (Peng et al. 1995). Figure 1A presents an example raw RR
 240 interval time series and 1B presents the integrated time series with the least-squares fit “trend” line plotted for
 241 box sizes of 64 data points.

244 [Figure 1 here]

246
247
248
249
250
251
252
253
254
255

2.6.3. Multiscale entropy

Multiscale entropy (MSE) analysis was performed as outlined by Costa et al. (2002) providing a measure of complexity of time series over multiple scales. The MSE analysis overcomes limitations of SampEn and ApEn which only measure the regularity of time series data on one scale, and therefore do not capture the structural and dynamical behaviour of the time series.

From the one-dimensional discrete time series, $\{\chi_1, \dots, \chi_1, \dots, \chi_N\}$, a coarse-grained time series were constructed, $\{y^{(\tau)}\}$, determined by the scale factor, τ , according to equation [5]:

$$[5] y_j^{(\tau)} = \frac{1}{\tau} \sum_{i=(j-1)\tau+1}^{j\tau} \chi_{i} \quad 1 \leq j \leq N/\tau$$

256
257
258
259
260
261
262
263
264
265
266

At one scale, the time series $\{y^{(1)}\}$ is the original time series of sample length. The length of the coarse-grained time series is equal to the length of the original time series divided by the scale factor, τ . The SampEn for each coarse-grained time series is calculated and plotted against the scale factor, τ , producing a MSE curve. The SampEn of each coarse-grained time series was computed using equation [2] and a template length $m = 2$ and $r = 0.2$ of the SD of the RR interval time series. The area under the MSE curve were calculated from scales 1 to 8 using equation [6] and is defined as the complexity index (CI-8) with higher CI values indicating greater complexity of the physiological signal.

$$[6] CI = \sum_{i=1}^{\tau} SampEn(i)$$

267
268
269
270
271
272
273
274
275

2.6.4. Poincare plot SD2

Poincare plots of RR interval times series were produced by plotting each RR interval as a function of the previous RR interval (Woo et al. 1992). Poincare plots were then analysed with an ellipse fitting procedure to derive the metrics SD1 (the standard deviation of the points perpendicular to the line of identity) and SD2 (the standard deviation along the line of identity; Brennan et al. 2001). Only SD2 was reported as SD1 is identical to RMSSD (Shaffer & Ginsberg 2017).

276
277

2.7. Heart rate variability – linear metric analysis

2.7.1. Time-domain metrics

The time-domain measures of heart rate variability quantify the amount of variability present within the RR interval time series.

278
279
280
281
282
283

The root mean square of successive differences between normal RR intervals (RMSSD) was calculated using equation [7]:

$$[7] RMSSD = \sqrt{\frac{1}{N-1} \sum_{n=1}^{N-1} (RR_{n+1} - RR_n)^2}$$

284
285
286
287

The standard deviation of normal RR intervals (SDNN) was calculated using equation [8]:

$$[8] SDNN = \sqrt{\frac{1}{N-1} \sum_{n=1}^N (RR_n - \overline{RR})^2}$$

288
289
290
291
292

The RMSSD and SDNN metrics were reported in milliseconds and natural logarithm transformed values, LnRMSSD and LnSDNN.

2.7.2. Frequency-domain metrics

The frequency-domain measures of heart rate variability provide an estimate of spectral power in frequency bands. The power spectrum was estimated using a parametric autoregressive based model, with the absolute power in

293
294
295

296 the low frequency power (LF) band (0.04 – 0.15 Hz) and high frequency power (HF) band (0.15 – 0.4 Hz)
 297 calculated, along with the LF/HF ratio. The absolute power in the LF and HF band is reported in ms^2 and natural
 298 logarithm transformed values (Ln).

300 2.8. Statistical analysis

301 Data are presented as individual values or mean \pm SD (unless specified otherwise). Statistical analyses were
 302 conducted using IBM SPSS Statistics 29 (IBM, Armonk, New York, USA). Visual inspection of Q-Q plots and
 303 Shapiro-Wilk statistics were used to check whether data were normally distributed.

304
 305 Day-to-day reliability of all heart rate complexity and variability metrics was assessed through a two-way random
 306 intraclass correlation coefficient (ICC_{2,1}) for absolute agreement, standard error of measurement (SEM), minimal
 307 detectable change (MDC) and Bias (being mean difference between day 1 and day 2). Upper and lower 95% limits
 308 of agreement (LOA) were calculated as the mean of differences between days \pm 1.96 x the standard deviation of
 309 the differences. Between day coefficient of variations (CVs) of all HRV metrics were calculated by dividing the
 310 SD of both days' measurement by the mean of both days measurement and multiplying by one hundred. Between
 311 participant CVs for all HRV metrics were calculated by dividing the SD of all participant measurement by the
 312 mean of all participant measurement and multiplying by one hundred. Paired samples *t*-tests were used to assess
 313 whether a significant difference in the complexity and variability metrics were present between days for each age
 314 group.

315
 316 Based on the ICCs, relative reliability was defined as: poor = ICC < 0.5, moderate = ICC \geq 0.5 to < 0.75, good =
 317 ICC \geq 0.75 to < 0.90 and excellent = ICC \geq 0.90 (Koo & Li 2016).

318
 319 Hedges' *g* effect sizes and the 95% confidence intervals were calculated to assess the differences between the two
 320 age groups (YG vs. OG) HRV metrics and interpreted as: 0.2 to 0.5 small effect, 0.5 to 0.8 medium effect, \geq 0.8
 321 large effect (Cohen 1992).

322
 323 Multiple linear regressions were performed to estimate the effect of participant age, sex and $\dot{V}O_{2\text{peak}}$ on all heart
 324 rate complexity and variability metrics. Males were set as the baseline reference level; therefore, positive beta
 325 coefficients indicate that being female will likely result in a higher value.

326
 327 The significance level was set at $P < 0.05$ in all cases.

329 3. RESULTS

330 3.1. Participant characteristics and anthropometrics

331 Data from forty-four older adults (34M; 10F) and twenty-two younger adults (16M; 6F) were included in the
 332 analysis. Table 1 presents participant anthropometrics and IET data.

333
 334 [Table 1 here]

336 3.2. Reliability of heart rate complexity and variability-based metrics

337 Based upon the ICCs the OG demonstrated poor reliability for the CI-8 and SD2 metric, moderate reliability for
 338 the RMSSD, SDNN, LnRMSSD, LnSDNN, LF(ms^2), HF(ms^2), LF(log), HF(log), ApEn, SampEn, DFA α , DFA
 339 α_1 and DFA α_2 metrics, and good reliability for the LF/HF metric (Table 2). By comparison, the YG demonstrated
 340 poor reliability for the ApEn, SampEn and SD2 metrics, moderate reliability for the LnSDNN, LF (ms^2), LF(log),
 341 DFA α_2 and CI-8 metrics, good reliability for the RMSSD, SDNN, LnRMSSD, HF(ms^2), HF(log), LF/HF and
 342 DFA α metrics and excellent reliability for the DFA α_1 metric (Table 3).

343
 344 [Table 2 here]

345 [Table 3 here]

347 3.3. Effect of age, sex and $\dot{V}O_{2\text{peak}}$ on heart rate complexity

348 There was a significant reduction in the ApEn ($P < 0.001$; Figure 2E), SampEn ($P = 0.031$; Figure 2F) and SD2
 349 ($P < 0.001$; Figure 2H) metrics with ageing (Table 5). There was no significant effect of age on the CI-8 ($P =$
 350 0.493; Figure 2G; Table 5).

351
 352 [Figure 2 here]

353

354 There was no significant effect of age on the DFA α_1 ($P = 0.107$; Figure 3B) and DFA α_2 ($P = 0.147$; Figure 3C)
 355 metrics (Table 5). The DFA α metric was significantly increased with ageing ($P = 0.029$; Figure 3A).
 356

357 There was a significant effect of sex ($P = 0.028$), but not of $\dot{V}O_{2\text{peak}}$ ($P = 0.822$) on DFA α_1 , with females
 358 presenting with lower values. There was no significant effect of sex or $\dot{V}O_{2\text{peak}}$ on the ApEn, SampEn, DFA α ,
 359 DFA α_2 , CI-8 and SD2 metrics ($P > 0.05$; Table 5).
 360

361 [Figure 3 here]
 362

363 3.4. Effect of age, sex and $\dot{V}O_{2\text{peak}}$ on heart rate variability

364 There was a significant reduction in RMSSD ($P < 0.001$; Figure 2A), SDNN ($P < 0.001$; Figure 2B), LF power
 365 ($P < 0.001$; Figure 2C) and HF power ($P < 0.001$; Figure 2D) metrics with ageing (Table 5).
 366

367 There was no significant effect of sex or $\dot{V}O_{2\text{peak}}$ on all linear HRV metrics ($P > 0.05$; Table 5).
 368

369 [Table 4 here]

370 [Table 5 here]
 371

372 4. DISCUSSION

373 4.1. Reliability of heart rate complexity and variability metrics

374 The current study provides new inter-day reliability data for a range of widely utilised time-domain, frequency-
 375 domain and nonlinear HRV metrics in healthy highly active younger and older adults. The primary findings of
 376 this investigation reveal all linear HRV metrics in both the younger adult and older adult groups to exhibit
 377 moderate to good inter-day reliability, as indicated by ICCs ranging from 0.56 to 0.88 (Tables 2 & 3). Similarly,
 378 the majority of nonlinear HRV metrics demonstrated moderate to excellent inter-day reliability with ICCs ranging
 379 from 0.55 to 0.93 (Tables 2 & 3). There were exceptions however, with ApEn, SampEn and SD2 metrics of the
 380 YG, and the SD2 metric of the OG exhibiting poor relative reliability, as shown by ICCs of less than 0.50 (Tables
 381 2 & 3). This variability in the inter-day reliability of HRV metrics can likely be attributed to the sensitivity of the
 382 ANS and the influence of various individual internal and external factors that can be challenging to control
 383 (Fatissou et al. 2016).
 384

385 It has been suggested that the assessment of test-retest reliability should not rely solely on ICCs (Weir et al. 2005).
 386 This viewpoint is supported by the current study, with the ApEn, SampEn and CI-8 HRV metrics displaying ICCs
 387 ranging from 0.37 to 0.69, indicating poor to moderate relative reliability (Tables 2 & 3). However, these metrics
 388 exhibited low SEM values (ranging from 0.06 to 0.20) and low between day CVs (ranging from 2.95% to 7.65%),
 389 which suggests high absolute retest reliability. This apparent contradiction can be explained by the homogeneous
 390 population recruited and low between participant CVs for these specific metrics, leading to low relative but high
 391 absolute reliability (Atkinson & Nevill 1998; Weir 2005). In contrast, the SD2 metric showed both low relative
 392 reliability (ICCs ranging from 0.33 to 0.44) and low absolute reliability (between day CVs of 18.13% to 20.42%
 393 and SEM values of 17.43 to 60.00). Similarly, the frequency-domain metrics LF, HF, and LF/HF also exhibited
 394 low absolute reliability (Tables 2 & 3). These findings indicate that specific HRV metrics may present significant
 395 challenges when used to detect intervention/treatment effects or individual changes over time. Consequently, the
 396 HRV metrics with low relative and absolute reliability may not be suitable in specific research contexts, especially
 397 those with limited sample sizes or small intervention/treatment effects.
 398

399 ICCs and SEM of the SampEn and DFA metrics for both age groups in the current study are comparable to those
 400 reported by Maestri et al. (2007a) who examined HRV inter-day reliability in healthy adults with a mean age of
 401 38 years (range 26 to 56 years). Accordingly, the LnRMSSD, LnSDNN, LnLF, and LnHF metrics of both age
 402 groups produced similar ICCs to those reported for healthy young students aged between 18 and 39 years (Uhlrig
 403 et al. 2020), in addition to comparable between day CVs and SEM to healthy trained young adults (aged $21.5 \pm$
 404 1.4 years; Al Haddad et al. 2011). The corroboration between reliability studies improves confidence in the
 405 expected retest error of HRV metrics. However, it also emphasises the high level of variance in certain HRV
 406 metrics (i.e., LF, HF, LF/HF and SD2), as well as the difficulty facing researchers in sufficiently powering studies
 407 which are utilising HRV measurements across multiple visits and/or during longitudinal studies.
 408

409 The study builds upon previous HRV reliability research providing inter-day reliability data for short-term resting
 410 HRV measurements for younger and importantly older adults across a range of widely utilised HRV metrics. The
 411 reliability data in tables 2 and 3 provides a resource for researchers to reference when calculating sample sizes for
 412 future HRV studies with healthy adult participants. Importantly, given the disparity in the reliability of different

413 HRV metrics (ICCs, 0.33 to 0.93; Between day CVs, 2.9 to 36.5; Tables 2 & 3), study sample size is recommended
414 to be based upon the chosen metric with the lowest reliability to reduce the likelihood of a type I or type II error
415 across all metrics. In addition, the reliability statistics also allow for the assessment of whether there is a true
416 intervention effect or individual change in HRV metrics within a study and not just a result of biological and
417 measurement error.

418

419 **4.2. Effect of age, sex and $\dot{V}O_{2peak}$ on heart rate complexity and variability**

420 The current study findings demonstrate a significant age-related decline in linear (RMSSD, LnRMSSD, SDNN,
421 LnSDNN, LF, HF) and nonlinear (ApEn, SampEn and SD2) HRV metrics (Table 4 & 5; Figure 2), corroborating
422 the findings of a broad body of literature which has assessed the effect of age on heart rate complexity and
423 variability (Kaplan et al. 1991; Iyengar et al. 1996; Jensen-Urstad et al. 1997; Umetani et al. 1998; Pikkujamsa et
424 al. 1999; Beckers et al. 2006; Goff et al. 2010; Voss et al. 2015; Hernandez-Vicente et al. 2020). An age-related
425 decrease in both the linear and nonlinear HRV metrics is expected, primarily driven by alterations in the ANS,
426 characterised by a decline in parasympathetic activity and an increase in sympathetic drive (Seals & Esler 2000).

427

428 Despite age-related differences in all other HRV metrics, there was no significant effect of age on the nonlinear
429 DFA $\alpha 1$ and $\alpha 2$ metrics (Table 5; Figures 3B & 3C). Mean DFA $\alpha 1$ and $\alpha 2$ values were close to 1.0 (i.e., 1/f or
430 pink noise), indicative of a healthy physiological signal of high complexity that is exhibiting both short and long-
431 range fractal-like correlations (Peng et al. 1995). These findings are comparable to previous research which also
432 found no age-related difference in the DFA $\alpha 1$ and $\alpha 2$ metric (Vuksanovic & Gal 2005; Schmitt & Ivanov 2007;
433 Wiersema et al. 2022). Seminal research exploring the effect of age on the fractal behaviour of RR interval time
434 series observed healthy older adults ($\alpha 2 = 0.75 \pm 0.17$) to have a significant decline in long-range fractal
435 correlations, in comparison to healthy younger adults ($\alpha 2 = 0.99 \pm 0.10$; Iyengar et al. 1996). The mean age of
436 the older group in the study of Iyengar et al. (1996) was greater than the older group of the current study (74 years
437 vs 59 years), which may partly explain the difference in findings between the studies, as well as the high activity
438 levels of the older participants of the current study. It is important to note that despite recruiting a homogenous
439 sample, several participants did produce $\alpha 1$ and $\alpha 2$ values closer to 0.5 and 1.5 (Figures 3B & 3C). Such between
440 participant variation is expected, occurring to differing extents for all HRV metrics (Tables 2 & 3) and highlights
441 the importance of also accounting for the inter-individual variability of HRV metrics when seeking to understand
442 the utility of HRV in different populations.

443

444 The findings of the current study demonstrate no significant age-related change in the nonlinear CI-8 metric
445 (Figure 2G; Table 5). Like the DFA $\alpha 1$ and $\alpha 2$ metrics, the CI-8 metric captures the structural and dynamical
446 behaviour of the RR interval time series over multiple scales (Costa et al. 2002). Accordingly, the *complexity*
447 (DFA and CI-8) of the study participants' RR interval time series is suggestive of their cardiovascular systems
448 ability to adapt to physiologic perturbations and respond quickly to challenges to maintaining homeostasis (Peng
449 et al. 2009; Manor & Lipsitz 2013). The mixed findings of the effect of age on different HRV metrics highlights
450 the necessity of employing multiple heart rate complexity and variability metrics when analysing RR interval
451 times series. If only specific time-domain, frequency-domain or non-linear HRV metrics are utilised, studies may
452 fail to capture different linear and nonlinear aspects of the signal, therefore potentially missing important
453 information on cardiac interval dynamics. However, the choice and combination of HRV metrics by
454 researchers is also likely to be dependent on the research context; with different HRV metrics better
455 suited to capturing specific properties and/or changes in cardiac interval dynamics, in addition to the
456 redundancy of combining HRV metrics which measure similar HRV properties (Maestri et al. 2007b).

457

458 The current study included male ($N = 50$) and female ($N = 16$) participants. Sex differences in HRV are well
459 documented and are influenced by physiological, hormonal, and neural factors (Koenig & Thayer 2016).
460 Moreover, sex-related differences in HRV may be more pronounced in younger adults, when compared to older
461 adults (Maria et al. 2023). It should be noted that the current study did not control for menstrual cycle phase or
462 hormone changes due to the menopause, which are known to effect HRV (Aubert et al. 2003; Maria et al. 2023).
463 Sex did not significantly predict the HRV metrics in the current study, except for the DFA $\alpha 1$ metric (Table 5).
464 The significant effect of sex indicates that females present with lower $\alpha 1$ value in comparison to males. Such
465 differences in $\alpha 1$ is suggestive of a notable change in the short-range fractal correlation properties of HRV and
466 an alteration in sympathetic and vagal activation (Tulppo et al. 2005).

467

468 While sex was not significantly predictive of the HRV metrics, the beta coefficients indicate a trend towards
469 females having higher values in HRV metrics primarily associated with parasympathetic activity (i.e., HF power
470 and RMSSD) in comparison to males. There is evidence to support an increase in parasympathetic modulation (as
471 indicated by absolute HF power) in females compared to males (Koenig & Thayer 2016). However, evidence is

472 argued to be inconclusive with heterogeneity in study findings, likely emanating from differences in study
473 methodology and analysis methods (Maria et al. 2023).

474
475 Aerobic physical activity has been shown to have positive effects on measures of HRV in both younger and older
476 adults, when compared to sedentary age matched individuals, through enhanced autonomic balance, improved
477 baroreflex sensitivity and cardiac adaptations (Aubert et al. 2003). To capture the effect of inherent biological
478 ageing on HRV (i.e., individuals unaffected by sedentary behaviour or underlying pathologies) all participants of
479 the current study were recruited to be in full health and regular exercisers closely matched for physical activity
480 levels and aerobic fitness (Table 1). Although the YG did present with a higher absolute aerobic fitness as
481 measured by $\dot{V}O_{2peak}$ (YG $\dot{V}O_{2peak} = 3.5 \pm 1.0 \text{ L}\cdot\text{min}^{-1}$ vs. OG $\dot{V}O_{2peak} = 3.0 \pm 0.8 \text{ L}\cdot\text{min}^{-1}$), $\dot{V}O_{2peak}$ was not
482 significantly predictive of any HRV metric (Table 5).

484 4.3. Limitations

485 The current study only assessed the reliability of HRV metrics derived from short-term RR interval measurements
486 in healthy active younger and older adults during free-breathing wakeful supine rest. Due to the sensitivity of the
487 ANS to various external and internal factors (Fatisson et al. 2016), caution is advised when extrapolating the
488 reliability data reported herein to HRV metrics derived from RR interval measurements performed under different
489 conditions. The current study was limited to the assessment of inter-day reliability and did not assess the intra-
490 day reliability of the HRV metrics. Given the sensitivity of the ANS, it is probable the inter-day variation in HRV
491 largely reflects biological error, whereas intra-day variation in HRV would likely provide a closer insight into the
492 measurement error.

493
494 The current study assessed a range of time-domain, frequency-domain and nonlinear HRV metrics, which are
495 extensively studied and widely accepted to provide valuable information regarding ANS function in ageing,
496 between sexes and in athletes (Koenig & Thayer 2016; Shaffer & Ginsberg 2017; Lundstrom et al. 2023).
497 However, it is important to highlight that the study does not provide a comprehensive list of available HRV
498 metrics. Notably, the study did not include HRV metrics from the major families of symbolic dynamics,
499 predictability, and empirical mode decomposition (Maestri et al. 2007b). Researchers should specifically consider
500 using the symbolic dynamic metric, one variation pattern (1VP) and empirical mode decomposition metric,
501 IMAI2. The IVP and IMAI2 metrics have been shown to provide additive predictive value independent to clinical
502 predictors when assessing chronic heart failure patients (Maestri et al. 2007b) and detect experimentally induced
503 changes in autonomic cardiovascular regulation in healthy individuals (Guzzetti et al. 2005).

504
505 The nonlinear HRV metric, ApEn, was included in the current study as a metric from the entropy family, which
506 can assess the irregularity or randomness of an RR interval time series (Pincus 1991). However, the calculation
507 of ApEn presents notable limitations due to its self-matching that may affect its interpretation (Richman &
508 Moorman 2000). ApEn exhibits sensitivity to data length, particularly in cases of short data sequences such as RR
509 interval time series, leading to potentially biased results due to its reliance on pattern identification within the
510 arbitrarily specified tolerance parameter, “*r*”. Moreover, ApEn's susceptibility to self-matching can cause relative
511 inconsistencies; meaning if the ApEn of a time series is higher than another time series, it should remain higher
512 under all conditions, however, it does not always remain higher (Richman & Moorman 2000). Despite ApEn
513 demonstrating high absolute retest reliability, researchers are advised to account for these limitations when using
514 ApEn for HRV analysis.

516 4.4. Conclusion

517 The current findings show that widely used HRV metrics derived from short-term (30-minutes) RR interval
518 measurements are reproducible between days in healthy, highly active younger and older adults. However, there
519 is a disparity in the inter-day reliability of different HRV metrics, with certain metrics presenting with a higher
520 level of variance (i.e., LF, HF, LF/HF and SD2). Both linear and nonlinear HRV metrics capture different aspects
521 of cardiac interval dynamics; therefore, researchers should not exclude metrics based solely on their reliability.
522 Instead, studies should be designed appropriately based upon the chosen HRV metrics to increase the probability
523 of detecting a true effect. This also study extends upon previous research by demonstrating a significant age-
524 related decline in the majority of linear and nonlinear HRV metrics assessed. However, the participants' sex and
525 $\dot{V}O_{2peak}$ did not significantly influence the HRV metrics.

526
527
528
529
530
531

532 **AUTHOR CONTRIBUTION STATEMENT**

533 CF, JH, and LM designed research. CF conducted experiments, data collection and data analysis. CF, JH and AM
534 wrote the manuscript. All authors read and approved the manuscript. All authors revised the manuscript.

535

536 **COMPLIANCE WITH ETHICAL STANDARDS**

537

538 **Ethical approval.**

539 The study was completed with full ethical approval from the University of Kent research ethics committee
540 (Proposal number: 21_2020_21), according to the 1964 Declaration of Helsinki standards and its later
541 amendments.

542 **Consent to participate.**

543 All participants provided written signed informed consent prior to testing.

544 **Consent to publication.**

545 All participants consented to having research findings published. All authors consented to publication of
546 manuscript.

547 **Conflicts of interest/Competing interests.**

548 The authors report no conflicts of interest or competing interests.

549

550

551 **DECLARATIONS**

552

553 **Funding.**

554 No funds, grants, or other support was received for conducting or submitting this manuscript.

555 **Financial interests.**

556 The authors declare they have no financial interests.

557 **Non-Financial interests.**

558 None

559 **Software availability**

560 Data analysis software application used (SPSS and MATLAB) openly available.

561

562

563 **ACKNOWLEDGEMENTS**

564 Not Applicable

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

582

583

584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638

REFERENCES

1. Atkinson G, Nevill AM (1998) Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Med.* 26:217-38. doi: 10.2165/00007256-199826040-00002. PMID: 9820922.
2. Aubert AE, Seps B, Beckers F (2003) Heart rate variability in athletes. *Sports Med.* 33:889-919. doi: 10.2165/00007256-200333120-00003. PMID: 12974657.
3. Al Haddad H, Laursen PB, Chollet D, Ahmaidi S, Buchheit M (2011) Reliability of resting and postexercise heart rate measures. *Int J Sports Med.* 32:598-605. doi: 10.1055/s-0031-1275356. PMID: 21574126.
4. Beaver WL, Wasserman K, Whipp BJ (1986) A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol.* 60:2020-7. 1986. doi: 10.1152/jappl.1986.60.6.2020. PMID: 3087938.
5. Brennan M, Palaniswami M, Kamen P (2001) Do existing measures of Poincaré plot geometry reflect nonlinear features of heart rate variability? *IEEE Trans Biomed Eng.* 48:1342-7. doi: 10.1109/10.959330. PMID: 11686633.
6. Beckers F, Verheyden B, Aubert AE (2006) Aging and nonlinear heart rate control in a healthy population. *Am J Physiol Heart Circ Physiol.* 290:H2560-70. doi: 10.1152/ajpheart.00903.2005. PMID: 16373585.
7. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, Carty C, Chaput JP, Chastin S, Chou R, Dempsey PC, DiPietro L, Ekelund U, Firth J, Friedenreich CM, Garcia L, Gichu M, Jago R, Katzmarzyk PT, Lambert E, Leitzmann M, Milton K, Ortega FB, Ranasinghe C, Stamatakis E, Tiedemann A, Troiano RP, van der Ploeg HP, Wari V, Willumsen JF (2020) World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med.* 54:1451-1462. doi: 10.1136/bjsports-2020-102955. PMID: 33239350.
8. Cohen J (1992) A power primer. *Psychol Bull.* 112:155-9. doi: 10.1037//0033-2909.112.1.155. PMID: 19565683.
9. Costa M, Goldberger AL, Peng CK (2002) Multiscale entropy to distinguish physiologic and synthetic RR time series. *Comput Cardiol.* 29:137-40. PMID: 14686448.
10. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P (2003) International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* 35:1381-95. doi: 10.1249/01.MSS.0000078924.61453.FB. PMID: 12900694.
11. Cipryan L, Litschmannova M (2013) Intra-day and inter-day reliability of heart rate variability measurement. *J Sports Sci.* 31:150-8. doi: 10.1080/02640414.2012.721931. PMID: 22963409.
12. de la Torre-Luque A, Bornas X, Balle M, Fiol-Veny A (2016) Complexity and nonlinear biomarkers in emotional disorders: A meta-analytic study. *Neurosci Biobehav Rev.* 68:410-422. doi: 10.1016/j.neubiorev.2016.05.023. PMID: 27267791.
13. Fatisson J, Oswald V, Lalonde F (2016) Influence diagram of physiological and environmental factors affecting heart rate variability: an extended literature overview. *Heart Int.* 11:e32-e40. doi: 10.5301/heartint.5000232. PMID: 27924215; PMCID: PMC5056628.
14. Goldberger AL, Rigney DR, Mietus J, Antman EM, Greenwald S (1988) Nonlinear dynamics in sudden cardiac death syndrome: heartrate oscillations and bifurcations. *Experientia.* 44:983-7. doi: 10.1007/BF01939894. PMID: 3197810.
15. Goff EA, Nicholas CL, Malaweera AS, Simonds AK, Trinder J, Morrell MJ (2010) The influence of age on heart rate variability during morning wakefulness. *Clin Auton Res.* 20:175-82. doi: 10.1007/s10286-009-0027-0. PMID: 19756828.
16. Gilgen-Ammann R, Schweizer T, Wyss T (2019) RR interval signal quality of a heart rate monitor and an ECG Holter at rest and during exercise. *Eur J Appl Physiol.* 119:1525-1532. doi: 10.1007/s00421-019-04142-5. PMID: 31004219.
17. Hansen AL, Johnsen BH, Sollers JJ 3rd, Stenvik K, Thayer JF (2004) Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur J Appl Physiol.* 93:263-72. doi: 10.1007/s00421-004-1208-0. PMID: 15338220.
18. Hernández-Vicente A, Hernando D, Santos-Lozano A, Rodríguez-Romo G, Vicente-Rodríguez G, Pueyo E, Bailón R, Garatachea N (2020) Heart Rate Variability and Exceptional Longevity. *Front Physiol.* 11:566399. doi: 10.3389/fphys.2020.566399. PMID: 33041862.
19. Iyengar N, Peng CK, Morin R, Goldberger AL, Lipsitz LA (1996) Age-related alterations in the fractal scaling of cardiac interbeat interval dynamics. *Am J Physiol.* 271(4 Pt 2):R1078-84. doi: 10.1152/ajpregu.1996.271.4.R1078. PMID: 8898003.

- 639 20. Jensen-Urstad K, Storck N, Bouvier F, Ericson M, Lindblad LE, Jensen-Urstad M (1997) Heart rate
640 variability in healthy subjects is related to age and gender. *Acta Physiol Scand.* 160:235-41. doi:
641 10.1046/j.1365-201X.1997.00142.x. PMID: 9246386.
- 642 21. Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ (1987) Decreased heart rate variability and its association
643 with increased mortality after acute myocardial infarction. *Am J Cardiol.* 59:256-62. doi: 10.1016/0002-
644 9149(87)90795-8. PMID: 3812275.
- 645 22. Kaplan DT, Furman MI, Pincus SM, Ryan SM, Lipsitz LA, Goldberger AL (1991) Aging and the
646 complexity of cardiovascular dynamics. *Biophys J.* 59:945-9. doi: 10.1016/S0006-3495(91)82309-8.
647 PMID: 2065195; PMCID: PMC1281262.
- 648 23. Koo TK, Li MY (2016) A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for
649 Reliability Research. *J Chiropr Med.* 15:155-63. doi: 10.1016/j.jcm.2016.02.012. PMID: 27330520.
- 650 24. Koenig J, Thayer JF (2016) Sex differences in healthy human heart rate variability: A meta-analysis.
651 *Neurosci Biobehav Rev.* 64:288-310. doi: 10.1016/j.neubiorev.2016.03.007. PMID: 26964804.
- 652 25. Lipsitz LA (1989) Altered blood pressure homeostasis in advanced age: clinical and research implications.
653 *J Gerontol.* 44:M179-83. doi: 10.1093/geronj/44.6.m179. PMID: 2681357.
- 654 26. Lipsitz LA, Goldberger AL (1992) Loss of 'complexity' and aging. Potential applications of fractals and
655 chaos theory to senescence. *JAMA.* 267:1806-9. PMID: 1482430.
- 656 27. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ (1998) Baroreflex sensitivity and heart-
657 rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic
658 Tone and Reflexes After Myocardial Infarction) Investigators. *Lancet.* 351:478-84. doi: 10.1016/s0140-
659 6736(97)11144-8. PMID: 9482439.
- 660 28. Lucía A, Sánchez O, Carvajal A, Chicharro JL (1999) Analysis of the aerobic-anaerobic transition in elite
661 cyclists during incremental exercise with the use of electromyography. *Br J Sports Med.* 33:178-85. doi:
662 10.1136/bjism.33.3.178. PMID: 10378070.
- 663 29. Lundstrom CJ, Foreman NA, Biltz G (2023) Practices and Applications of Heart Rate Variability
664 Monitoring in Endurance Athletes. *Int J Sports Med.* 44:9-19. doi: 10.1055/a-1864-9726. PMID: 35853460.
- 665 30. Maestri R, Pinna GD, Porta A, Balocchi R, Sassi R, Signorini MG, Dudziak M, Raczak G (2007a) Assessing
666 nonlinear properties of heart rate variability from short-term recordings: are these measurements reliable?
667 *Physiol Meas.* 28:1067-77. doi: 10.1088/0967-3334/28/9/008. PMID: 17827654.
- 668 31. Maestri R, Pinna GD, Accardo A, Allegrini P, Balocchi R, D'Addio G, Ferrario M, Menicucci D, Porta A,
669 Sassi R, Signorini MG, La Rovere MT, Cerutti S (2007b) Nonlinear indices of heart rate variability in
670 chronic heart failure patients: redundancy and comparative clinical value. *J Cardiovasc Electrophysiol.*
671 18:425-33. doi: 10.1111/j.1540-8167.2007.00728.x. PMID: 17284264.
- 672 32. Manor B, Lipsitz LA (2013) Physiologic complexity and aging: implications for physical function and
673 rehabilitation. *Prog Neuropsychopharmacol Biol Psychiatry.* 45:287-93. doi: 10.1016/j.pnpbp.2012.08.020.
674 PMID: 22985940; PMCID.
- 675 33. Mosley E, Laborde S, Kavanagh E (2018) Coping related variables, cardiac vagal activity and working
676 memory performance under pressure. *Acta Psychol (Amst).* 191:179-189. doi:
677 10.1016/j.actpsy.2018.09.007. PMID: 30308441.
- 678 34. De Maria B, Parati M, Dalla Vecchia LA, La Rovere MT (2023) Day and night heart rate variability using
679 24-h ECG recordings: a systematic review with meta-analysis using a gender lens. *Clin Auton Res.* 2023
680 Aug 4. doi: 10.1007/s10286-023-00969-3. PMID: 37541968.
- 681 35. Pincus SM (1991) Approximate entropy as a measure of system complexity. *Proc Natl Acad Sci U S A.*
682 88:2297-301. doi: 10.1073/pnas.88.6.2297. PMID: 11607165.
- 683 36. Peng CK, Buldyrev SV, Havlin S, Simons M, Stanley HE, Goldberger AL (1994) Mosaic organization of
684 DNA nucleotides. *Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics.* 49:1685-9. doi:
685 10.1103/physreve.49.1685. PMID: 9961383.
- 686 37. Peng CK, Havlin S, Stanley HE, Goldberger AL (1995) Quantification of scaling exponents and crossover
687 phenomena in nonstationary heartbeat time series. *Chaos.* 5:82-7. doi: 10.1063/1.166141. PMID: 11538314.
- 688 38. Pikkujämsä SM, Mäkikallio TH, Sourander LB, Räihä IJ, Puukka P, Skyttä J, Peng CK, Goldberger AL,
689 Huikuri HV (1999) Cardiac interbeat interval dynamics from childhood to senescence: comparison of
690 conventional and new measures based on fractals and chaos theory. *Circulation.* 100:393-9. doi:
691 10.1161/01.cir.100.4.393. PMID: 10421600.
- 692 39. Peng CK, Costa M, Goldberger AL (2009) Adaptive data analysis of complex fluctuations in physiologic
693 time series. *Adv Adapt Data Anal.* 1:61-70. doi: 10.1142/S1793536909000035. PMID: 20041035.

- 694 40. Pallarés JG, Morán-Navarro R, Ortega JF, Fernández-Elías VE, Mora-Rodríguez R (2016) Validity and
695 Reliability of Ventilatory and Blood Lactate Thresholds in Well-Trained Cyclists. *PLoS One*. 11:e0163389.
696 doi: 10.1371/journal.pone.0163389. PMID: 27657502.
- 697 41. Guzzetti S, Borroni E, Garbelli PE, Ceriani E, Della Bella P, Montano N, Cogliati C, Somers VK, Malliani
698 A, Porta A (2005) Symbolic dynamics of heart rate variability: a probe to investigate cardiac autonomic
699 modulation. *Circulation*. 112:465-70. doi: 10.1161/CIRCULATIONAHA.104.518449. PMID: 16027252.
- 700 42. Richman JS, Moorman JR (2000) Physiological time-series analysis using approximate entropy and sample
701 entropy. *Am J Physiol Heart Circ Physiol*. 278:H2039-49. doi: 10.1152/ajpheart.2000.278.6.H2039. PMID:
702 10843903.
- 703 43. Seals DR, Esler MD (2000) Human ageing and the sympathoadrenal system. *J Physiol*. 528:407-17. doi:
704 10.1111/j.1469-7793.2000.00407.x. PMID: 11060120.
- 705 44. Schwab JO, Eichner G, Schmitt H, Weber S, Coch M, Waldecker B (2003) The relative contribution of the
706 sinus and AV node to heart rate variability. *Heart*. 89:337-8. doi: 10.1136/heart.89.3.337. PMID: 12591853.
- 707 45. Stein PK, Domitrovich PP, Hui N, Rautaharju P, Gottdiener J (2005) Sometimes higher heart rate variability
708 is not better heart rate variability: results of graphical and nonlinear analyses. *J Cardiovasc Electrophysiol*.
709 16:954-9. doi: 10.1111/j.1540-8167.2005.40788.x. PMID: 16174015.
- 710 46. Schmitt DT, Ivanov PCh (2007) Fractal scale-invariant and nonlinear properties of cardiac dynamics remain
711 stable with advanced age: a new mechanistic picture of cardiac control in healthy elderly. *Am J Physiol*
712 *Regul Integr Comp Physiol*. 293:R1923-37. doi: 10.1152/ajpregu.00372.2007. PMID: 17670859.
- 713 47. Shaffer F, Ginsberg JP (2017) An Overview of Heart Rate Variability Metrics and Norms. *Front Public*
714 *Health*. 5:258. doi: 10.3389/fpubh.2017.00258. PMID: 29034226.
- 715 48. Schaffarczyk M, Rogers B, Reer R, Gronwald T (2022) Validity of the Polar H10 Sensor for Heart Rate
716 Variability Analysis during Resting State and Incremental Exercise in Recreational Men and Women.
717 *Sensors (Basel)*. 22:6536. doi: 10.3390/s22176536. PMID: 36081005.
- 718 49. Task force (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical
719 use. Task Force of the European Society of Cardiology and the North American Society of Pacing and
720 Electrophysiology. *Circulation*. 93:1043-65. PMID: 8598068.
- 721 50. Tulppo MP, Kiviniemi AM, Hautala AJ, Kallio M, Seppänen T, Mäkikallio TH, Huikuri HV (2005)
722 Physiological background of the loss of fractal heart rate dynamics. *Circulation*. 112:314-9. doi:
723 10.1161/CIRCULATIONAHA.104.523712. PMID: 16009791.
- 724 51. Umetani K, Singer DH, McCraty R, Atkinson M (1998) Twenty-four hour time domain heart rate variability
725 and heart rate: relations to age and gender over nine decades. *J Am Coll Cardiol*. 31:593-601. doi:
726 10.1016/s0735-1097(97)00554-8. PMID: 9502641.
- 727 52. Uhlig S, Meylan A, Rudolph U (2020) Reliability of short-term measurements of heart rate variability:
728 Findings from a longitudinal study. *Biol Psychol*. 154:107905. doi: 10.1016/j.biopsycho.2020.107905.
729 PMID: 32505705.
- 730 53. Vuksanović V, Gal V (2005) Nonlinear and chaos characteristics of heart period time series: healthy aging
731 and postural change. *Auton Neurosci*. 121:94-100. doi: 10.1016/j.autneu.2005.06.004. PMID: 16055389.
- 732 54. Voss A, Schroeder R, Heitmann A, Peters A, Perz S (2015) Short-term heart rate variability--influence of
733 gender and age in healthy subjects. *PLoS One*. 10:e0118308. doi: 10.1371/journal.pone.0118308. PMID:
734 25822720.
- 735 55. Whipp BJ, Davis JA, Wasserman K (1989) Ventilatory control of the 'isocapnic buffering' region in rapidly-
736 incremental exercise. *Respir Physiol*. 76:357-67. doi: 10.1016/0034-5687(89)90076-5. PMID: 2501844.
- 737 56. Woo MA, Stevenson WG, Moser DK, Trelease RB, Harper RM (1992) Patterns of beat-to-beat heart rate
738 variability in advanced heart failure. *Am Heart J*. 123:704-10. doi: 10.1016/0002-8703(92)90510-3. PMID:
739 1539521.
- 740 57. Weir JP (2005) Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM.
741 *J Strength Cond Res*. 19:231-40. doi: 10.1519/15184.1. PMID: 15705040.
- 742 58. Williams DP, Cash C, Rankin C, Bernardi A, Koenig J, Thayer JF (2015) Resting heart rate variability
743 predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation.
744 *Front Psychol*. 6:261. doi: 10.3389/fpsyg.2015.00261. PMID: 25806017.
- 745 59. Wiersema JM, Kamphuis AEP, Rohling JHT, Kervezee L, Akintola AA, Jansen SW, Slagboom PE, van
746 Heemst D, van der Spoel E (2022) The association between continuous ambulatory heart rate, heart rate
747 variability, and 24-h rhythms of heart rate with familial longevity and aging. *Aging (Albany NY)*. 14:7223-
748 7239. doi: 10.18632/aging.204219. PMID: 35980264.

749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798

FIGURE CAPTIONS

Fig. 1. (A) Example raw RR interval time series; (B) The integrated RR interval time series, with the least-squares fit representing the “trend” in each box (red lines) and the vertical lines indicating the box size of $n = 64$ data points. The RR interval data presented produced a DFA $\alpha = 1.04$ (DFA α calculated over box sizes 4 to ≤ 64 ; data were from a younger male participant aged 18 years).

Fig. 2. Comparisons between the younger and older groups complexity and variability metrics (A) Root mean square of successive differences between normal RR intervals; (B) Standard deviation of normal RR intervals; (C) Low frequency power; (D) High frequency power; (E) Approximate entropy; (F) Sample entropy; (G) Complexity index under 8 scales; (H) Standard deviation of points along the line of identity of the Poincare plot (* $P < 0.05$; ** $P < 0.001$; Data points are the mean of both days for each individual participant).

Fig. 3. Comparisons between the younger and older groups detrended fluctuation analysis metrics (A) DFA α (box sizes 4 to ≤ 64 data points); (B) DFA α_1 (box sizes of $4 \leq n \leq 16$ data points); (C) DFA α_2 (box sizes of $16 \leq n \leq 64$ data points; * $P < 0.05$; Data points are the mean of both days for each individual participant).

799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845

Table 1 Participant characteristics, anthropometrics and IET data (mean \pm SD)

	OG	YG
<i>N</i>	44 (34M; 10F)	22 (16M; 6F)
Age (years)	58.6 \pm 5.1	21.9 \pm 3.7
Height (cm)	173.8 \pm 8.6	177.3 \pm 9.8
Mass (kg)	72.3 \pm 12.1	74.1 \pm 12.1
Fat Mass (%)	22.0 \pm 7.2	16.1 \pm 9.1
Lean Body Mass (%)	78.0 \pm 7.2	83.9 \pm 9.1
Lean Body Mass (kg)	56.3 \pm 10.1	61.9 \pm 10.9
Lean Body Mass Index (kg.m ²)	18.5 \pm 2.1	19.3 \pm 1.9
Systolic BP (mmHg)	130.6 \pm 7.9	126.1 \pm 6.0
Diastolic BP (mmHg)	80.3 \pm 9.6	73.4 \pm 7.8
Absolute $\dot{V}O_{2peak}$ (L.min ⁻¹)	3.0 \pm 0.8	3.5 \pm 1.0
Relative $\dot{V}O_{2peak}$ (ml.kg ⁻¹ .min ⁻¹)	40.9 \pm 7.6	47.2 \pm 12.8
Power at $\dot{V}O_{2peak}$ (W)	277.2 \pm 68.2	318.1 \pm 94.4
Relative $\dot{V}O_2$ at GET (ml.kg ⁻¹ .min ⁻¹)	27.2 \pm 6.7	31.5 \pm 10.3
Power at GET (W)	162.1 \pm 47.7	193.0 \pm 71.6
Relative $\dot{V}O_2$ at RCP (ml.kg ⁻¹ .min ⁻¹)	34.3 \pm 7.1	38.4 \pm 10.9
Power at RCP (W)	215.3 \pm 56.6	242.4 \pm 80.3
Exercise time per week (hours)	9.9 \pm 4.7	13.2 \pm 4.8
MET hours per week	85.9 \pm 49.4	104.1 \pm 52.4

Abbreviations: OG = older group; YG = younger group; BP = blood pressure; $\dot{V}O_{2peak}$ = peak oxygen uptake; $\dot{V}O_2$ = oxygen uptake; GET = gas exchange threshold; RCP = respiratory compensation point; MET = metabolic equivalents.

Table 2 Older group day-to-day reliability of RR interval complexity and variability metrics.

	Between Day CV (%)	Between Participant CV (%)	ICC2,1	SEM	MDC	Bias	SD Bias	Lower 95% LOA	Upper 95% LOA	<i>P</i>
HR (bpm)	4.36	11.64	0.79	2.89	8.00	-0.20	4.08	-8.21	7.80	0.74
RRi (s)	4.13	11.81	0.83	0.05	0.15	<0.01	0.08	-0.15	0.15	0.83
RMSD (ms)	17.25	44.11	0.61	10.70	29.66	0.71	15.13	-28.95	30.37	0.76
LnRMSD	5.09	11.92	0.57	0.28	0.77	0.01	0.39	-0.76	0.77	0.89
SDNN (ms)	14.8	28.0	0.62	10.06	27.88	-4.09	14.23	-31.97	23.79	0.06
LnSDNN	3.77	6.74	0.53	0.19	0.52	-0.08	0.26	-0.60	0.44	0.05
LF (ms ²)	29.22	83.08	0.69	349.65	969.18	-84.91	494.48	-1054.09	884.27	0.28
HF (ms ²)	28.91	87.28	0.65	239.02	662.54	30.81	338.03	-631.72	693.35	0.53
LF (Ln)	4.87	10.86	0.69	0.39	1.07	-0.16	0.55	-1.24	0.91	0.06
HF (Ln)	5.80	14.73	0.62	0.53	1.46	-0.03	0.74	-1.49	1.42	0.79
LF/HF (ratio)	27.07	112.92	0.88	1.16	3.23	-0.25	1.65	-3.48	2.97	0.33
ApEn	2.95	6.45	0.60	0.06	0.17	-0.02	0.09	-0.18	0.15	0.27
SampEn	7.57	14.10	0.65	0.17	0.48	0.04	0.24	-0.44	0.51	0.31
DFA α	7.76	13.95	0.55	0.10	0.27	-0.02	0.14	-0.29	0.25	0.34
DFA α_1	9.60	19.88	0.55	0.14	0.39	-0.05	0.20	-0.44	0.34	0.13
DFA α_2	8.78	16.40	0.57	0.11	0.31	-0.01	0.16	-0.32	0.30	0.67
CI-8	6.08	9.93	0.43	1.37	3.78	0.15	1.93	-3.64	3.93	0.61
SD2	18.13	42.61	0.33	17.43	48.30	1.31	24.64	-46.99	49.61	0.74

Abbreviations: RMSD = root mean square of successive differences of normal RR intervals; SDNN = standard deviation of normal RR intervals; LF = absolute power in low frequency band; HF = absolute power in high frequency band; ApEn = approximate entropy; SampEn = sample entropy; DFA = detrended fluctuation analysis CI-8 = complexity index under 8 scales; SD2 = standard deviation of points along the line of identity of the Poincare plot; CV = coefficient of variation; ICC = intraclass correlation coefficient; MDC = minimal detectable change; LOA = limits of agreement.

865

866

Table 3 Younger group day-to-day reliability of RR interval complexity and variability metrics.

	Between Day CV (%)	Between Participant CV (%)	ICC2,1	SEM	MDC	Bias	SD Bias	Lower 95% LOA	Upper 95% LOA	<i>P</i>
HR (bpm)	6.23	13.92	0.67	4.97	13.77	1.14	7.03	-12.63	14.91	0.46
RRi (s)	6.22	13.75	0.71	0.08	0.21	-0.01	0.11	-0.22	0.20	0.69
RMSSD (ms)	17.88	46.46	0.81	15.48	42.91	-3.44	21.89	-46.35	39.47	0.47
LnRMSSD	4.42	11.29	0.79	0.22	0.60	-0.03	0.31	-0.63	0.58	0.70
SDNN (ms)	18.96	39.05	0.64	24.18	67.02	-0.73	34.19	-67.75	66.29	0.92
LnSDNN	4.34	8.08	0.59	0.24	0.67	0.03	0.34	-0.64	0.70	0.68
LF (ms ²)	30.72	72.82	0.56	1186.69	3289.34	-370.70	1678.23	-3660.04	2918.64	0.31
HF (ms ²)	36.48	91.22	0.75	1015.31	2814.30	-230.05	1435.87	-3044.35	2584.25	0.46
LF (Ln)	4.43	9.68	0.72	0.41	1.13	-0.03	0.58	-1.16	1.11	0.83
HF (Ln)	5.38	13.38	0.78	0.45	1.24	-0.04	0.63	-1.28	1.20	0.77
LF/HF (ratio)	24.58	71.85	0.80	0.54	1.50	0.04	0.77	-1.46	1.54	0.80
ApEn	3.52	5.33	0.37	0.07	0.18	-0.003	0.09	-0.18	0.18	0.87
SampEn	7.65	12.74	0.49	0.20	0.55	-0.10	0.28	-0.64	0.45	0.11
DFA α	6.42	16.69	0.84	0.06	0.18	-0.02	0.09	-0.20	0.16	0.35
DFA α_1	6.52	22.86	0.93	0.08	0.21	-0.005	0.11	-0.21	0.21	0.88
DFA α_2	8.98	17.68	0.69	0.10	0.26	-0.04	0.13	-0.30	0.22	0.17
CI-8	7.48	13.56	0.69	1.59	4.41	-0.82	2.25	-5.22	3.59	0.10
SD2	20.42	64.69	0.44	60.00	166.32	-10.77	84.86	-177.09	155.54	0.45

Abbreviations: RMSSD = root mean square of successive differences of normal RR intervals; SDNN = standard deviation of normal RR intervals; LF = absolute power in low frequency band; HF = absolute power in high frequency band; ApEn = approximate entropy; SampEn = sample entropy; DFA = detrended fluctuation analysis CI-8 = complexity index under 8 scales; SD2 = standard deviation of points along the line of identity of the Poincare plot; CV = coefficient of variation; ICC = intraclass correlation coefficient; MDC = minimal detectable change; LOA = limits of agreement.

867

868

869

870

871

872

873

874

875

876

877

878

879

880

881

882

883

Table 4 Mean HRV metrics for age groups and effect size comparisons.

	YG N = 22	OG N = 44	Hedges' g	Hedges' g Lower 95% CI	Hedges' g Upper 95% CI
HR (bpm)	61.75±75	54.24±6.28	1.12	0.57	1.66
RRi (s)	1.00±0.14	1.13±0.13	1.03	0.49	1.57
RMSSD (ms)	72.56±33.64	38.60±16.95	1.51	0.93	2.08
LnRMSSD	4.18±0.45	3.56±0.38	1.52	0.95	2.10
SDNN (ms)	97.40±38.16	58.40±16.33	1.66	1.08	2.25
LnSDNN	4.51±0.33	4.03±0.24	1.77	1.17	2.36
LF (ms ²)	2197.61±1445.90	763.81±582.24	1.48	0.91	2.05
HF (ms ²)	1958.89±1692.68	431.53±345.17	1.49	0.92	2.07
LF (Ln)	7.45±0.67	6.39±0.64	1.62	1.04	2.20
HF (Ln)	7.17±0.91	5.73±0.76	1.75	1.16	2.34
LF/HF ratio	1.71±1.16	2.71±2.97	-0.39	-0.91	0.12
ApEn	1.54±0.08	1.48±0.10	0.82	0.29	1.35
SampEn	2.16±0.28	2.01±0.28	0.57	0.05	1.09
DFA α	0.93±0.15	1.01±0.14	-0.64	-1.16	-0.12
DFA α_1	0.97±0.22	1.05±0.21	-0.43	-0.95	0.09
DFA α_2	0.94±0.17	1.01±0.17	-0.46	-0.98	0.05
CI-8	18.36±2.25	18.13±1.52	0.12	-0.39	0.64
SD2	110.84±29.67	49.21±10.08	1.87	1.27	2.48

Abbreviations: YG = younger group; OG = older group; HR = heart rate; RRi = time between two successive R-waves of an ECG; RMSSD = root mean square of successive differences between normal RR intervals; SDNN = standard deviation of normal RR intervals; LF = absolute power in low frequency band; HF = absolute power in high frequency band; ApEn = approximate entropy; SampEn = sample entropy; DFA = detrended fluctuation analysis; CI-8 = complexity index under 8 scales; SD2 = standard deviation of points along the line of identity of the Poincare plot; data are mean ± SD of both days measurements.

Table 5 Multiple linear regression model statistics.

	Overall Regression Model			Age (years)		Sex (M/F) [F]		VO _{2peak} (L.min ⁻¹)	
	Adjusted R ²	F (3, 62)	P	β [95% CI]	t P	β [95% CI]	t P	β [95% CI]	t P
HR (bpm)	0.267	8.884	<0.001	-0.236 [-0.335, -0.138]	4.800 <0.001	-0.091 [-5.518, 5.336]	0.034 0.973	-2.121 [-4.896, 0.653]	1.528 0.135
RRi (s)	0.197	6.305	<0.001	0.003 [0.001, 0.005]	4.033 <0.001	-0.002 [-0.108, 0.103]	0.044 0.965	0.032 [-0.002, 0.086]	1.167 0.248
RMSSD (ms)	0.362	13.270	<0.001	-0.853 [-1.188, -0.519]	5.097 <0.001	15.550 [-2.915, 34.010]	1.683 0.097	2.107 [-7.332, 11.550]	0.446 0.657
LnRMSSD	0.347	12.520	<0.001	-0.015 [-0.021, -0.009]	4.980 <0.001	0.255 [-0.083, 0.592]	1.509 0.136	0.051 [-0.121, 0.223]	0.592 0.556
SDNN (ms)	0.379	14.230	<0.001	-1.019 [-1.375, -0.662]	5.721 <0.001	1.126 [-18.520, 20.770]	0.115 0.909	1.736 [-8.309, 11.780]	0.345 0.731
LnSDNN	0.412	16.190	<0.001	-0.012 [-0.017, -0.008]	5.986 <0.001	0.022 [-0.206, 0.250]	0.196 0.846	0.035 [-0.081, 0.152]	0.603 0.549
LF (ms ²)	0.303	9.998	<0.001	-35.730 [-51.200, -20.270]	4.624 <0.001	-7.820 [-862.100, 846.500]	0.018 0.985	114.200 [-328.400, 556.800]	0.517 0.608
HF (ms ²)	0.353	12.280	<0.001	-41.560 [-57.410, -25.700]	5.246 <0.001	404.900 [-470.800, 1281.000]	0.925 0.359	-75.550 [-529.200, 378.100]	0.333 0.740
LF (Ln)	0.367	13.000	<0.001	-0.026 [-0.036, -0.016]	5.129 <0.001	-0.158 [-0.724, 0.407]	0.559 0.578	0.099 [-0.194, 0.392]	0.675 0.502
HF (Ln)	0.431	16.620	<0.001	-0.037 [-0.049, -0.024]	5.913 <0.001	0.449 [-0.243, 1.143]	1.298 0.199	0.052 [-0.307, 0.411]	0.289 0.773
LF/HF ratio	0.064	2.425	0.075	0.023 [-0.016, 0.061]	1.187 0.239	-1.921 [-4.035, 0.192]	1.819 0.074	-0.442 [-1.537, 0.654]	0.807 0.423
ApEn	0.202	6.478	<0.001	-0.002 [-0.003, -0.001]	3.917 <0.001	0.008 [-0.057, 0.073]	0.243 0.809	-0.023 [-0.056, 0.012]	1.366 0.177
SampEn	0.122	4.003	0.001	-0.004 [-0.008, -0.0004]	2.203 0.031	0.132 [-0.073, 0.337]	1.291 0.202	-0.032 [-0.137, 0.073]	0.611 0.544
DFA α	0.142	4.573	0.005	0.002 [0.0002, 0.004]	2.242 0.029	-0.079 [-0.187, 0.029]	1.469 0.147	0.017 [-0.038, 0.072]	0.621 0.537
DFA α1	0.167	5.350	0.002	0.002 [-0.0005, 0.005]	1.635 0.107	-0.171 [-0.323, -0.019]	2.245 0.028	0.009 [-0.069, 0.086]	0.226 0.822
DFA α2	0.020	1.443	0.239	0.002 [-0.0006, 0.004]	1.469 0.147	-0.035 [-0.162, 0.091]	0.557 0.579	0.018 [-0.047, 0.082]	0.551 0.584
CI-8	-0.026	0.457	0.714	-0.009 [-0.038, 0.018]	0.689 0.493	-0.207 [-1.746, 1.332]	0.269 0.788	-0.369 [-1.156, 0.418]	0.937 0.352
SD2	0.482	20.230	<0.001	-1.501 [-1.933, -1.070]	6.969 <0.001	8.167 [-15.650, 31.980]	0.686 0.495	-8.328 [-20.670, 4.012]	1.350 0.182

Abbreviations: VO_{2peak} = peak oxygen uptake; HR = heart rate; RRi = time between two successive R-waves of an ECG; RMSSD = root mean square of successive differences between normal RR intervals; SDNN = standard deviation of normal RR intervals; LF = absolute power in low frequency band; HF = absolute power in high frequency band; ApEn = approximate entropy; SampEn = sample entropy; DFA = detrended fluctuation analysis; CI-8 = complexity index under 8 scales; SD2 = standard deviation of points along the line of identity of the Poincare plot; data are mean ± SD of both days measurements.

894
895
896
897
898
899

Table 5 Continued.

	Age x Sex		Age x $\dot{V}O_{2peak}$		Sex x $\dot{V}O_{2peak}$	
	β [95% CI]	t P	β [95% CI]	t P	β [95% CI]	t P
HR (bpm)	0.144 [-0.1564, 0.445]	0.961 0.341	0.116 [-0.039, 0.271]	1.489 0.142	-6.782 [-15.070, 1.507]	1.637 0.107
RRi (s)	-0.003 [-0.009, 0.003]	0.993 0.325	-0.002 [-0.005, 0.001]	1.123 0.266	0.123 [-0.039, 0.286]	1.513 0.136
RMSSD (ms)	-0.998 [-1.989, -0.007]	2.015 0.049	-0.190 [-0.703, 0.323]	0.742 0.461	16.900 [-10.410, 44.210]	1.238 0.221
LnRMSSD	-0.013 [-0.032, 0.006]	1.364 0.178	-0.003 [-0.012, 0.007]	0.528 0.599	0.173 [-0.349, 0.696]	0.662 0.511
SDNN (ms)	-0.026 [-1.138, 1.085]	0.048 0.962	-0.094 [-0.669, 0.481]	0.327 0.745	26.260 [-4.367, 56.890]	1.716 0.092
LnSDNN	< 0.001 [-0.013, 0.013]	0.030 0.976	< -0.001 [-0.007, 0.006]	0.155 0.877	0.280 [-0.077, 0.637]	1.568 0.122
LF (ms ²)	-14.900 [-61.150, 31.360]	0.645 0.522	-15.950 [-40.280, 8.387]	1.313 0.194	1273.000 [-3.202, 2548.000]	1.998 0.050
HF (ms ²)	-34.490 [-82.220, 13.250]	1.447 0.153	-3.728 [-28.840, 21.380]	0.2970 0.767	551.800 [-764.800, 1869.000]	0.839 0.405
LF (Ln)	-0.003 [-0.034, 0.028]	0.178 0.859	-0.004 [-0.021, 0.012]	0.536 0.594	0.925 [0.074, 1.775]	2.117 0.034
HF (Ln)	-0.020 [-0.059, 0.018]	1.054 0.297	-0.002 [-0.022, 0.018]	0.201 0.841	0.267 [-0.801, 1.335]	0.501 0.618
LF/HF ratio	-0.025 [-0.145, 0.094]	0.421 0.676	-0.029 [-0.092, 0.034]	0.925 0.359	1.150 [-2.144, 4.443]	0.699 0.487
ApEn	0.002 [-0.001, 0.006]	1.259 0.213	0.001 [-0.001, 0.003]	1.302 0.198	-0.065 [-0.166, 0.035]	1.300 0.199
SampEn	-0.007 [-0.019, 0.004]	1.262 0.212	-0.001 [-0.007, 0.005]	0.353 0.726	0.070 [-0.249, 0.389]	0.439 0.662
DFA α	0.005 [-0.001, 0.113]	1.724 0.089	0.001 [-0.002, 0.004]	0.629 0.532	0.006 [-0.161, 0.173]	0.072 0.943
DFA α_1	0.009 [0.001, 0.018]	2.244 0.029	0.001 [-0.004, 0.005]	0.289 0.773	0.129 [-0.098, 0.357]	1.135 0.261
DFA α_2	0.001 [-0.006, 0.009]	0.389 0.698	0.001 [-0.003, 0.005]	0.514 0.609	-0.097 [-0.297, 0.103]	0.971 0.336
CI-8	-0.033 [-0.120, 0.054]	0.769 0.445	-0.008 [-0.053, 0.037]	0.337 0.738	1.458 [-0.939, 3.856]	1.217 0.229
SD2	-1.281 [-2.577, 0.016]	1.979 0.053	-0.148 [-0.830, 0.534]	0.436 0.665	-9.758 [-45.520, 26.000]	0.547 0.587

Abbreviations: $\dot{V}O_{2peak}$ = peak oxygen uptake; HR = heart rate; RRi = time between two successive R-waves of an ECG; RMSSD = root mean square of successive differences between normal RR intervals; SDNN = standard deviation of normal RR intervals; LF = absolute power in low frequency band; HF = absolute power in high frequency band; ApEn = approximate entropy; SampEn = sample entropy; DFA = detrended fluctuation analysis; CI-8 = complexity index under 8 scales; SD2 = standard deviation of points along the line of identity of the Poincare plot; data are mean \pm SD of both days measurements.

900
901
902
903