



Cardiac autonomic function, cardiovascular risk and physical activity in adolescents

Journal:	<i>International Journal of Sports Medicine</i>
Manuscript ID	IJSM-01-2017-6118-pb
Manuscript Type:	Physiology & Biochemistry
Key word:	Cardiovascular disease, heart rate variability, CVD risk factors, risk factor gap
Abstract:	<p>The aims of this study were to investigate in adolescents: 1) the relationships of physical activity (PA) and cardiorespiratory fitness (CRF) to traditional CVD risk factors, rest and recovery autonomic function; and 2) whether autonomic function strengthens the associations between PA, CRF and CVD risk. Fifty-four (22 girls) adolescents had traditional CVD risk factors, rest and recovery autonomic function evaluated. CRF was measured using a steep ramp cycle test and PA was assessed with accelerometers. Resting HRV (and RMSSD30) and heart rate recovery (T30, HHR_T) were used. Clustered traditional (CVDR_{trad}) and autonomic (CVDR_{auto}) risk scores were created and added to form a composite clustered CVD risk score (CVDR_{com}). PA and CRF were significantly and negatively associated to traditional CVD risk factors. Moderate (MPA) and vigorous (VPA) were positively related to resting RMSSD, and negatively related to T30 and HHR_T (all $P < 0.05$). RMSSD30 recovered faster in the high compared to low median split for VPA. Stronger associations for CVDR_{com} compared to CVDR_{trad} were observed for MPA (CVDR_{com}: $r^2 = 0.32$, $P < 0.001$; CVDR_{trad}: $r^2 = 0.17$, $P = 0.002$), and VPA (CVDR_{com}: $r^2 = 0.18$, $P = 0.001$; CVDR_{trad}: $r^2 = 0.06$, $P = 0.08$). These findings strengthen the proposed additional beneficial effects of PA on autonomic function above traditional CVD risk factors.</p>

SCHOLARONE™
Manuscripts

Cardiac autonomic function, cardiovascular risk and physical activity in adolescents

Abstract

The aims of this study were to investigate in adolescents: 1) the relationships of physical activity (PA) and cardiorespiratory fitness (CRF) to traditional CVD risk factors, rest and recovery autonomic function; and 2) whether autonomic function strengthens the associations between PA, CRF and CVD risk. Fifty-four (22 girls) adolescents had traditional CVD risk factors, rest and recovery autonomic function evaluated. CRF was measured using a steep ramp cycle test and PA was assessed with accelerometers. Resting HRV (and RMSSD₃₀) and heart rate recovery (T₃₀, HHR_τ) were used. Clustered traditional (CVDR_{trad}) and autonomic (CVDR_{auto}) risk scores were created and added to form a composite clustered CVD risk score (CVDR_{com}). PA and CRF were significantly and negatively associated to traditional CVD risk factors. Moderate (MPA) and vigorous (VPA) were positively related to resting RMSSD, and negatively related to T₃₀ and HHR_τ (all $P < 0.05$). RMSSD₃₀ recovered faster in the high compared to low median split for VPA. Stronger associations for CVDR_{com} compared to CVDR_{trad} were observed for MPA (CVDR_{com}: $r^2=0.32$, $P < 0.001$; CVDR_{trad}: $r^2=0.17$, $P=0.002$), and VPA (CVDR_{com}: $r^2=0.18$, $P=0.001$; CVDR_{trad}: $r^2=0.06$, $P=0.08$). These findings strengthen the proposed additional beneficial effects of PA on autonomic function above traditional CVD risk factors.

Key-words: Cardiovascular disease, heart rate variability, CVD risk factors, risk factor gap

Introduction

The pathobiological process of atherosclerosis starts during childhood and is related to 'traditional' cardiovascular disease (CVD) risk factors such as blood lipids, blood pressure (BP) and body composition [3]. Physical activity (PA) and cardiorespiratory fitness (CRF) confer CVD risk reduction during childhood by modifying individual or clustered CVD risk factors [18]. However, in adults the summed improvements in traditional CVD risk factors accounts for ~ 60% of the reduction in CVD risk [24], meaning there is a 40% 'risk factor gap' in the explanation of PA benefits [19]. The autonomic and arterial systems have been proposed as components of the risk factor gap and may be considered as 'novel' risk factors [19]. While arterial function has recently been added to a clustered score of traditional CVD risk factors in an attempt to improve the associations between PA and CVD risk in children [14], the influence of autonomic function above traditional CVD risk factors is unknown.

Assessment of cardiac autonomic function by measuring rest and recovery HRV as well as heart rate recovery (HRR) provides distinct and complementary information [11]. While positive relationships between PA and CRF with resting HRV have been demonstrated in youth [9, 16, 27], further understanding about the potential relationships of PA and CRF to cardiac autonomic function during recovery following exercise is needed. Similarly, the effects of PA intensity on HRV and HRR is not clear. In adolescents, one study reported positive effects for moderate to vigorous (MVPA) but not VPA [27], whereas in another study with pre-adolescents, VPA but not MPA, presented stronger effects [9]. However, none of these studies measured cardiac autonomic recovery following exercise nor combined measures of cardiac autonomic function with traditional CVD risk factors.

The aims of this study were: 1) to investigate the relationship of PA intensity and CRF to traditional CVD risk factors, as well as novel CVD risk factors using measurements of autonomic function at rest and recovery; and 2) to investigate whether adding autonomic

1
2
3 function measures to a clustered score of traditional CVD risk factors strengthens the
4
5 associations between PA, CRF and CVD risk.
6
7

8 **Methods**

9 *Participants*

10 Participants were recruited from two secondary schools in the South West of England. The
11
12 volunteers were informed about the study via an assembly and study information sheets were
13
14 distributed. A flow diagram of the recruitment process with the final number of participants
15
16 included in the study is presented in Figure 1. Participant descriptive data are presented in
17
18 Tables 1 and 2. Exclusion criteria included an existing musculoskeletal injury, presence of
19
20 cardiometabolic disease, taking medications, and showing any contraindications to exercise.
21
22 Before the study commenced, all participants and their parents/guardians provided written
23
24 assent and consent, respectively. The study received ethics approval from the institutional
25
26 Ethics Committee (Ref No: 141022/B/07).
27
28
29
30
31

32 *Study design*

33 This is a cross-sectional study where participants completed three visits to a school-based
34
35 laboratory over a one-week period as follows:
36
37
38

39 Visit 1: Participants had stature, body mass, sitting height, and waist circumference (WC)
40
41 measured followed by triceps and subscapular skinfolds assessment using standard
42
43 procedures. Peak height velocity (PHV) was used as an indicator of somatic maturity [23]
44
45 and participants were classified as pre (-1 year), circa (-1 to +1 year), or post (+1 year) PHV.
46
47 Body fat percentage (%BF) was obtained using validated age and sex-specific equations from
48
49 skinfold measures [30].
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Visit 2: Participants reported to the laboratory in a fasted state (>10 hours) and lay supine for
4
5 10-min. Resting heart rate was recorded followed by measurements of BP. Next, a fingertip
6
7 capillary blood sample was collected to measure lipid profile and glucose concentration.
8
9

10
11 Visit 3: A cycle test to exhaustion was performed to determine CRF. Following exhaustion,
12
13 participants sat for 10-min for assessment of HRR. At the end of this session, participants
14
15 were given an accelerometer and instructed to wear the device for seven consecutive days for
16
17 PA measurements.
18

19 20 *Autonomic function*

21
22 A 10-min resting period of heart rate measurements (Polar Team2, Kempele, Finland)
23
24 obtained during the second visit was used to calculate HRV. Participants were asked to pace
25
26 their breathing frequency at 12 cycles per minute using a metronome. Data were downloaded
27
28 and error correction conducted in the software polar ProTrainer 5. No files presented more
29
30 than 3% of error. Data were then transferred into Kubios v 2.2 (Biosignal Analysis and
31
32 Medical Imaging Group at the Department of Applied Physics, University of Kuopio,
33
34 Kuopio, Finland) to calculate time and frequency domain of HRV. The final 5 min period of
35
36 the data was filtered with a smooth-priors filter and used to calculate HRV. The time domain
37
38 index obtained to reflect the vagal activity was the RMSSD (square root of the mean of the
39
40 sum of the squares of differences between adjacent RR intervals). For the frequency domain,
41
42 a Fast Fourier Transformation was applied and the area under the low (LF: 0.04 - 0.15 Hz)
43
44 and high frequency (HF: 0.15 to 0.50 Hz) bands calculated in absolute (ms^2) and normalized
45
46 units (nu), as well as a ratio (LF/HF). The HF band reflects vagal modulations while the LF
47
48 band indicates both sympathetic and parasympathetic influences. All data collection and
49
50 analyses procedures were conducted in accordance with published guidelines [31]. HRV
51
52 measurements performed by our group have been demonstrated to be reliable (CV: 17.6%)
53
54
55
56
57 [5].
58
59
60

1
2
3 Heart rate recovery after the cycle test was recorded (Polar Team2, Kempele, Finland) and
4 downloaded for later analysis. The same procedures of error correction for the HRV data
5 were applied. The time decay of the heart rate in the first 30 s (T_{30}) post exercise was
6 measured as an index of vagal reactivation [17]. A single-exponential function was also used
7 to model HRR during the 10 min period and the time constant (τ) was used as an indicator of
8 the HRR. For this purpose, beat-by-beat heart rate was interpolated into 1 s. The HRR_{τ} is
9 known to be dependent of both parasympathetic reactivation and sympathetic withdrawal [8].
10 During recovery, the $RMSSD_{30}$ was obtained by calculating RMSSD every 30 s throughout
11 the full 10-min recovery period. $RMSSD_{30}$ was then transformed into a natural logarithm and
12 a median filter was applied [15]. $RMSSD_{30}$ is known to reflect the time course of the vagal
13 reactivation after exercise.
14
15
16
17
18
19
20
21
22
23
24
25
26
27

28 *Traditional cardiovascular disease risk factors*

29 Blood pressure was measured three times after the 10 min resting period (A&D Medical Co.,
30 LTD, Japan). The average of the two closest systolic and diastolic blood pressure values was
31 retained for analysis and mean arterial pressure (MAP) calculated. The observed coefficient
32 of variation between the measurements of systolic and diastolic BP were all <4%. Capillary
33 blood samples were used to determine total cholesterol (TC), high-density lipoprotein (HDL),
34 TAG and glucose (CardioChek[®] PA, PTS Diagnostics, USA). All measurements were
35 performed in duplicate and the average value retained for analysis. The observed %CV were
36 5.1, 5.7, 7.4 and 4.3% for TC, HDL, TAG and glucose, respectively.
37
38
39
40
41
42
43
44
45
46
47

48 *Cardiorespiratory fitness*

49 Peak oxygen uptake (peak VO_2) was estimated using a validated steep ramp test performed
50 on an electromagnetic brake cycle ergometer (Lode, The Netherlands) [6]. After three
51 minutes of warm-up at 25 W, participants started the test, which consisted of a predetermined
52 increment in work-rate per minute. The work-rate increments were chosen according to
53
54
55
56
57
58
59
60

1
2
3 participant stature: 60 W if <120 cm, 90 W if between 120 and 150 cm, and 120 W if >150
4
5 cm. The peak power (W) obtained at the end of the ramp test was used to estimate peak VO_2
6
7 in $\text{mL}\cdot\text{min}^{-1}$. Participants were asked to maintain a pedalling frequency of 80 rpm. The
8
9 protocol ended when participants dropped the pedalling frequency for 5 s below 60 rpm
10
11 despite strong verbal encouragement. Maximal effort was considered when participants
12
13 showed subjective signs of intense effort (e.g., unsteady cycling, sweating, and clear
14
15 unwillingness to continue despite encouragement). This method has been shown to have
16
17 strong ($R^2=0.92$) criterion-related validity with directly measured CRF [6]. CRF was
18
19 subsequently normalised for body mass using a ratio standard ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and an
20
21 allometric method. The latter was calculated from the sample specific exponent ($\beta=0.58$)
22
23 obtained from log-linear regression to allometrically scale CRF for body mass.
24
25
26

27 28 *Physical activity*

29
30 Habitual PA was measured using a wrist-worn accelerometer (GENEAciV, Activinsights Ltd,
31
32 UK). The device was set to record the activities for seven consecutive days at a frequency of
33
34 100 Hz starting in the morning following the third visit. Participants were instructed to wear
35
36 the device on their non-dominant wrist, including during sleeping hours and water activities.
37
38 The device was then retrieved and the raw acceleration data transformed into epochs of 60s
39
40 using specific software. The 60 s epoch files were then used to calculate the time spent
41
42 performing sedentary time (ST) and light (LPA), moderate (MPA) and vigorous (VPA) PA
43
44 using validated cut-off points [26]. Participants were included in the final analysis with a
45
46 minimum of three days with >12 h per day of wear time [28].
47
48
49

50 51 *Statistical analyses*

52
53 All data are presented as mean and SD unless otherwise stated. Normality of distribution was
54
55 checked using Shapiro Wilk's test and skewed data were transformed prior to analysis. A
56
57 clustered traditional CVD risk score ($\text{CVDR}_{\text{trad}}$) was calculated as the sum of the following
58
59
60

1
2
3 sex-specific standardized z-scores: fasted glucose, fasted triglycerides (TAG), fasted high-
4
5 density lipoprotein (HDL), %BF and blood pressure ($[\text{SBP}+\text{DBP}]/2$) [13]. A clustered
6
7 autonomic risk score ($\text{CVDR}_{\text{auto}}$) was created by adding the sex-specific standardized z-
8
9 scores of resting RMSSD, T_{30} and HRR_{τ} . Z-scores were inverted when appropriate. In order
10
11 to explore the effects of PA, ST and CRF beyond the traditional CVD risk factors, the
12
13 $\text{CVDR}_{\text{trad}}$ was combined to the $\text{CVDR}_{\text{auto}}$ to produce a composite CVD risk score (CVDR_{com}).
14
15 This is in accordance with a recent study that has included novel CVD risk factors into a
16
17 composite CVD risk score [14].
18
19

20
21 Separate linear regressions were performed for PA intensities, ST and CRF as the predictor
22
23 variables. For this purpose, a base model adjusting for sex and maturation was created and
24
25 the predictors inserted separately. The outcome variables included were: the traditional CVD
26
27 risk-factors (BMI, %BF, WC, MAP, TC, HDL, TAG and glucose), non-traditional CVD risk-
28
29 factors (HRV and HHR) and the clustered CVD risk scores ($\text{CVDR}_{\text{trad}}$, $\text{CVDR}_{\text{auto}}$ and
30
31 CVDR_{com}). The following variables were log transformed prior to entry into the model: VPA,
32
33 RMSSD, T_{30} , HRR_{τ} , HDL, WC, BMI and TAG. %BF did not present a significant
34
35 relationship to the outcome variables after adjusting for PA/CRF and was not included as a
36
37 covariate.
38
39
40
41

42
43 In order to test the effects of PA intensities and CRF on the time course of parasympathetic
44
45 reactivation as measured by the RMSSD_{30} , participants were divided into groups below and
46
47 above the sex-specific median split for PA intensities and CRF. Median splits were chosen
48
49 aiming to create equal sample sizes between the CRF groups as well as no thresholds are
50
51 available in the literature for the allometric scaled CRF used in the present investigation.
52

53
54 Repeated measures ANCOVA controlling for sex and maturation were used to examine a
55
56
57
58
59
60

1
2
3 time (0 to 600 s) by group (above or below median split) interaction effect for RMSSD₃₀. The
4
5 alpha level was set at 0.05 for all analyses which were performed using SPSS version 22.
6
7

8 **Results**

9 *Traditional CVD risk factors*

10 Regression coefficients for PA intensities, ST, CRF and traditional CVD risk factors after
11
12 adjusting for sex and maturation are presented in Table 3. ST and LPA were not significantly
13
14 associated to any of the traditional CVD risk factors. By contrast, MPA was significantly and
15
16 negatively associated to BMI, %BF, WC and MAP. VPA was significantly and negatively
17
18 associated to %BF. Both ratio and allometric scaled CRF were significantly and negatively
19
20 associated to BMI, %BF and WC. However, the associations between allometric scaled CRF
21
22 and the body composition variables were weaker compared to the ratio scaled CRF.
23
24
25
26
27

28 *Non-traditional CVD risk factors*

29 Regression coefficients for the PA intensities, CRF and non-traditional risk factors after
30
31 adjusting for sex and maturation are presented in Table 3. ST, LPA and CRF (ratio standard
32
33 and allometrically scaled) were not significantly related to any of the rest or recovery
34
35 autonomic indices. Both MPA and VPA were significantly and positively related to RMSSD
36
37 and significantly and negatively related to HRR_τ and T₃₀. The time course of parasympathetic
38
39 reactivation using the RMSSD₃₀ is presented in Figure 2. There was no group (below vs.
40
41 above) by time interaction for ST, LPA, MPA, or CRF for RMSSD₃₀ (all $P>0.05$). In
42
43 contrast, there was a group by time interaction ($P=0.01$) for VPA, with the above the median
44
45 group presenting a higher RMSSD₃₀ after the first 60 s of recovery after exercise (all
46
47 $P<0.05$).
48
49
50
51

52 *Clustered CVD risk scores*

53 Regression coefficients for the PA intensities, CRF and the CVD risk scores after adjusting
54
55 for sex and maturation are presented in Table 3. ST time and LPA were not significantly
56
57
58
59
60

1
2
3 related to any of the clustered CVD risk scores. MPA was significantly and negatively
4 associated to $CVDR_{trad}$, $CVDR_{auto}$ and $CVDR_{com}$. VPA was significantly and negatively
5 associated to $CVDR_{auto}$ and $CVDR_{com}$, but not with $CVDR_{trad}$ ($P=0.08$). While ratio scaled
6
7 CRF was negatively related to $CVDR_{trad}$, allometrically-scaled CRF was not associated to
8
9 any of the clustered CVD risk scores. After combining $CVDR_{trad}$ and $CVDR_{auto}$ to form
10
11 $CVDR_{com}$ the coefficient of determination increased from 17 to 32 % and 6 to 18 % for MPA
12
13 and VPA, respectively.
14
15
16
17
18

19 Discussion

20 The key findings of the present investigation were: 1) MPA and VPA were related to
21 traditional CVD risk factors, as well as rest and recovery autonomic function, 2) Girls and
22 boys performing VPA above the median split presented faster $RMSSD_{30}$; 3) MPA and VPA
23 were strongly and negatively related to CVD risk when rest and recovery autonomic indices
24 were added to the traditional $CVDR$ score; and 4) CRF was only significantly and negatively
25 related to traditional CVD risk score, however, when allometrically scaled the significant
26 relationship disappeared.
27
28
29
30
31
32
33
34
35
36

37 In the current investigation, ST and LPA were not significantly related to the health outcomes
38 which corroborates with previous literature [13], and supports the focus of current PA
39 guidelines on time spent performing MVPA to promote CVD health in adolescents. Both
40 MPA and VPA were negatively associated to %BF highlighting the benefits of PA on CVD
41 risk via lower body composition scores. Although these observations are in accordance with
42 the literature [18], causality cannot be inferred as increased %BF may lead to reduced MPA
43 and VPA [22]. The lack of significant associations between PA intensities and blood lipids is
44 in accordance with the literature [12]. Importantly, in adolescents with favourable lipoprotein
45 profile, body composition have been associated to CVD risk [21]. Altogether, our present
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 results highlight the important role of PA on cardiovascular health, via negative associations
4
5 with body composition in this population, independent of blood lipid profile.
6
7

8
9 In addition to the traditional CVD risk factors, MPA and VPA were significantly related to
10
11 resting HRV. This is reflected in other studies reporting the associations of combined MVPA
12
13 and resting HRV in youth [16, 27]. In contrast to the present results, Radtke, Kriemler, Eser,
14
15 Saner and Wilhelm (27) did not find associations between VPA and resting HRV in 15 years
16
17 old adolescents. Discrepancies between the results might lie in the different levels of total
18
19 MVPA performed by the participants, and different cut-off points used to define PA
20
21 intensities, as this has been shown to affect the interpretation of PA results [2]. Significant
22
23 associations between CRF and resting autonomic function were not observed in the present
24
25 study which is in line with the literature showing no consensus for this relationship [7, 16].
26
27 Interestingly, when CRF was allometrically scaled the positive association between CRF and
28
29 RMSSD approached significance ($P=0.06$). As allometric models are superior in controlling
30
31 for the effect of body size on CRF, this indicates that body size has a confounding effect on
32
33 the CRF-HRV associations when using the ratio standard method and needs further
34
35 investigation.
36
37
38

39
40 A novel finding in the current study is the significant and negative associations of HRR_{τ} and
41
42 T_{30} to MPA and VPA. These observations show that MPA and VPA are related to neural
43
44 control of vagal modulation (measured by resting HRV), as well as vagal tonus in adolescents
45
46 [11]. The group above the median split of VPA, which equated to 2 and 12 min per day for
47
48 girls and boys respectively, presented faster $RMSSD_{30}$ with no observed differences between
49
50 the MPA and CRF groups (see Figure 2). This is the first study to investigate the effects of
51
52 PA intensity and CRF on parasympathetic reactivation throughout 10 min of recovery. In
53
54 contrast to MPA and VPA, CRF, LPA and ST had no significant associations with HRR and
55
56
57
58
59
60

1
2
3 parasympathetic reactivation. As parasympathetic reactivation provides CVD prognostic
4 information [10, 20, 25], the present results highlights the important role of VPA and novel
5 mechanisms by which this intensity is associated with cardiovascular health in adolescents.
6
7 Possible explanations for the observed associations are the hemodynamic and hormonal
8 alterations occurring during and after VPA. These alterations may be related to an increased
9 catecholamine response, increased cardiac output, redistribution of blood flow to skin and
10 muscles, and the higher demand on the respiratory muscles amongst others, posing important
11 stresses on the autonomic and cardiovascular systems.
12
13
14
15
16
17
18
19

20
21 Further to the traditional and non-traditional CVD risk factors, a traditional CVD risk score
22 (CVDR_{trad}) as it is known to differentiate CVD risk in children was used [1]. The observed
23 MPA but not VPA relationship possibly reflects the observed association of MPA to BP
24 which was not found for VPA. Similarly, CRF was negatively and significantly related to
25 CVDR_{trad}, which is in line with evidence of associations between CVD profile and CRF in
26 youth [29]. However, when CFR was expressed using an allometric model, the observed
27 significance of the relationship disappeared. This observation shows that scaling for body
28 size has an important influence on the CRF-CVDR_{trad} association, which is not typically
29 accounted for in the literature [29]. Future studies should account for the confounding effects
30 of body size in youth because CRF normalized by body mass, using a ratio standard
31 approach, might reflect differences in body size occurring during adolescence rather than the
32 'true' CRF score.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48

49 A recent study has included endothelial function into a CVD risk score in children and
50 examined the associations between MPA, VPA and this new composite CVD score [14]. The
51 authors did not present the changes in the PA relationships to the risk score after the inclusion
52 of endothelial function to the composite risk score so additional explanation could not be
53
54
55
56
57
58
59
60

1
2
3 inferred. In contrast, our current data show that after combining RMSSD, T_{30} and HRR_{τ} to
4 the $CVDR_{\text{trad}}$ score, providing a composite risk score ($CVDR_{\text{com}}$), the coefficient of
5 determination rose from 17 to 32% for MPA and 6% to 18% for VPA. This increase in the
6 strength of the association shows that in adolescents, MPA and VPA have important
7 influences on autonomic function. This is in accordance with a recent study showing
8 improvements in HRV but not traditional CVD risk factors in this population following an
9 exercise intervention [4]. Altogether, these results contribute to the possible role of PA on
10 CVD risk in adolescents via autonomic function beyond changes in traditional CVD risk
11 factors.
12
13
14
15
16
17
18
19
20
21

22
23
24 Some limitations must be considered when interpreting the current findings. For instance, the
25 possible influence of the menstrual cycle in females was not possible to be taken into
26 consideration. Specific types of sedentary behaviours (e.g. TV viewing) were not examined
27 in the current study and the 60 s epoch used for the PA analyses may have underestimated the
28 PA status of the participants. Additionally, the participants were recruited using a
29 convenience sample and therefore sample bias might be present and the maturation measure
30 applied might have misclassified some of the participants. Finally, the cross-sectional design
31 limits causality between the observed associations.
32
33
34
35
36
37
38
39
40
41

42 **Conclusions**

43 This is the first study to examine the associations of PA intensities and CRF to cardiac
44 autonomic function together with traditional CVD risk factors in this population.
45 Complementary to the observed relationships between PA and traditional CVD risk factors,
46 the current study highlights the strong associations of MPA and VPA to autonomic function
47 at rest and recovery in adolescents but not CRF, ST and LPA. Additionally, this is the first
48 study to demonstrate the associations between VPA and a more rapid 10 min parasympathetic
49 reactivation. A two and a threefold increase in the association between MPA, VPA and the
50
51
52
53
54
55
56
57
58
59
60

1
2
3 composite CVD risk score after adding autonomic function to the traditional CVD risk score
4
5 was observed. These are novel findings that extend the current literature about a potential role
6
7 of the autonomic function on our understanding of CVD risk reduction. These results provide
8
9 information for health policy advocating the importance of MPA and VPA during
10
11 adolescence. Although our study provides original data, longitudinal studies are warranted to
12
13 clarify the causality of the relationships.
14
15

16 17 18 **References**

- 19 [1] *Andersen LB, Riddoch C, Kriemler S, Hills AP.* Physical activity and cardiovascular risk
20 factors in children. *Br J Sports Med.* 2011;45(11):871-6.
21 [2] *Banda JA, Haydel KF, Davila T, Desai M, Bryson S, Haskell WL, Matheson D, Robinson*
22 *TN.* Effects of varying epoch lengths, wear time algorithms, and activity cut-points on
23 estimates of child sedentary behavior and physical activity from accelerometer data.
24 *PLoS One.* 2016;11(3):e0150534.
25 [3] *Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA.*
26 Association between multiple cardiovascular risk factors and atherosclerosis in
27 children and young adults. The Bogalusa Heart Study. *N Engl J Med.*
28 1998;338(23):1650-6.
29 [4] *Bond B, Cockcroft EJ, Williams CA, Harris S, Gates PE, Jackman SR, Armstrong N,*
30 *Barker AR.* Two weeks of high-intensity interval training improves novel but not
31 traditional cardiovascular disease risk factors in adolescents. *Am J Physiol Heart Circ*
32 *Physiol.* 2015;309(6):H1039-47.
33 [5] *Bond B, Williams CA, Barker AR.* The reliability of a single protocol to determine
34 endothelial, microvascular and autonomic functions in adolescents. *Clin Physiol*
35 *Funct Imaging.* 2016: Ahead of Print.
36 [6] *Bongers BC, De Vries SI, Helders PJM, Takken T.* The steep ramp test in healthy children
37 and adolescents: reliability and validity. *Med Sci Sports Exerc.* 2013;45(2):366-71.
38 [7] *Brunetto AF, Roseguini BT, Silva BM, Hirai DM, Guedes DP.* Effects of gender and
39 aerobic fitness on cardiac autonomic responses to head-up tilt in healthy adolescents.
40 *Pediatr Cardiol.* 2005;26(4):418-24.
41 [8] *Buchheit M, Papelier Y, Laursen PB, Ahmadi S.* Noninvasive assessment of cardiac
42 parasympathetic function: postexercise heart rate recovery or heart rate variability?
43 *Am J Physiol Heart Circ Physiol.* 2007;293(1):H8-10.
44 [9] *Buchheit M, Platat C, Oujaa M, Simon C.* Habitual physical activity, physical fitness and
45 heart rate variability in preadolescents. *Int J Sports Med.* 2007;28(3):204-10.
46 [10] *Dewey FE, Freeman JV, Engel G, Oviedo R, Abrol N, Ahmed N, Myers J, Froelicher*
47 *VF.* Novel predictor of prognosis from exercise stress testing: heart rate variability
48 response to the exercise treadmill test. *Am Heart J.* 2007;153(2):281-8.
49 [11] *Dewland TA, Androne AS, Lee FA, Lampert RJ, Katz SD.* Effect of acetylcholinesterase
50 inhibition with pyridostigmine on cardiac parasympathetic function in sedentary
51 adults and trained athletes. *Am J Physiol Heart Circ Physiol.* 2007;293(1):H86-92.
52 [12] *Ekelund U, Anderssen SA, Froberg K, Sardinha LB, Andersen LB, Brage S.* Independent
53 associations of physical activity and cardiorespiratory fitness with metabolic risk
54
55
56
57
58
59
60

- factors in children: the European youth heart study. *Diabetologia*. 2007;50(9):1832-40.
- [13] *Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A*. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. *JAMA*. 2012;307(7):704-12.
- [14] *Fussenich LM, Boddy LM, Green DJ, Graves LE, Fowweather L, Dagger RM, McWhannell N, Henaghan J, Ridgers ND, Stratton G, Hopkins ND*. Physical activity guidelines and cardiovascular risk in children: a cross sectional analysis to determine whether 60 minutes is enough. *BMC Public Health*. 2016;16(1):67.
- [15] *Goldberger JJ, Le FK, Lahiri M, Kannankeril PJ, Ng J, Kadish AH*. Assessment of parasympathetic reactivation after exercise. *Am J Physiol Heart Circ Physiol*. 2006;290(6):H2446-52.
- [16] *Gutin B, Howe C, Johnson MH, Humphries MC, Snieder H, Barbeau P*. Heart rate variability in adolescents: relations to physical activity, fitness, and adiposity. *Med Sci Sports Exerc*. 2005;37(11):1856-63.
- [17] *Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, Takeda H, Inoue M, Kamada T*. Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J Am Coll Cardiol*. 1994;24(6):1529-35.
- [18] *Janssen I, Leblanc AG*. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. *Int J Behav Nutr Phys Act*. 2010;7:40.
- [19] *Joyner MJ, Green DJ*. Exercise protects the cardiovascular system: effects beyond traditional risk factors. *J Physiol*. 2009;587(Pt 23):5551-8.
- [20] *Lahiri MK, Chicos A, Bergner D, Ng J, Banthia S, Wang NC, Subacius H, Kadish AH, Goldberger JJ*. Recovery of heart rate variability and ventricular repolarization indices following exercise. *Ann Noninvasive Electrocardiol*. 2012;17(4):349-60.
- [21] *McGill HC, McMahan CA, Zieske AW, Malcom GT, Tracy RE, Strong JP, Group fitPDoAiYR*. Effects of nonlipid risk factors on atherosclerosis in youth with a favorable lipoprotein profile. *Circulation*. 2001;103(11):1546-50.
- [22] *Metcalf BS, Hosking J, Jeffery AN, Voss LD, Henley W, Wilkin TJ*. Fatness leads to inactivity, but inactivity does not lead to fatness: a longitudinal study in children (EarlyBird 45). *Arch Dis Child*. 2011;96(10):942-7.
- [23] *Mirwald RL, Baxter-Jones AD, Bailey DA, Beunen GP*. An assessment of maturity from anthropometric measurements. *Med Sci Sports Exerc*. 2002;34(4):689-94.
- [24] *Mora S, Cook N, Buring JE, Ridker PM, Lee IM*. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. *Circulation*. 2007;116(19):2110-8.
- [25] *Peçanha T, Bartels R, Brito LC, Paula-Ribeiro M, Oliveira RS, Goldberger JJ*. Methods of assessment of the post-exercise cardiac autonomic recovery: A methodological review. *Int J Cardiol*. 2016.
- [26] *Phillips LR, Parfitt G, Rowlands AV*. Calibration of the GENEActiv accelerometer for assessment of physical activity intensity in children. *J Sports Sci Med*. 2013;16(2):124-8.
- [27] *Radtke T, Kriemler S, Eser P, Saner H, Wilhelm M*. Physical activity intensity and surrogate markers for cardiovascular health in adolescents. *Eur J Appl Physiol*. 2013;113(5):1213-22.
- [28] *Rich C, Geraci M, Griffiths L, Sera F, Dezateux C, Cortina-Borja M*. Quality control methods in accelerometer data processing: defining minimum wear time. *PLoS One*. 2013;8(6):e67206.

- 1
2
3 [29] Ruiz JR, Huybrechts I, Cuenca-Garcia M, Artero EG, Labayen I, Meirhaeghe A,
4 Vicente-Rodriguez G, Polito A, Manios Y, Gonzalez-Gross M, Marcos A, Widhalm K,
5 Molnar D, Kafatos A, Sjostrom M, Moreno LA, Castillo MJ, Ortega FB.
6 Cardiorespiratory fitness and ideal cardiovascular health in European adolescents.
7 Heart. 2014;101(10):766-73.
8
9 [30] Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Loan MDV, Bembien
10 DA. Skinfold Equations for Estimation of Body Fatness in Children and Youth. Hum
11 Biol. 1988;60(5):709-23.
12 [31] Task-Force. Heart rate variability: standards of measurement, physiological
13 interpretation, and clinical use. Circulation. 1996;93(5):1043-65.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2 Table 1: Characteristics of the participants according to sex.
3

4 Table 2: Fitness and physical activity characteristics of the participants
5

6 Table 3: Standardised regression coefficients
7
8
9

10 Figure 1: Flow chart of recruitment and sample size in the final analysis
11

12 Figure 2: Parasympathetic reactivation for the groups above (solid circles) and below (open circles) the sex
13 specific median split of: A) VPA($\text{min}\cdot\text{day}^{-1}$); B) MPA ($\text{min}\cdot\text{day}^{-1}$); and C) CRF ($\text{mL}\cdot\text{kg}^{-0.58}\cdot\text{min}^{-1}$). * $P<0.05$
14 for the comparison between groups from 60 to 600 s (except at 510 s, $P=0.053$). Values are mean and SD.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Table 1: Characteristics of the participants according to sex.

	All (n=53)	Female (n=23)	Male (n=31)
Demographic characteristics			
Age (y)	13.1±0.8	12.9±0.8	13.1±0.9
Stature (cm)	156.6±9.6	153.8±7.8	158.6±10.5
Body mass (kg)	49.2±12.1	50.1±13.8	48.5±11.0
Pubertal status			
Pre (n (%))	18(33.3)	1(4)	17(56)
Circum (n (%))	27(50)	15(65)	12(39)
Post (n (%))	9(16.7)	7(31)	2(5)
Traditional CVD risk factors			
BMI (kg·m ⁻²)	19.9±3.5	21.0±4.3	19.1±2.6
Body fat (%)	20±7.6	23.2±6.9	17.8±7.4
WC (cm)	68.5±9.5	68.3±10	68.5±8.9
SPB (mmHg)	113±9	113±9	113±9
DPB (mmHg)	68±7	70±7	67±7
TC (mmol·L ⁻¹)	3.4±0.5	3.4±0.6	3.4±0.5
HDL (mmol·L ⁻¹)	1.4±0.3	1.3±0.4	1.4±0.3
TAG (mmol·L ⁻¹)	0.7±0.2	0.8±0.3	0.8±0.5
Glucose (mmol·L ⁻¹)	4.3±0.4	4.2±0.5	4.3±0.4
Non-traditional CVD risk factors			
RMSSD (ms)	77.8±40.4	64.7±32.4	87.6±43.4
HF (ln)	7.8±1.1	7.5±1.1	8.0±1.0
LF (ln)	7.1±0.9	6.8±0.9	7.4±0.9
HF (nu)	64.9±16.2	65.2±15.4	64.7±17.0
LF (nu)	34.8±16.2	34.6±15.4	34.9±17.0
LF/HF	0.7±0.7	0.6±0.5	0.7±0.9
T ₃₀ (s)	192±88.8	193.3±57.5	191.1±107.4
HRR τ (s)	70.8±29.6	66.1±15.8	74.4±36.5

BMI, body mass index; WC, waist circumference; SPB, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein; TAG, triglycerides; RMSSD, square root of the mean of the sum of the squares of differences between adjacent RR intervals; HF, High-frequency; LF, Low-frequency.

Table 2: Fitness and physical activity characteristics of the participants

	All (n=54)	Girls (n=22)	Boys (n=31)
CRF ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	46.3±8.1	42.5±9.2	49.2±5.9
CRF ($\text{mL} \cdot \text{kg}^{-0.58} \cdot \text{min}^{-1}$)	235.1±36.1	215.9±35.9	249.4±29.3
ST ($\text{min} \cdot \text{day}^{-1}$)	329.6±110.4	330.7±105.5	328.9±115.7
LPA ($\text{min} \cdot \text{day}^{-1}$)	278.7±112.2	305.2±104.7	259.1±115.2
MPA ($\text{min} \cdot \text{day}^{-1}$)	104.6±33.4	92.0±36.0	114.0±28.5
VPA ($\text{min} \cdot \text{day}^{-1}$)	11.2±10.4	5.5±6.3	15.4±11.0
MVPA ($\text{min} \cdot \text{day}^{-1}$)	115.8±41.1	97.6±39.8	129.4±37.2

CRF, cardiorespiratory fitness; ST, sedentary time; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate to vigorous physical activity.

For Peer Review

Table 3: Standardised regression coefficients

	ST (min·day ⁻¹)		LPA (min·day ⁻¹)		MPA (min·day ⁻¹)		VPA (min·day ⁻¹)		CRF (ml·kg·min ⁻¹)		CRF (ml·kg ^{-0.58} ·min ⁻¹)	
	β (P)	r ²	β (P)	r ²	β (P)	r ²	β (P)	r ²	β (P)	r ²	β (P)	r ²
Traditional CVD risk factors												
BMI (kg·m ²)	-0.017 (0.90)	0.01	0.029 (0.83)	0.01	-0.276 (0.04)	0.07	-0.229 (0.15)	0.03	-0.706 (<0.001)	0.41	-0.430 (0.004)	0.13
%BF (%)	0.022 (0.86)	0.01	0.024 (0.85)	0.01	-0.310 (0.02)	0.09	-0.384 (0.01)	0.10	-0.701 (<0.001)	0.40	-0.602 (<0.001)	0.25
WC (cm)	-0.050 (0.72)	0.01	0.091 (0.52)	0.01	-0.316 (0.03)	0.09	-0.300 (0.07)	0.06	-0.765 (<0.001)	0.48	-0.496 (0.001)	0.17
MAP (mmHg)	-0.023 (0.87)	0.01	0.142 (0.30)	0.02	-0.326 (0.02)	0.09	0.182 (0.27)	0.02	-0.191 (0.20)	0.03	-0.170 (0.29)	0.02
TC (mmol·L ⁻¹)	0.062 (0.66)	0.01	0.062 (0.66)	0.03	0.041 (0.78)	0.01	-0.162 (0.34)	0.02	-0.09 (0.56)	0.01	-0.236 (0.15)	0.04
HDL (mmol·L ⁻¹)	0.177 (0.20)	0.03	-0.169 (0.23)	0.02	0.209 (0.15)	0.04	0.037 (0.83)	0.01	0.138 (0.37)	0.02	-0.026 (0.87)	0.01
TAG (mmol·L ⁻¹)	-0.145 (0.28)	0.02	-0.024 (0.86)	0.01	-0.158 (0.26)	0.02	-0.204 (0.22)	0.03	-0.135 (0.37)	0.01	-0.08 (0.63)	0.01
Glucose (mmol·L ⁻¹)	-0.106 (0.45)	0.01	-0.046 (0.75)	0.01	-0.184 (0.21)	0.03	-0.165 (0.34)	0.02	-0.189 (0.22)	0.03	-0.097 (0.57)	0.01
Non-traditional CVD risk factors												
RMSSD (ms)	-0.005 (0.97)	0.01	0.006 (0.96)	0.01	0.402 (0.003)	0.14	0.453 (0.005)	0.13	0.237 (0.11)	0.05	0.299 (0.06)	0.06
HRR τ (s)	-0.139 (0.33)	0.02	0.149 (0.30)	0.02	-0.311 (0.03)	0.08	-0.406 (0.02)	0.11	-0.034 (0.83)	0.01	0.109 (0.22)	0.01
T ₃₀ (s)	-0.111 (0.42)	0.02	0.100 (0.49)	0.01	-0.356 (0.02)	0.11	-0.433 (0.01)	0.12	0.081 (0.61)	0.01	0.207 (0.22)	0.03
Clustered CVD risk scores												
CVDR _{trad}	-0.152 (0.28)	0.02	0.041 (0.78)	0.01	-0.447 (0.002)	0.17	-0.340 (0.08)	0.06	-0.438 (0.004)	0.15	-0.277 (0.11)	0.05
CVDR _{auto}	-0.115 (0.42)	0.01	0.102 (0.48)	0.01	-0.483 (0.001)	0.20	-0.563 (0.001)	0.20	-0.070 (0.67)	0.01	0.020 (0.91)	0.01
CVDR _{com}	-0.142 (0.32)	0.02	0.041 (0.78)	0.01	-0.601 (<0.001)	0.32	-0.540 (0.001)	0.18	-0.313 (0.05)	0.08	-0.153 (0.38)	0.02

Standardised regression coefficients are adjusted for maturation and sex. ST, sedentary time; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; CRF, cardiorespiratory fitness; BMI, body mass index; BF, body fat; WC, waist circumference; MAP, mean arterial pressure; TC, total cholesterol; HDL, high-density lipoprotein; TAG, triglycerides; RMSSD, square root of the mean of the sum of the squares of differences between adjacent RR intervals;

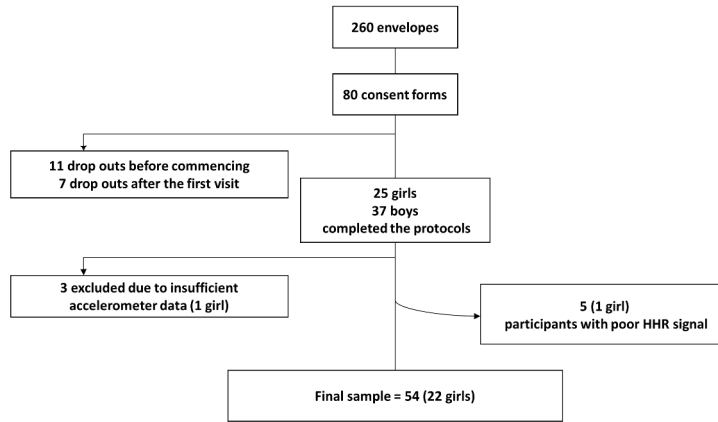


Figure 1: Flow chart of recruitment and sample size in the final analysis
the final number of participant
338x190mm (230 x 230 DPI)

Peer Review

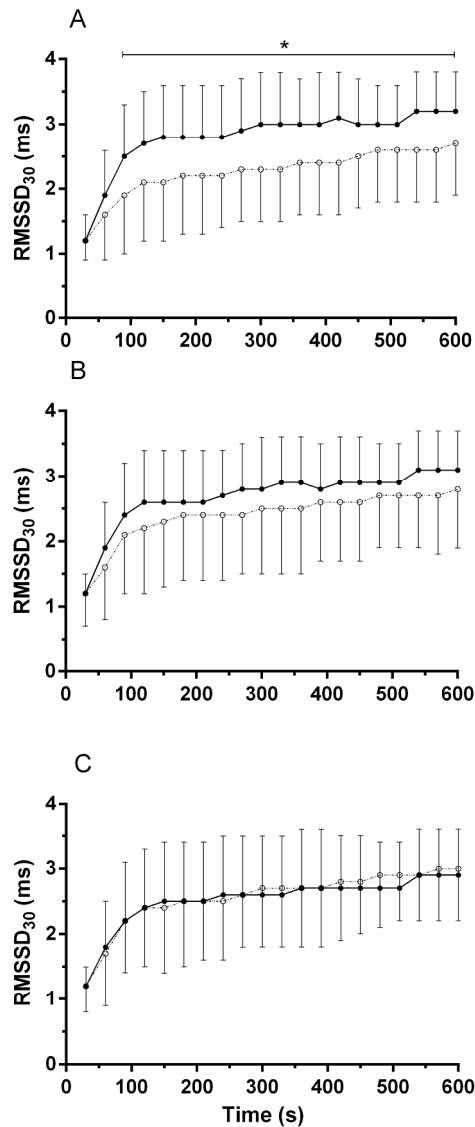


Figure 2: Parasympathetic reactivation for the groups above (solid circles) and below (open circles) the sex specific median split of: A) VPA(min·day⁻¹); B) MPA (min·day⁻¹); and C) CRF (mL·kg⁻¹·min⁻¹). *P<0.05 for the comparison between groups from 60 to 600 s (except at 510 s, P=0.053). Values are mean and SD.

The time course of parasympath
211x446mm (300 x 300 DPI)