1 **Inter-day reliability of heart rate complexity and variability metrics in healthy highly**
2 **active younger and older adults.**
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4 Original Investigation
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6 Christopher R. J. Fennell, Alexis R. Mauger, James G. Hopker,
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9 School of Sport and Exercise Sciences, University of Kent, Canterbury, Kent, England.
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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
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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
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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; $\dot{V}O_{2peak}$ = 40.9 ± 7.6 ml.kg⁻¹.min⁻¹) and twenty-two
52 younger adults (16M, 6F; 22 ± 4 years; $\dot{V}O_{2peak}$ = 47.2 ± 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 $\alpha1$ ($P = 0.107$), $\alpha2$
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 $\alpha1$, $\alpha2$ and CI-8.
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67
68 **KEYWORDS:** complexity; ageing; reproducibility; heart rate.

69
70 **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
$\dot{V}O_{2peak}$	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

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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 arrythmias, 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; Uhlig 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 $\geq$ 2years. 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 ± 1.24% (range 0.05 to 4.33%) of total RR intervals and OG, 7.5 ± 10.6 RR intervals or 0.46
192 ± 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 ± 0.94%
193 (range 0.00 to 3.03%) of total RR intervals and OG, 6.7 ± 12.1 RR intervals or 0.42 ± 0.76% (range 0.00 to 4.10%)
194 of total RR intervals.

195

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

2.6.1. Approximate and Sample entropy

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

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, for both ApEn and SampEn analysis (Kaplan et al. 1991). ApEn was calculated as shown by equation [1] and SampEn by equation [2], where N is the number of data points in the time series, m is the length of the template, Ai is the number of matches of the ith template of length m + 1 data points, and Bi is the number of matches of the ith template of length m data points:

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

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

2.6.2. Detrended fluctuation analysis

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

$$[3] \; y(k) = \sum_{j=1}^{k} (RR_j - \overline{RR}), \qquad k = 1, \ldots, N$$

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

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

The DFA computation [4] is repeated across all box sizes to provide a relationship between $F(n)$, the average fluctuation as a function of box size, and the box size, n, the number of RR interval data points in a box. The slope of the double log plot, log F(n) vs log n, determines the scaling exponent α. DFA α was calculated with box sizes ranging from 4 to ≤ 64 data points. DFA α1 was calculated over box sizes of $4 \leq n \leq 16$ data points (i.e., scaling exponent calculated over short time scales) and DFA α2 was calculated over box sizes of $16 \leq n \leq 64$ data points (i.e., scaling exponent calculated over long time scales), as used previously by Peng et al. (1995).

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

[Figure 1 here]

246
247 ### 2.6.3. Multiscale entropy
248 Multiscale entropy (MSE) analysis was performed as outlined by Costa et al. (2002) providing a measure of
249 complexity of time series over multiple scales. The MSE analysis overcomes limitations of SampEn and ApEn
250 which only measure the regularity of time series data on one scale, and therefore do not capture the structural and
251 dynamical behaviour of the time series.
252
253 From the one-dimensional discrete time series, $\{\chi_1,...., \chi_I,...., \chi_N\}$, a coarse-grained time series were constructed,
254 $\{y^{(\tau)}\}$, determined by the scale factor, τ, according to equation [5]:
255

256
$$[5] \; y_j^{(\tau)} = \frac{1}{\tau} \sum_{i-(j-1)\tau+1}^{j\tau} \chi_i, \; 1 \leq j \leq N/\tau$$

257
258
259 At one scale, the time series $\{y^{(1)}\}$ is the original time series of sample length. The length of the coarse-grained
260 time series is equal to the length of the original time series divided by the scale factor, τ. The SampEn for each
261 coarse-grained time series is calculated and plotted against the scale factor, τ, producing a MSE curve. The
262 SampEn of each coarse-grained time series was computed using equation [2] and a template length $m = 2$ and $r =$
263 0.2 of the SD of the RR interval time series. The area under the MSE curve were calculated from scales 1 to 8
264 using equation [6] and is defined as the complexity index (CI-8) with higher CI values indicating greater
265 complexity of the physiological signal.
266

267
$$[6] \; CI = \sum_{i=1}^{\tau} SampEn(i)$$

268
269 ### 2.6.4. Poincare plot SD2
270 Poincare plots of RR interval times series were produced by plotting each RR interval as a function of the previous
271 RR interval (Woo et al. 1992). Poincare plots were then analysed with an ellipse fitting procedure to derive the
272 metrics SD1 (the standard deviation of the points perpendicular to the line of identity) and SD2 (the standard
273 deviation along the line of identity; Brennan et al. 2001). Only SD2 was reported as SD1 is identical to RMSSD
274 (Shaffer & Ginsberg 2017).
275
276 ### 2.7. Heart rate variability – linear metric analysis
277 ### 2.7.1. Time-domain metrics
278 The time-domain measures of heart rate variability quantify the amount of variability present within the RR
279 interval time series.
280
281 The root mean square of successive differences between normal RR intervals (RMSSD) was calculated using
282 equation [7]:
283

284
$$[7] \; RMSSD = \sqrt{\frac{1}{N-1} \sum_{n=1}^{N-1} (RR_{n+1} - RR_n)^2}$$

285
286 The standard deviation of normal RR intervals (SDNN) was calculated using equation [8]:
287

288
$$[8] \; SDNN = \sqrt{\frac{1}{N-1} \sum_{n=1}^{N} (RR_n - \overline{RR})^2}$$

289
290 The RMSSD and SDNN metrics were reported in milliseconds and natural logarithm transformed values,
291 LnRMSSD and LnSDNN.
292
293 ### 2.7.2. Frequency-domain metrics
294 The frequency-domain measures of heart rate variability provide an estimate of spectral power in frequency bands.
295 The power spectrum was estimated using a parametric autoregressive based model, with the absolute power in

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).
299
300 **2.8. Statistical analysis**
301 Data are presented as individual values or mean ± 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 (ICC2,1) 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 ± 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 ≥ 0.5 to < 0.75, good =
317 ICC ≥ 0.75 to < 0.90 and excellent = ICC ≥ 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, ≥ 0.8
321 large effect (Cohen 1992).
322
323 Multiple linear regressions were performed to estimate the effect of participant age, sex and $\dot{V}O_{2peak}$ 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.
328
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]
335
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 α1 and DFA α2 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 α2 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 α1 metric (Table 3).
343
344 [Table 2 here]
345 [Table 3 here]
346
347 **3.3. Effect of age, sex and $\dot{V}O_{2peak}$ 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 $\alpha1$ ($P = 0.107$; Figure 3B) and DFA $\alpha2$ ($P = 0.147$; Figure 3C)
355 metrics (Table 5). The DFA α metric was significantly increased with ageing ($P = 0.029$; Figure 3A).

357 There was a significant effect of sex ($P = 0.028$), but not or $\dot{V}O_{2peak}$ ($P = 0.822$) on DFA $\alpha1$, with females
358 presenting with lower values. There was no significant effect of sex or $\dot{V}O_{2peak}$ on the ApEn, SampEn, DFA α,
359 DFA $\alpha2$, CI-8 and SD2 metrics ($P > 0.05$; Table 5).

361 [Figure 3 here]

3.4. Effect of age, sex and $\dot{V}O_{2peak}$ 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).

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

369 [Table 4 here]
370 [Table 5 here]

4. DISCUSSION
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 (Fatisson et al. 2016).

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.

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 (Uhlig
403 et al. 2020), in addition to comparable between day CVs and SEM to healthy trained young adults (aged 21.5 ±
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.

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 $\alpha1$ and $\alpha2$ metrics (Table 5; Figures 3B & 3C). Mean DFA $\alpha1$ and $\alpha2$ 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 $\alpha1$ and $\alpha2$ 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 ($\alpha2 = 0.75 \pm 0.17$) to have a significant decline in long-range fractal
435 correlations, in comparison to healthy younger adults ($\alpha2 = 0.99 \pm 0.10$; 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 $\alpha1$ and $\alpha2$ 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 $\alpha1$ and $\alpha2$ 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 $\alpha1$ metric (Table 5).
464 The significant effect of sex indicates that females present with lower $\alpha1$ value in comparison to males. Such
465 differences in $\alpha1$ 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 ± 1.0 L.min^{-1} vs. OG $\dot{V}O_{2peak}$ = 3.0 ± 0.8 L.min^{-1}), $\dot{V}O_{2peak}$ was not
482 significantly predictive of any HRV metric (Table 5).
483

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.
515

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.
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AUTHOR CONTRIBUTION STATEMENT

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

COMPLIANCE WITH ETHICAL STANDARDS

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

Consent to participate.
All participants provided written signed informed consent prior to testing.

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

Conflicts of interest/Competing interests.
The authors report no conflicts of interest or competing interests.

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Non-Financial interests.
None

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

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Not Applicable

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752 **FIGURE CAPTIONS**
753 **Fig. 1. (A)** Example raw RR interval time series; **(B)** The integrated RR interval time series, with the least-squares
754 fit representing the "trend" in each box (red lines) and the vertical lines indicating the box size of n = 64 data
755 points. The RR interval data presented produced a DFA α = 1.04 (DFA α calculated over box sizes 4 to $\leq$ 64;
756 data were from a younger male participant aged 18 years).
757
758 **Fig. 2.** Comparisons between the younger and older groups complexity and variability metrics **(A)** Root mean
759 square of successive differences between normal RR intervals; **(B)** Standard deviation of normal RR intervals;
760 **(C)** Low frequency power; **(D)** High frequency power; **(E)** Approximate entropy; **(F)** Sample entropy; **(G)**
761 Complexity index under 8 scales; **(H)** Standard deviation of points along the line of identity of the Poincare plot
762 (* $P < 0.05$; ** $P < 0.001$; Data points are the mean of both days for each individual participant).
763
764 **Fig. 3.** Comparisons between the younger and older groups detrended fluctuation analysis metrics **(A)** DFA α
765 (box sizes 4 to $\leq$ 64 data points); **(B)** DFA α1 (box sizes of $4 \leq$ n ≤ 16 data points); **(C)** DFA α2 (box sizes of
766 $16 \leq$ n ≤ 64 data points; * $P < 0.05$; Data points are the mean of both days for each individual participant).
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Table 1 Participant characteristics, anthropometrics and IET data (mean ± SD)

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

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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	*P*
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
RMSSD (ms)	17.25	44.11	0.61	10.70	29.66	0.71	15.13	-28.95	30.37	0.76
LnRMSSD	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 α1	9.60	19.88	0.55	0.14	0.39	-0.05	0.20	-0.44	0.34	0.13
DFA α2	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: 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.

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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	P
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 α1	6.52	22.86	0.93	0.08	0.21	-0.005	0.11	-0.21	0.21	0.88
DFA α2	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.

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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 α1	0.97±0.22	1.05±0.21	-0.43	-0.95	0.09
DFA α2	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]		V̇O$_{2peak}$ (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: V̇O$_{2peak}$ = 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.

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Table 5 Continued.

	Age x Sex		Age x V̇O$_{2peak}$		Sex x V̇O$_{2peak}$	
	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 α1	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 α2	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: V̇O$_{2peak}$ = 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.

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