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1 **ABSTRACT**

2

3 **Background:** Heart rate variability (HRV) has been promoted as a non-invasive method of
4 evaluating autonomic influence on cardiac rhythm. Although female subjects predominate in
5 the walking studies, no study to date has examined the influence of the duration of a moderate
6 intensity walking physical activity bout on HRV in this population. **Methods:** Twelve healthy
7 physically active middle-aged women undertook two conditions; 20min (W20) and 60min
8 (W60) bouts of walking on a treadmill. Resting HRV measures were obtained prior to (-1 h),
9 and 1 h and 24 h after the walking bouts. **Results:** Mean NN interval (i.e., normal-to-normal
10 intervals between adjacent QRS complexes) was significantly lower ($p=0.017$) at +1h in W60
11 (832, 686-979ms) compared with W20 (889, 732-1046ms). A borderline main effect for time
12 was observed for both the SDNN intervals in W60 ($p=0.056$), and for low frequency (LF_{abs})
13 power in W60 ($p=0.047$), with post-hoc tests revealing a significant increase between -1 h
14 (51, 33-69 ms and 847, 461-1556 ms²) and +1h (65, 34-97ms and 1316, 569-3042 ms²) for
15 SDNN and LF_{abs} power, respectively, but no increase at +24h compared with -1 h.
16 **Conclusions:** It appears that a walking bout of 60 min duration does alter cardiac autonomic
17 influence in healthy active women, and this alteration is not evident after 20 min of walking.
18 Given the rather subtle effect, further studies with larger sample sizes are required to explore
19 the nature of the changes in cardiac autonomic influence following a prolonged bout of
20 walking.

21

22 **Key words**

23 Physical Activity; Cardiac Autonomic Function; Respiratory Sinus Arrhythmia

1 Introduction

2 Public health recommendations state that every adult should undertake moderate-intensity
3 aerobic physical activity for a minimum of 30 min on five days each week or vigorous-
4 intensity aerobic activity for a minimum of 20 min on three days each week. The
5 recommendation of moderate intensity could be met by walking briskly with a noticeable
6 acceleration of heart rate, and could be accumulated from multiple bouts lasting ten or more
7 minutes [{Haskell, 2007 276 /id;Department of Health, 2005 277 /id}](#).

8
9 Various outcomes have been shown to acutely change following a single bout of moderate
10 intensity physical activity [{Shephard, 2001 282 /id;Thompson, 2001 283 /id}](#) and some of
11 these changes have been reported for up to 48 h. Changes that are thought to be of benefit to
12 health include a reduction in blood pressure [{Brownley, 2003 284 /id;Ishikawa-Takata, 2003](#)
13 [285 /id;Taylor-Tolbert, 2000 281 /id}](#), improved glucose tolerance [{Wojtaszewski, 2002 286](#)
14 [/id}](#) and improved lipid metabolism [{Gill, 2003 287 /id}](#). However, other recent
15 investigations have shown that single bouts of moderate intensity physical activity appear to
16 have little effect on fasting plasma triglyceride homeostasis [{Magkos, 2007 278 /id}](#), and that
17 to get some metabolic improvements would require unfeasible amounts of physical activity
18 for most sedentary individuals [{Magkos, 2007 279 /id}](#). More recently, heart rate variability
19 (HRV) (i.e. the oscillation in the interval between consecutive heartbeats [{Electrophysiology,](#)
20 [1996 237 /id}](#)) outcomes have been examined acutely following a single bout of physical
21 activity, since HRV has been promoted as a non-invasive method of evaluating cardiac
22 autonomic influence.

23
24 Cross sectional studies have revealed that in fit and healthy individuals, HRV is known to be
25 higher (i.e., greater parasympathetic activity at rest) [{De Meersman, 1993 27 /id}](#).

1 Intervention studies in healthy male subjects have not only demonstrated that chronic physical
2 training has a positive influence on cardiac autonomic influence as assessed by HRV
3 outcomes [{Genovesi, 2007 229 /id;Melanson, 2000 240 /id;Melanson, 2001 239 /id;Seals,
4 1989 241 /id}](#), but also that a single bout of moderate intensity physical activity acutely alters
5 HRV outcomes in the hours after the physical activity bout [{Convertino, 1991 306 /id;Pober,
6 2004 233 /id;Raczak, 2005 232 /id}](#). More specifically, high frequency (HF) oscillations of
7 HRV (representing parasympathetic nervous activity [{Electrophysiology, 1996 237 /id}](#)) were
8 shown to increase and the low to high frequency (LF/HF) ratio (assessed to estimate the
9 overall sympathovagal balance [{Pagani, 1986 305 /id}](#)) was shown to decrease [{Pober, 2004
10 233 /id}](#). Recently, Raczak et al [{Raczak, 2005 232 /id}](#) have confirmed these findings at
11 one-hour post physical activity. Such acute changes following moderate intensity physical
12 activity would therefore indicate that parasympathetic modulation is increased and
13 sympathetic modulation is reduced, suggesting a more favourable autonomic influence on the
14 heart.

15
16 A recent meta-analysis has shown the predominance of female subjects in the walking studies,
17 reflecting the intuitive appeal of this activity for women [{Murphy, 2007 290 /id}](#). In contrast,
18 most of the previous HRV investigations have been conducted on male subjects in order to
19 observe the acute response to either high or moderate intensity physical activity bouts as a
20 primary objective. No studies have assessed the influence of the duration of a moderate
21 intensity walking bout on the HRV response in middle-aged healthy women. To our
22 knowledge, only investigations including elderly healthy women have been conducted to
23 determine both the effect of physical activity level [{Reland, 2004 227 /id}](#) and dynamic
24 physical activity on HRV [{Perini, 2000 243 /id}](#).

25

1 Given that walking appears to be a popular activity among women, it is important to explore
2 whether HRV outcomes could be acutely altered following a walking bout in women, and
3 whether the duration of the walking bout has any additional influence. Therefore, the aims of
4 the present study were: 1) to examine the influence of a moderate intensity walking bout on
5 the post-walking HRV response in middle-aged healthy women, and 2) to assess whether the
6 post-walking HRV response is altered by the duration of the walking bout (i.e., 20 min or 60
7 min).

8

9 **Methods**

10 *Participants*

11 Twelve females (age 31 ± 12 yr; height 163 ± 0.05 cm; mass 63.2 ± 8.2 kg; BMI 23.9 ± 3.2)
12 volunteered to participate. All participants were non-smokers who completed moderate
13 intensity physical activity each week (5 ± 2 h per week) who had no history of diabetes,
14 hypertension or cardiovascular disease. Prior to participation, all participants completed a
15 health screening procedure and were fully informed of the nature of the study. Participants
16 then provided written consent to participate. The University Research Ethics Committee
17 approved all procedures.

18

19 *Study Design*

20 It has been previously reported that adult recreational walkers self-select a pace which elicits
21 moderate intensity physical activity (55-69% HR_{max} , 40-59% VO_2 Reserve) that meets current
22 recommendations for health with “brisk” walking speeds from $5.61 \text{ km}\cdot\text{h}^{-1}$ to $6.44 \text{ km}\cdot\text{h}^{-1}$
23 | [{Murphy, 2006 291 /id;Murtagh, 2002 289 /id}](#). Therefore, following a familiarisation visit,
24 participants undertook two conditions in a counterbalanced order: a 20 min bout of walking
25 (W20) and a 60 min bout of walking (W60). The second day of the testing was separated

1 from the first by a minimum of 72 h and a maximum of one week. Walking was performed
2 on a flat motorised treadmill (Ergo ELG 70, Woodway, Weil am Rhein, Germany) at 6.5
3 km·h⁻¹. For each condition resting HRV was assessed once prior to (-1 h) and twice
4 following (+1 h; +24 h) the walking bouts. For the hour prior to and following the walking
5 bout, participants sat quietly and read. Time of the day for testing was controlled across
6 conditions within participants through standardised visit times in order to reduce the influence
7 of circadian variation. Participants were requested not to eat or drink anything, other than
8 water, in the final 3 h before each assessment of HRV and to perform no physical activity,
9 beyond normal lifestyle activities, between the +1 h and +24 h time points. Each subject was
10 weighed before and after the walking bout and the change in body mass was calculated. For
11 the group as a whole, participants lost 100 g and 400 g following W20 and W60 respectively.
12 The equivalent fluid volume was then determined and the subject was required to consume
13 this fluid immediately on completion of the walking bout. This rehydration strategy has been
14 used in previous studies and has been shown to be effective at restoring plasma volume
15 | [{James, 1998 311 /id}](#).

16

17 *HRV Outcome Measures*

18 | According to the Task Force of the European Society of Cardiology [{Electrophysiology, 1996](#)
19 | [237 /id}](#) there are a variety of indices that are used to assess HRV, which can be divided into
20 | two major categories: time domain indices and frequency domain indices. In the present
21 | investigation the included time domain indices are the mean NN interval (MNN) (i.e., normal-
22 | to-normal intervals between adjacent QRS complexes) and the standard deviation of the NN
23 | intervals (SDNN) (i.e., the square root of variance, reflecting all the cyclic components
24 | responsible for variability in the period of recording). The included frequency domain indices

1 are: high frequency (HF) (represents some parasympathetic activity), low frequency (LF)
2 (includes both sympathetic and parasympathetic influences) and very low frequency (VLF).

3

4 *Procedures*

5 During the HRV assessment points at -1 h, +1 h and +24 h, subjects sat quietly for 20 min and
6 controlled their breathing frequency (BF). BF was set at 0.20 Hz (12 breath.min⁻¹), with each
7 breath comprising 2 s of inspiration and 3 s of expiration. Previous studies {Gamelin, 2006
8 365 /id;Vanderlei, 2008 363 /id} have shown that the RR interval assessed using the Polar
9 transmitter is as reliable as that obtained by ECG. Therefore, subjects wore a chest strap
10 consisting of two electrodes and a transmitter (Polar Electro Oy, Kempele, Finland) and the
11 data were transmitted directly to a PC via an interface (Advantage, Polar Electro Oy,
12 Kempele, Finland). NN interval data were collected over the final 5 min of the 20 min period
13 and stored for subsequent analysis (Precision Performance 2.1, Polar Electro Oy, Kempele,
14 Finland). The data were presented graphically and visually inspected to identify any spurious
15 beats. No spurious beats were identified, and this was confirmed with the error detection
16 algorithm in the Precision Performance analysis programme (Polar Electro Oy, Kempele,
17 Finland) which filters the data using median and moving average based methods in order to
18 identify artefacts in the signal. The MNN and SDNN were then calculated and a power
19 spectrum analysis was undertaken (using autoregressive modelling with a fixed model order
20 of 18). For the HF component, which is synchronous with respiration, a frequency band of
21 0.16 to 0.24 Hz was selected. For the LF component, which typically ranges between 0.03
22 and 0.15 Hz and is normally observed at ~0.1 Hz, a frequency band of 0.04 to 0.16 Hz was
23 selected. Finally, for the VLF component, a frequency band of 0.00 to 0.04 Hz was selected.
24 For the HF and LF components, power was expressed both in absolute values of power with
25 the units of measurements of ms² (HF_{abs} and LF_{abs}) and in normalised units (HF_{nu} and LF_{nu}).

1 The normalisation procedure involves dividing the HF or LF power (ms^2) by the total spectral
2 power minus the VLF component (also in ms^2) and the result is therefore a dimensionless
3 ratio. The representation of LF and HF in normalised units emphasizes the controlled and
4 balanced behaviour of the two branches of the autonomic nervous system and is thought to
5 minimise the influence of changes in total power on the HF and LF powers [{Strano, 1998 300](#)
6 [/id}](#).

8 *Data Analysis*

9 Interactions between condition (walking duration; 20 min or 60 min) and time (-1 h, +1 h, +24
10 h) were explored using 2 x 3 repeated measures ANOVA. Whether or not a significant
11 interaction was present, we were also interested in main effects for time, since we were
12 interested in the response following each condition. One-way repeated measures ANOVA
13 were conducted for each condition to examine effects over time. When significant main
14 effects were observed, post-hoc t-tests were conducted to locate the differences. Differences
15 were considered significant when $p < 0.05$ and of borderline significance when the p-value
16 approached 0.05. In all cases the actual p-values are presented. As the data for the spectral
17 parameters were positively skewed (prior to normalisation), these data were transformed via a
18 natural logarithmic function prior to analysis. This is consistent with the approach of Bernardi
19 and colleagues [{Bernardi, 1997 366 /id}](#) who, having found that the data for the spectral
20 parameters were skewed, transformed the data with a logarithmic transformation. Data in
21 table 1 are therefore presented as mean (68% confidence interval) as it is not possible to
22 ‘back-transform’ a log transformed standard deviation into the original measurement unit.
23 The presentation of data as mean (68% confidence interval) is consistent with the normal
24 convention of presenting data as mean (one standard deviation).

25

1 Results

2 **Time-domain measures.** Data for MNN and SDNN are presented in Table 1. A borderline
3 significant ($p = 0.05$) interaction (condition \times time) for MNN was observed (Figure 1).
4 Although no difference between conditions was evident at -1 h and $+24$ h, MNN was
5 significantly lower (6%) at $+1$ h in W60 condition (832, 686-979 ms) compared with W20
6 condition (889, 732-1046 ms) ($p = 0.017$). A borderline main effect for time ($p = 0.057$) was
7 observed for SDNN in W60 condition (Figure 2), with post-hoc tests revealing a significant
8 increase between -1 h (51, 33-69 ms) and $+1$ h (65, 34-97 ms). However, by $+24$ h, SDNN
9 was not significantly elevated from the -1 h value (Figure 2).

10

11 **Frequency-domain measures.** Data for frequency-domain outcomes are presented in Table
12 1. A significant main effect for time ($p = 0.047$) was observed for LF_{abs} in W60 condition,
13 with post-hoc tests revealing a significant increase between -1 h (847, 461-1556 ms^2) and $+1$
14 h (1316, 569-3042 ms^2). However, by $+24$ h, LF_{abs} was not significantly elevated from the -1
15 h value. A main effect for time was not observed for HF_{abs} , Total Power, LF/HF , or LF_{nu} or
16 HF_{nu} . The two factor ANOVA revealed no significant interaction (condition \times time) for any
17 of the spectral parameters of HRV or SDNN outcomes.

18

19 Discussion

20 To the best of our knowledge, the present investigation is the first to explore the influence of
21 the duration of a moderate intensity walking bout on HRV response in physically active
22 women. Previous studies have examined the effects of a single bout of moderate intensity
23 endurance running [{Raczak, 2005 232 /id}](#) or cycling [{Mourot, 2004 231 /id;Pober, 2004 233](#)
24 [/id}](#) exercise on HRV response in males. However, considering that walking appears to
25 confer similar benefits to those from higher intensity physical activity in women [{Brown,](#)

1 | [2007_304 /id](#)}, it would be interesting to know whether HRV is acutely altered following a
2 walking bout, and whether there are differences in the response according to the duration of
3 the bout.

4
5 Moderate-intensity walking of 20 min duration did not acutely alter HRV in active middle-
6 aged women in the present study, suggesting no acute changes in cardiac parasympathetic or
7 sympathetic influence. Walking of 60 min duration had some influence on selected HRV
8 outcomes at +1 h, in particular MNN, SDNN and LF_{abs}, but changes in these outcomes alone
9 make it difficult to draw clear conclusions about the nature of the change in the cardiac
10 autonomic influence. Regardless of the walking duration, at two time points following the
11 walking bout (+1 h and +24 h) the outcomes of HF_{abs}, HF_{nu}, LF_{nu} and LF/HF ratio remained
12 stable when compared with values recorded prior to the walking bout (-1 h). Changes in these
13 outcomes would have provided a clearer indication of the nature of the change in cardiac
14 autonomic influence. The findings of the present study suggest that the acute effects of a 20
15 min bout of walking on post walking HRV in women are different to those observed
16 following higher intensity submaximal physical activity previously reported in males, where a
17 shift towards a greater parasympathetic influence has been observed for up to 24 h [{Pober,](#)
18 [2004_233 /id](#)}. The acute effects of a 60 min bout of walking are not as pronounced as those
19 observed following higher intensity exercise, but some changes in cardiac autonomic
20 influence are evident. Direct comparisons with previous studies are difficult due to
21 differences in participant group (i.e., males in previous studies) and physical activity intensity.
22 When studying the chronic effects following exercise training interventions, previous studies
23 have shown differences between men and women, with females having longer QT interval
24 when corrected for HR [{Stramba-Badiale, 1997_309 /id}](#), lower HRV (expressed as lower
25 SDNN) [{Genovesi, 2007_229 /id}](#), and lower QT-interval rate adaptation during recovery

1 | [{Chauhan, 2002 230 /id}](#) compared to males; these sex differences also appear to be greater in
2 | the young and middle-aged population [{Zhang, 2007 228 /id}](#).

3 |
4 | The 6% lower MNN at +1 h in W60 condition (832, 686-978 ms) compared with W20
5 | condition (889, 732-1046 ms) might have been due to a number of factors (Figure 1). It is
6 | well known that at rest a decrease in NN interval is often associated with a reduced
7 | parasympathetic influence and possibly an increased sympathetic influence. However, in the
8 | present study there was no clear evidence that the parasympathetic or sympathetic influences
9 | were altered to a greater extent following W60 compared with W20. Although the LF_{abs} was
10 | increased at +1 h following W60, the LF_{abs} component is thought to represent both
11 | sympathetic and parasympathetic influence. The lower MNN (i.e., higher HR) at +1 h in the
12 | W60 condition might simply reflect a higher HR at the end of the W60 condition compared
13 | with the W20 condition [{Tulppo, 1998 310 /id}](#). Although within a participant, HR at a fixed
14 | walking speed should be similar across the two conditions once a physiological steady state
15 | has been reached, it is possible that the end of bout HR was higher in the longer duration W60
16 | condition due to cardiovascular drift, where, in order to maintain cardiac output, an increased
17 | duration may have led to an increased HR to compensate for a reduced stroke volume [{Coyle,](#)
18 | [2001 307 /id}](#). The longer duration may have resulted in reduced stroke volume through
19 | increased core temperature and an associated reduction in central blood volume leading to
20 | reduced venous return and consequently reduced stroke volume [{Coyle, 2001 307 /id}](#). A
21 | greater reduction in body mass was observed following W60 (0.4 kg) compared with the W20
22 | (0.1 kg) condition. Even if only a proportion of the 0.4 kg mass loss was attributable to
23 | plasma volume reduction, some influence on end-exercise heart rate is likely.

24 |

1 The increase in SDNN between -1 h (51, 33-69 ms) and +1 h (65, 34-97 ms) in the W60
2 condition is difficult to explain (Figure 2). It has been previously suggested that SDNN is not
3 a reliable autonomic marker because of its dependence on the length of recording period
4 [{Electrophysiology, 1996 237 /id}](#). It is therefore inappropriate to compare SDNN outcomes
5 obtained from recordings of different durations, although the repeated measures design in the
6 present study suggests that our analysis is appropriate. It is also recognised that SDNN is
7 influenced by the MNN. Although we made our measurements at rest, MNN was influenced
8 by the walking bout, and the influence differed depending on the walking bout duration.
9 Interestingly, if the change in MNN is accounted for when interpreting the change in SDNN
10 (using a coefficient of variation value; i.e., $[SDNN/MNN]*100$), the change in SDNN is even
11 more pronounced, resulting in a significant change ($p < 0.05$) in the W60 condition. Although
12 LF_{abs} was also increased at +1 h following W60, compared with the value recorded at -1 h,
13 interpretation of the LF component of the power spectrum is controversial as LF_{abs} is affected
14 by sympathetic as well as parasympathetic influences [{Houle, 1999 367 /id}](#). Furthermore,
15 changes in LF_{abs} disappeared when the data were expressed in normalised units, where the
16 controlled and balanced behaviour of the two branches of the autonomic nervous system are
17 more appropriately reflected [{Electrophysiology, 1996 237 /id}](#). The increase in the LF_{abs} is
18 therefore unlikely to provide a clear explanation for the increase in the SDNN following the
19 W60 condition.

20

21 In interpreting the findings, some potential limitations to the present study should be
22 acknowledged. A lack of acute change in some outcome measures following walking, even in
23 the 60 min duration condition, should be treated with caution. Whilst it may be tempting to
24 conclude that walking bouts of the durations examined in the present study do not acutely
25 modify the autonomic influence on the heart towards a greater parasympathetic influence,

1 there are reasons why such a conclusion might be unsafe. Firstly, it is possible that a lack of
2 statistical power, as a partial consequence of the modest sample size ($n = 12$), might be
3 responsible for the lack of change in some outcomes (e.g., HF_{abs} ; LF/HF) following the
4 walking bouts. However, the observation of significant changes for some variables (i.e.,
5 MNN, SDNN, LF_{abs}) suggests that this is an unlikely explanation. Secondly, the middle-aged
6 healthy active female population may have been at differing phases of their menstrual cycle.
7 Although previous studies have shown that cardiac autonomic function can be influenced by
8 multiple factors, such as age, body mass index, and menstrual cycle [{Vallejo, 2005 294 /id}](#),
9 the influence of menstrual cycle is contested. Some research indicates that sympathetic
10 nervous activity in the luteal phase is greater than in the follicular phase, likely due to the
11 increase in serum progesterone [{Nakagawa, 2006 42 /id;Princi, 2005 296 /id}](#), whilst other
12 studies have reported similar HRV regardless of menstrual cycle phase and endogenous
13 female sex hormone levels [{Leicht, 2003 298 /id}](#). Although we did not monitor menstrual
14 cycle phase or whether participants were taking oral contraceptives, the repeated measures
15 design, along with the short duration of the study, suggests that the findings were unlikely to
16 be heavily influenced by this contested confounding variable.

17

18 Although it has been established that vigorous-intensity activities may have greater cardio-
19 protective benefits than moderate-intensity physical activities [{Swain, 2006 301 /id}](#), the
20 relative role of the repeated acute effect, as opposed to the chronic effects of prolonged
21 physical training periods, has not been established. Recently it has been shown that a walking
22 programme in women chronically shifted the autonomic balance to a greater parasympathetic
23 predominance [{Sakuragi, 2006 308 /id}](#). Consequently, a period of walking training seems to
24 be effective in chronically shifting autonomic balance. It remains to be firmly concluded
25 whether a single walking bout may be enough to acutely modify the autonomic influence on

1 the heart towards a greater parasympathetic predominance in healthy active women.

2

3 In summary, a single bout of walking of 20 min duration does not appear to acutely modify
4 resting cardiac autonomic influence in healthy middle-aged active women. Although a 60
5 min bout of walking did influence the cardiac autonomic influence, the nature of the
6 autonomic shift remains to be firmly established. Further studies with larger sample sizes are
7 required to confirm these findings, and determine the nature of the shift of the autonomic
8 influence on the heart.

9

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13

14