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Fitness, Fatness, Physical Activity and Autonomic Function in Mid-life

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ABSTRACT

Purpose: Although low cardiorespiratory fitness (CRF), physical inactivity and obesity are associated with impaired autonomic function, they are also extensively interrelated. The present study aimed to assess the extent to which they contribute to autonomic function independently of each other. **Methods:** At age of 46 yrs, 1383 men and 1761 women without cardiorespiratory diseases and diabetes underwent assessments of vagally mediated heart rate (HR) variability (root mean square of successive differences in R-R interval, rMMSD), peak HR during a submaximal step test (CRF) and 60-s HR recovery (HRR). Moderate-to-vigorous physical activity (MVPA, ≥3.5 METs, 2 weeks) was measured by wrist-worn accelerometer and body fat percentage (Fat%) by bioimpedance. **Results:** In men, CRF and Fat% were significantly associated with higher rMSSD (standardized β=0.31 and -0.16) and HRR (β=0.19 and -0.18), whereas higher MVPA was linked with higher HRR (β=0.13) when including CRF, MVPA and Fat% in the initial regression. After adjustments for other lifestyle and cardiometabolic factors, CRF remained significantly associated with rMMSD (β=0.24) and HRR (β=0.14), as did MVPA with HRR (β=0.11). In women, CRF was associated with rMSSD (β=0.23) and HRR (β=0.15), and MVPA (β=0.17) and Fat% (β=-0.07) with HRR, when CRF, MVPA and Fat% were adjusted for each other. After further adjustments, CRF remained a significant determinant of rMSSD (β=0.20) and HRR (β=0.13), as did MVPA with HRR (β=0.15). The final models explained 23% and 21% of variation in rMSSD and HRR in men, and 10% and 12% in women, respectively. **Conclusion:** Cardiorespiratory fitness was a more important determinant of cardiac autonomic function than MVPA and body fat. Furthermore, MVPA, but not body fat was independently associated with cardiac autonomic function in both men and women.

Key Words: exercise; body composition; heart rate variability; heart rate recovery; baroreflex

Introduction

Paragraph 1 – Impaired cardiorespiratory fitness (CRF), physical inactivity and obesity are important risk factors for many cardiometabolic diseases (28). One potential mechanism for the increased risk related to these factors is impaired autonomic function, manifested as decreased vagal and elevated sympathetic activity. Higher CRF (4, 10, 13, 18, 35) and physical activity (PA) (4, 12, 13, 18, 22, 31, 35) and more optimal body weight and composition (10, 12, 13, 35) have been associated with better cardiac autonomic function as measured by heart rate (HR) variability (HRV) and post-exercise heart rate recovery (HRR). Autonomic function is known to be related to several cardiometabolic risk factors (39), and its enhancement with improved CRF and PA is beneficial in reducing cardiovascular risk, independently of traditional risk markers (17).

Paragraph 2 – Several studies have assessed the individual contributions of CRF, PA and anthropometric measures to autonomic regulation of the activity of the sinoatrial node (4, 10, 12, 13, 18, 22, 31, 35). Although these factors display evident inter-relationships, there are rather few studies examining their association with autonomic function independently of each other and, as far as we are aware, none conducted in population-based samples of adults. Knowledge about the independent relationship of these factors with autonomic function could help in targeting life style interventions and be important in the primary prevention of autonomic dysfunction and related cardiometabolic diseases. Methodologically, objective PA measurements and detailed measures of body composition have been less extensively reported in large-scale epidemiological studies. Finally, despite well-known sex-differences in autonomic function (16), few studies have assessed sex-differences in the relationship between cardiac autonomic activity and CRF, PA and body composition (12, 13, 31). Previously, Rennie et al. identified a significant association between HRV and PA in men but this relationship was lacking in women (31).

Paragraph 3 – We aimed here to assess the extent to which CRF, PA and body fat proportion (Fat%) would be associated with cardiac autonomic function independently of each other and established cardiometabolic risk factors in men and women. We hypothesized that CRF could be the most important factor the underlying the variation in cardiac autonomic function regardless of PA and Fat%, even though these factors may have independent associations with autonomic function. Furthermore, we tested the hypothesis that sex would modify the association of autonomic function to CRF, PA and Fat%.

Methods

Paragraph 4 – Subjects: All those individuals living in northern Finland whose expected date of birth fell between January 1st and December 31st 1966 (96.3% of all 1966 births, n = 12,058 live births) were included in the prospective NFBC1966-study. Since their mother's recruitment during her first visit to the maternity health centers, data have been collected on their health, lifestyle and socioeconomic status. The study was conducted according to the Declaration of Helsinki and approved by the Ethical Committee of the Northern Ostrobothnia Hospital District in Oulu, Finland. The study participants provided their written informed consent for the study.

Paragraph 5 – Protocol: Postal surveys inquiring about the participant's health status and lifestyle, including an invitation to attend a clinical examination, were sent in 2012-2014 to subjects who were living at known addresses in Finland (n=10,321). The response rate to the postal surveys was 66% (n=6,825). A total of 5,861 (57%) subjects participated in the clinical examinations in one of the three laboratory units (Oulu, southern and northern Finland). between April 2012 and March 2014 (Figure 1). The subjects entered the laboratory between 7:00 and 11:00 a.m. after overnight fasting (12 hours) and abstained from smoking and drinking coffee during the examination day. Venous blood samples were drawn for the analysis of

glycemic and lipid status. Serum glucose was analyzed using an enzymatic hexokinase/glucose-6-phosphate dehydrogenase method. Total cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol, and triglycerides were determined with an enzymatic assay method. The concentrations of glycated and total hemoglobin were measured using immunochemical assay methods. The ratio is reported as percent hemoglobin A1c (NGSP). The samples were analyzed in NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO 15189) (All methods: Advia 1800; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Seated systolic (SBP) and diastolic blood pressures (DBP) were measured three times (the two lowest values averaged, Omron M10, Omron Healthcare, Kyoto, Japan) after 15 minutes of rest. After the anthropometric measurements, including body composition (Fat%) by bioimpedance (InBody720, InBody, Seoul, Korea), and other examinations, the participants had a light meal 60-90 min before the assessments of cardiovascular autonomic function and performance of the submaximal exercise test. Subsequently, the two-week monitoring of PA was initiated. On a separate day, an oral glucose tolerance test was conducted according to the recommendations of the World Health Organization in those participants without medication for diabetes.

Paragraph 6 – Inclusions/Exclusions: A total of 4,537 subjects successfully underwent HRV recording, submaximal exercise test with HRR assessment, PA and bioimpedance measurements. Further exclusions are described in Figure 1. The final population included 1383 men and 1761 women for HRV and HRR, and 709 men and 805 women for BRS. Based on the questionnaire, approximately 4% of women did not have an active menstrual cycle, whereas 28% had undergone hysterectomy and/or were on hormone therapy.

Paragraph 7 – Lifestyle factors: Based on the questionnaire, subjects were defined as non-, ex- and current smokers. The amount of alcohol consumed per day was estimated from the questions concerning the frequency and the usual amount of beverage consumed on one

occasion. Total sitting time during waking hours was established by asking the subjects how many hours on average they sat on weekdays (at work, at home, in a vehicle and elsewhere) and the total sum of sitting hours was used. Finally, the subjects were asked how tired they typically felt in the morning during the first half hour after awakening (very tired, somewhat tired, somewhat rested or well rested).

Paragraph 8 – Physical activity monitoring: PA was objectively measured with a wrist-worn Polar Active device (Polar Electro Oy, Kempele Finland). Participants were asked to wear the Polar Active monitor for 24 hours every day for at least 14 days, also while sleeping, on the non-dominant wrist. The first day when activity monitors were given was excluded from the analysis. An eligible day was considered as at least 600 min/day wearing time during waking hours. Participants with four or more eligible days were included in the analyses. In the final dataset, mean (SD) for eligible days was 13.6 (1.2), ranging from 4 to 19 days and including weekends. Polar Active provides daily PA based on estimated metabolic equivalent (MET) values every half minute (26). Daily averages of duration spent in different PA levels (min/day) were calculated in all participants using the cutoff values provided by the manufacturer (very light: 1–2 MET, light: 2–3.5 MET, moderate: 3.5–5 MET, vigorous: 5–8 MET and very vigorous >8 MET). The three highest activity levels were combined as moderate-to-vigorous physical activity (MVPA), which was the primary PA variable, and the two highest as vigorous PA.

Paragraph 9 – Values obtained from the wrist-worn PA monitor have been shown to correlate ($R^2 = 0.74$) with a doubly labelled water technique when assessing energy expenditure during exercise training intervention (21). The amount of MVPA measured by the wrist-worn Polar Active is higher compared to hip worn accelerometers when using standard cutoffs of 3 MET and 6 MET for moderate and vigorous PA, respectively (26). However, the differences

between Polar Active and hip-worn monitors declines when using the cutoffs values provided by the Polar Active manufacturer (26).

Paragraph 10 – Measurement of resting cardiovascular autonomic function: Each participant sat in a chair to allow instrumentation and was provided with a review of the protocol. A heart rate (HR) monitor (RS800CX, Polar Electro Oy, Kempele, Finland) was used to record R-R intervals (RRi). In half of the participants (Oulu laboratory unit), spontaneous baroreflex sensitivity (BRS) was also assessed. Standard lead-II ECG (Cardiolife, Nihon Kohden, Tokyo, Japan), breathing frequency (MLT415/D, Nasal Temperature Probe, ADInstruments, Bella Vista, New South Wales, Australia), and blood pressure (BP) by finger photoplethysmography (Nexfin, BMEYE Medical Systems, Amsterdam, the Netherlands) were recorded with a sampling frequency of 1,000 Hz (PowerLab 8/35, ADInstruments). The finger cuff was adjusted so that SBP and DBP assessed by finger photoplethysmography (left arm, supported by an arm sling) did not differ by more than 10 mmHg from the values measured by the automated sphygmomanometer (right arm, Omron M10). Physiological calibration of finger BP was then turned off. After these procedures (5-10 min), there was at least a 1-min period allowing stabilization of HR before the recording of 3 min in the seated position while breathing spontaneously. An 1-min stabilization period has been documented to suffice for robust HRV measurements from even as short as a 1-min recording (11). The first 150 s of 3-min recording were analyzed. Spontaneous breathing was allowed because it requires less familiarization and co-operation with the participant and breathing frequency has been reported to exert only a modest impact on the present main HRV variable, root mean square of successive differences in RRi (rMSSD, ms), has been reported (29). Conversely, a low breathing frequency may overestimate BRS (36) despite its good reproducibility during spontaneous breathing (27).

Paragraph 11 – Heart rate variability: Artifacts and ectopic beats were removed and replaced by the local average (Hearts 1.2, University of Oulu, Oulu, Finland). Sequences with ≥10 consecutive beats of noise or ectopic beats were deleted. The RRi series with ≥80% accepted data were included in the analyses. A total of 5,679 subjects took part in the RRi recordings and of these, 5,473 (96%) had eligible HRV data. Mean HR (HR_{REST}) and root mean square of successive differences in RRi (rMSSD, ms), a robust measure of cardiac vagal activity (29), were analyzed.

Paragraph 12 – Baroreflex sensitivity: Continuous ECG, BP and respiration signals were imported to a custom-made stand-alone Matlab-based software (Biosignal Processing Team, University of Oulu, Oulu, Finland) where RRi and SBP values were extracted. Artifacts and ectopic beats were replaced using linear interpolation (<5% for accepted recording) and thereafter, resampled at 2 Hz and detrended (<0.04 Hz, Savitzky-Golay method). A fast Fourier transform (Welch method, segments of 128 samples with 50% overlap) was performed to analyze low frequency (LF, 0.04-0.15 Hz) power of RRi and SBP oscillations for subsequent analysis of BRS by the alpha method, if sufficient coherence (≥0.5) between LF oscillations in RRi and SBP was verified. Out of 2,726 recordings, BRS was successfully calculated in 2,599 subjects (95%).

Paragraph 13 – Cardiorespiratory fitness: CRF was measured by a submaximal 4-min single-step test with a stepping rate of 23 ascents per minute paced by metronome and expressed as peak HR during the step test (HR_{STEP}) (33). In a previous sub-study (n=124) of NFBC1966 at the age of 31, the correlation between HR_{STEP} and directly measured maximal oxygen uptake during a maximal cycle ergometer test was -0.52 (33). Stepping was performed without shoes on a bench adjusted to a height of 33 cm for women and 40 cm for men. Heart rate was measured during and 90 s after the stepping in a seated position (RS800CX). The population was divided into CRF sex-wise tertiles and percentiles according to HR_{STEP}. The

participants who terminated the test due to exhaustion were placed in the lowest tertile or percentile. Out of 5,861 participants, 5,019 successfully performed the test, 237 terminated the test due to exhaustion (test duration > 60 s), 40 terminated the test due to some reason other than exhaustion, 534 did not perform the test due to impaired health status (e.g. musculoskeletal problems, elevated blood pressure or exercise-induced angina pectoris) or unwillingness, and in 31 there were technical problems with HR recording.

Paragraph 14 –Heart rate recovery after exercise: The HR recording was transformed into moving 10-beat median data that was visually inspected for noise and ectopic beats. The peak HR of the test was determined as 10-beat median at the time of cessation of the stepping. Subsequently, the median HR at 60 s after the stepping was registered and HRR calculated (peak HR – HR at 60 s post-exercise). Additionally, the steepest 30-s slope during 60 s of recovery was calculated from the median HR data. The HRR at 60 s (bpm) and the HRR slope (bpm/s) were also normalized by peak HR.

Paragraph 15 – Statistical analysis: The distributions of the dependent variables were first assessed by analyzing the skewness of the data by visual inspection of histograms. In the case of skewed distributions (|skewness|>1; rMSSD and BRS) (14), the variable was transformed into its natural logarithm (ln), which eliminated skewness in the dependent variables. Thereafter, these transformed variables were verified to be Gaussian. One-way ANOVA was used to compare the groups and sexes and Bonferroni's *post hoc* test to account for multiple testing. Sex-differences in categorical variables were analyzed using Chi-square test. Interactions of CRF, MVPA and Fat%, in tertiles, with sex in their associations with cardiac autonomic function were assessed by ANCOVA. The linearity and collinearity of the associations were assessed by the linear and quadratic regression models with continuous and by contrasts estimated by ANOVA with categorical independent variables. The main explanatory variables (CRF, MVPA and Fat%) were transformed into categorical (tertiles) or

percentiles (continuous) for each sex before ANOVA and Pearson correlation analyses. Subsequently, multivariate linear regression analysis (enter method) was employed where the association analyses of CRF, MVPA, Fat% and all together (in percentiles) with autonomic function were adjusted for the potential contributing factors (enter method: smoking, alcohol consumption, sitting time, tiredness in the morning, brachial systolic and diastolic blood pressure, glycated hemoglobin, fasting plasma glucose, serum total and high-density lipoprotein cholesterol and triglycerides). Low-density lipoprotein cholesterol was excluded from the covariates due to its significant collinearity with total cholesterol (variance inflation factor > 5). No significant collinearity was observed between CRF, MVPA and Fat%. ANCOVA was used to assess interactions between the tertiles of CRF, MVPA and Fat%. The data were analyzed using SPSS software (IBM SPSS Statistics 21, IBM Corp., New York). A p-value <0.05 was considered significant.

Results

Paragraph 16 **–** The characteristics of the study population are presented in Table 1. In the univariate analysis, CRF, MVPA and Fat% were linearly associated with cardiac autonomic function (Figures 2 and 3, see Tables, Supplemental Digital Contents 1, Correlations between autonomic function, CRF, MVPA and Fat%, and 2-3, Autonomic function across the tertiles of CRF, MVPA and Fat%). In both sexes, CRF (Figures 2a-e and 3a-e) and MVPA (Figures 2f-j and 3f-j) were significantly and positively associated with rMSSD, BRS and HRR and inversely related to HR_{REST}. Similarly, Fat% was significantly and inversely associated with rMSSD, BRS and HRR and positively associated with HR_{REST} in both sexes (Figures 2k-o and 3k-o). Significant interactions between CRF and sex were observed in their associations with HR_{REST} ($p<0.001$) and rMSSD ($p<0.002$) and between Fat% and sex with HR_{REST} ($p<0.001$), rMSSD ($p<0.001$), HRR60s ($p<0.001$) and HRR_{SLOPE} ($p=0.004$), with men manifesting a

clearer trend across the tertiles (see Figure, Supplementary Digital Content 4, Sex-interactions in the associations of autonomic function to CRF, MVPA and Fat%).

Paragraph 17 **–** In men, when assessing the contributions of CRF, MVPA and Fat% to autonomic function separately after adjustments for covariates, all associations remained significant, except for the association of MVPA with rMSSD and BRS (Table 2). The standardized β-values were consistently greater with CRF and autonomic function than with MVPA or Fat% (Table 2), and remained greater also when including all CRF, MVPA and Fat% together in the initial block of regression (Table 2). After further adjustment for covariates, CRF was associated with all cardiac autonomic function variables (Table 2), with MVPA being significantly related only to HRR variables but not to HR_{REST}, or BRS (Table 2). An unexpected but statistically significant negative association was observed between MVPA and rMSSD when including CRF, MVPA and Fat% in the same regression model. However, no significant interactions or collinearity were present in the associations of these variables to HRV.

Paragraph 18 **–** In women, associations of CRF, MVPA and Fat%, when analyzed separately, remained significant after adjustments for covariates, except for MVPA with rMSSD and BRS (Table 3). Similar to the findings in men, the standardized β-values of CRF to autonomic function were higher than those with MVPA and Fat% (Table 3). When including all CRF, MVPA and Fat% in the same model that adjusted for potential covariates, CRF was still associated with all indexes of autonomic function, whereas MVPA remained significant determinant of HRR but not HR_{REST}, rMSSD or BRS (Table 3). Fat% was not significantly related to rMSSD, BRS or HRR in this model. The relationship between Fat% and HR_{REST} became negative when CRF and MVPA were included in the same model. However, no significant interactions or collinearity between CRF, MVPA and Fat% were observed in this respect.

Discussion

Paragraph 19 – In this study, CRF was the most significant factor accounting for the variation in cardiac autonomic function; its contribution was greater than objectively measured MVPA and body composition in middle-aged men and women. However, MVPA was associated with HRR, regardless of CRF, body composition and several cardiometabolic risk factors in both men and women, whereas no independent contribution of Fat% to autonomic function was observed. The present results suggest that CRF should be the primary target in the prevention of abnormalities in cardiac autonomic function and related cardiometabolic diseases.

Paragraph 20 – Previous studies in different populations have shown that impaired CRF is a more significant cardiovascular risk factor than either overweight or abdominal obesity (6, 24) or physical inactivity (25, 28, 32). One plausible explanation for our finding concerning the strong association between CRF and cardiac autonomic function is that genetic and lifelong environmental effects on autonomic function are better integrated with CRF than MVPA and body composition in the current cross-sectional setting. First, an important factor underlying CRF is stroke volume; this is known to improve with aerobic training via increased left ventricular dimensions and contractility as well as an increased plasma volume (1, 9). These factors are also major determinants of cardiac autonomic function (1, 5). Secondly, while exercise training increases CRF, the adaptations of CRF i.e. central hemodynamics and functional properties of the myocardium to exercise are individual and may even be absent (3, 30). Training-induced improvement in CRF has been suggested to be positively associated with pre-training cardiac vagal activity (15). Therefore, it can be speculated that among those with high CRF, high cardiac vagal activity may have contributed to the CRF response to PA. Whether this explanation is true cannot be determined in the present cross-sectional study.

Paragraph 21 – It has been suggested that up to 50% of CRF is explained by genetic factors (37). Nonetheless, physical exercise remains the most potentially modifiable means of

249 improving CRF, body composition and cardiometabolic risk factors (19). In the present study,
250 objectively measured MVPA was independently associated with cardiac autonomic function,
251 particularly with HRR. This suggests that the prevailing PA contributes to cardiac autonomic
252 function regardless of CRF. It is noteworthy that PA was measured continuously over a period
253 of about 2 weeks, and therefore it can be considered as representative of the overall current PA
254 level. It can also be speculated that PA affects autonomic function via mechanisms not shared
255 with CRF. Our findings on the associations between MVPA and HRR are supported by
256 Buchheit et al. who reported a stronger association between training load and HRR than CRF
257 and HRR (4). Methodologically, it is also possible that the measurement error of CRF leaves
258 room for the association between PA and autonomic function. For example, if a subject has
259 high true maximal HR, he/she potentially has a high absolute HR during submaximal step test,
260 CRF may be underestimated despite high PA. It has been shown that inclusion of PA into the
261 regression model for maximal oxygen uptake significantly improves the accuracy of the CRF
262 estimation by the peak HR during the submaximal stepping test (33).

263 ***Paragraph 22 –*** The present study showed that Fat% was significantly associated with
264 cardiac autonomic function independently of CRF and MVPA. However, these associations
265 disappeared after further adjustments for other lifestyle and cardiometabolic risk factors. This
266 may not nullify the contribution of Fat% to autonomic function but rather emphasizes that there
267 are potent mediators, such as glycemic and lipid profile and BP accompanying obesity (20),
268 that also underlie this relationship. Fat% had a consistently stronger association with these
269 cardiometabolic risk markers than either CRF or MVPA among both men and women in the
270 present study (data not shown). Our findings support the previous reports stating that CRF and
271 PA seem to provide important prognostic information than can be ascertained from overweight
272 and obesity (2, 28) – obesity is not related with increased cardiometabolic risk in the presence
273 of good CRF or PA. In this study, CRF and PA were more strongly associated with cardiac

autonomic function than Fat%. It may be that body fatness alone is not as detrimental as either low CRF or physical inactivity for cardiac autonomic function, which is known to be a significant risk factor for cardiovascular morbidities and mortality in population-based samples (7, 23, 38).

Paragraph 23 – In this study, a significant interaction with sex was observed in the associations of CRF and Fat%, but not MVPA, with cardiac autonomic function (see Figure, Supplementary Digital Content 4, Sex-interactions in the associations of autonomic function to CRF, MVPA and Fat%). The associations of these factors with autonomic function were linear but stronger in men than women. While the sex-differences in autonomic function have been well-documented (16, 22), the between-tertile differences were greater in men than women which was reflected also in the correlations coefficients (see Table, Supplementary Digital Content 1, Correlations between autonomic function, CRF, MVPA and Fat%). The reason why men seem to benefit more than women from greater CRF and lower Fat% in terms of autonomic regulation remains unknown. For instance, Gutin et al. reported more deleterious effects of adiposity on autonomic function in adolescent women than men (13). It remains unclear why this association seems to reverse opposite at mid-life. The previous findings by Rennie et al. show that sex-differences impact the relationship between PA and autonomic function but this was not confirmed in the present study (31). Differences in PA assessments (questionnaire vs. accelerometer) may partly explain these contrasting findings.

Study limitations

Paragraph 24 – The HRV analysis is considered less reproducible from short-term laboratory measurements than longer-term ambulatory recordings (8). For example, the time elapsing since the previous meal may affect the quantification of autonomic function, which was relatively short but controlled and optimized, taking into account the other competing

objectives of the NFBC1966-study. Spontaneous breathing may confound the spectral analysis of BRS, whereas rMMSD is a more robust measure of cardiac vagal activity regardless of the breathing pattern (29). The objective PA measurements were based on wrist-worn accelerometry with known limitations regarding PA without arm movement and arm movement without significant PA (26). Yet, the ability present PA method to identify the fulfillment of daily PA recommendation is comparable to hip-worn devices (26). Also, it remains unclear, how well does the current PA level represent longer term PA which may have contributed more to the current CRF. This may be one factor explaining the stronger association of autonomic function to CRF than to PA. The CRF was estimated by the submaximal step test HR, which includes bias caused by individual differences in maximal HR (34) and does not fully concur with the direct measurement of maximal oxygen uptake (33). In addition, HR in the step test *per se* reflects cardiac autonomic function during submaximal exercise; this may partly explain the strong association between autonomic measures at rest and estimated CRF (40). Furthermore, we cannot establish the causality in the present observations due to the study's cross-sectional design. More detailed information about diet and clinical status, especially concerning disorders other than those used for exclusions, would have strengthened the interpretation of the findings. Finally, the population does not fully represent the whole NFBC1966 due incomplete attendance to the measurements at age of 46 yrs and the exclusions of individuals with cardiorespiratory and metabolic diseases and medications affecting autonomic function.

Conclusion

Paragraph 25 – Cardiorespiratory fitness was a stronger determinant of cardiac autonomic function than moderate-to-vigorous physical activity and body fat proportion. Nonetheless, moderate-to-vigorous physical activity, but not body fat proportion was independently

associated with cardiac autonomic function in men and women. The present results suggest that primary prevention of abnormalities in autonomic function and related cardiometabolic risk should focus on improving cardiorespiratory fitness.

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CONFLICT OF INTEREST

None. The results of the present study do not constitute endorsement by ACSM.

FIGURE LEGENDS

Figure 1. The selection of the study population from the Northern Finland Birth Cohort 1966. Antihypertensive medication included β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, diuretics and calcium channel blockers. *HRV* heart rate variability, *CRF* cardiorespiratory fitness, *HRR* heart rate recovery, *Fat%* body fat proportion, *PA* physical activity and *BRS* baroreflex sensitivity measurement successfully performed.

Figure 2. Correlations of cardiorespiratory fitness (CRF, a-e) as evaluated by peak heart rate during the step test (HR_{STEP}), daily amount of moderate-to-vigorous physical activity (MVPA, f-j) and body fat percentage (Fat%, k-o) to cardiac autonomic function in men. *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R interval, *BRS* baroreflex sensitivity, *HRR* heart rate recovery. Percentiles of HR_{STEP}, MVPA and Fat% and natural logarithm of BRS and rMSSD were used in Pearson correlation analyses.

Figure 3. Correlations of cardiorespiratory fitness (CRF, a-e) as evaluated by peak heart rate during the step test (HR_{STEP}), daily amount of moderate-to-vigorous physical activity (MVPA, f-j) and body fat percentage (Fat%, k-o) to cardiac autonomic function in women. *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R interval, *BRS* baroreflex sensitivity, *HRR* heart rate recovery. Percentiles of HR_{STEP}, MVPA and Fat% and natural logarithm of BRS and rMSSD were used in Pearson correlation analyses.

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476

477 **Supplemental Digital Contents**

478 Supplement1.pdf

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Table 1. Characteristics of the study population.

		Men	Women
		n=1383	n=1761
Lifestyle			
Smoking status, n	Non-smoker	589 (43%)	905 (51%)†
	Ex-smoker	410 (30%)	423 (24%)
	Current smoker	384 (28%)	433 (25%)
Alcohol consumption, $g \cdot d^{-1}$		8 (2-19)	3 (1-8)†
Tiredness in the morning, n	Very tired	30 (2%)	60 (3%)*
	Somewhat tired	363 (26%)	473 (27%)
	Somewhat rested	768 (56%)	889 (51%)
	Well rested	222 (16%)	339 (19%)
Sitting time on weekdays, self-report, h/day		8.0 (3.2)	7.3 (3.2)†
MVPA, mean min/day		76 (56-99)	60 (43-80)
VPA, mean min/day		28 (18-42)	31 (20-45)
Clinical and laboratory measurements			
Systolic blood pressure, mmHg		127 (13)	117 (15)†
Diastolic blood pressure, mmHg		84 (10)	80 (10)†
Body mass index, $kg \cdot m^{-2}$		26.5 (3.6)	25.3 (4.1)†
Waist-hip-ratio		0.97 (0.06)	0.86 (0.05)†
Body fat, %		22.0 (6.5)	31.3 (7.7)†
HbA1c, %		5.5 (0.3)	5.4 (0.3)†
Plasma glucose, $mmol \cdot L^{-1}$		5.6 (0.5)	5.2 (0.4)†
Total cholesterol, $mmol \cdot L^{-1}$		5.6 (1.0)	5.1 (0.8)†
LDL cholesterol, $mmol \cdot L^{-1}$		3.8 (0.9)	3.2 (0.8)†

HDL cholesterol, $mmol \cdot L^{-1}$	1.4 (0.3)	1.7 (0.4)†
Triglycerides, $mmol \cdot L^{-1}$	1.2 (0.9-1.7)	0.9 (0.7-1.1)†
Cardiovascular autonomic function at rest		
HR_{REST}, bpm	71 (12)	71 (10)
rMSSD, ms	21 (14-31)	25 (18-36)†
BRS, $ms \cdot mmHg^{-1}$	7.1 (5.1-9.9)	6.6 (4.6-8.7)†
Cardiorespiratory fitness by step test		
HR_{STEP}, bpm	145 (15)	149 (15)†
Incomplete test due to fatigue, n	21 (2%)	52 (3%)*
Duration, s	146 (39)	135 (34)
Heart rate recovery after exercise test		
HRR_{60s}, bpm	39 (11)	44 (11)†
HRR_{60s}, %	28 (9)	30 (8)†
HRR_{SLOPE}, $bpm \cdot s^{-1}$	0.96 (0.32)	1.12 (0.34)†
HRR_{SLOPE}, $\% \cdot s^{-1}$	0.68 (0.26)	0.76 (0.27)†

The values are absolute or relative (%) number of cases, means (SD) or median (1^{st}-3^{rd} quartile) and p value for sex-difference. *MVPA* moderate-to-vigorous physical activity, *VPA* vigorous physical activity, *HbA1c* glycated hemoglobin, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R intervals, *BRS* baroreflex sensitivity, * $p<0.01$ and † $p<0.001$ compared to men.

Table 2. Multivariate analysis of cardiorespiratory fitness (CRF) by peak heart rate during submaximal step test (HR_{STEP}), moderate-to-vigorous physical activity (MVPA) and relative amount of body fat (Fat%) as determinants of autonomic function in men.

		HR_{REST}	rMSSD, ln	BRS, ln	HRR_{60s}	HRR_{60s}, %	HRR_{SLOPE}	$HRR_{SLOPE\%}$
Covariates	R^2	0.22	0.18	0.19	0.17	0.23	0.19	0.23
Smoking	β	0.05*	-0.06*	-0.02	-0.10‡	-0.08†	-0.08†	-0.07†
Alcohol consumption	β	0.05	-0.07†	-0.01	-0.04	-0.05	-0.06*	-0.07†
Tiredness in the morning	β	0.06†	-0.06*	0.00	-0.04	-0.05*	-0.06*	-0.07†
Sitting time on week days	β	-0.03	0.04	-0.01	-0.03	-0.05	-0.06*	-0.07†
Systolic blood pressure	β	-0.26‡	0.19‡	0.09	0.23‡	0.26‡	0.26‡	0.27‡
Diastolic blood pressure	β	0.52‡	-0.42‡	-0.42‡	-0.42‡	-0.46‡	-0.40‡	0.43‡
HbA1c	β	-0.01	0.02	-0.07	0.01	0.01	0.00	0.00
Plasma glucose	β	0.10‡	-0.07*	-0.09*	-0.07†	-0.10‡	-0.11‡	-0.13‡
Total cholesterol	β	0.08†	-0.09†	-0.07	-0.12‡	-0.13‡	-0.14‡	-0.14‡
HDL cholesterol	β	-0.06*	0.05	0.10*	0.13‡	0.13‡	0.13‡	0.14‡
Triglycerides	β	0.10†	-0.12‡	-0.05	-0.06	-0.07*	-0.06*	-0.07*
CRF (HR_{STEP}) + Covariates	R^2	0.41	0.23	0.21	0.20	0.42	0.24	0.42

	β (CRF)	-0.48‡	0.24‡	0.17‡	0.18‡	0.48‡	0.24‡	0.48‡
MVPA + Covariates	R^2	0.22	0.18	0.19	0.19	0.26	0.21	0.26
	β (MVPA)	-0.09‡	0.00	0.05	0.15‡	0.20‡	0.15‡	0.19‡
Fat% + Covariates	R^2	0.23	0.19	0.20	0.18	0.26	0.21	0.27
	β (Fat%)	0.13‡	-0.12‡	-0.14‡	-0.13‡	-0.21‡	-0.18‡	-0.24‡
CRF, MVPA & Fat% (Initial block)	R^2	0.35	0.15	0.13	0.15	0.38	0.20	0.39
	β (CRF)	-0.56‡	0.31‡	0.22‡	0.19‡	0.49‡	0.25‡	0.48‡
	β (MVPA)	0.01	-0.05*	-0.10	0.13‡	0.11‡	0.12‡	0.10‡
	β (Fat%)	0.04†	-0.16‡	-0.22‡	-0.18‡	-0.14‡	-0.21‡	-0.17‡
CRF, MVPA & Fat% + Covariates	R^2	0.41	0.23	0.21	0.21	0.42	0.25	0.43
	β (CRF)	**-0.50‡**	**0.24‡**	**0.14‡**	**0.14‡**	**0.44‡**	**0.19‡**	**0.44‡**
	β (MVPA)	0.02	**-0.06***	0.01	**0.11‡**	**0.09‡**	**0.10‡**	**0.08‡**
	β (Fat%)	-0.04	-0.05	**-0.09***	-0.06	-0.04	**-0.10†**	**-0.08†**

The values are R^2 and standardized coefficients β (per percentile). *HbA1c* glycated hemoglobin, *HDL* high-density lipoprotein, *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R intervals, *BRS* baroreflex sensitivity, *HRR* heart rate recovery. *$p<0.05$, †$p<0.01$ and ‡ $p<0.001$.

Table 3. Multivariate analysis of cardiorespiratory fitness (CRF) by peak heart rate during submaximal stepping-test (HR_{STEP}), moderate-to-vigorous physical activity (MVPA) and relative amount of body fat (Fat%) as determinants of autonomic function in women.

		HR_{REST}	rMSSD, ln	BRS, ln	HRR_{60s}	HRR_{60s}, %	HRR_{SLOPE}	$HRR_{SLOPE\%}$
Covariates	R^2	0.12	0.08	0.14	0.08	0.13	0.11	0.15
Smoking	β	-0.03	-0.02	-0.01	-0.03	-0.01	-0.02	-0.01
Alcohol consumption	β	0.04	-0.01	0.05	-0.01	-0.01	-0.03	-0.02
Tiredness in the morning	β	0.04	-0.05*	-0.03	-0.08†	-0.07†	-0.05*	-0.05*
Sitting time on week days	β	-0.05*	0.04	-0.09†	0.06*	0.04	0.00	-0.01
Systolic blood pressure	β	-0.29‡	0.20‡	0.01	0.11*	0.17‡	0.16†	0.20‡
Diastolic blood pressure	β	0.48‡	-0.37‡	-0.34‡	-0.21‡	-0.33‡	-0.25‡	-0.34‡
HbA1c	β	-0.01	0.00	-0.04	-0.06*	-0.05*	-0.06*	-0.04*
Plasma glucose	β	0.03	0.00	-0.05	-0.05*	-0.09‡	-0.09‡	-0.12‡
Total cholesterol	β	0.00	0.01	-0.01	-0.03	-0.04	-0.05	-0.05*
HDL cholesterol	β	-0.01	-0.04	-0.02	0.06*	0.08†	0.08†	0.10‡
Triglycerides	β	0.13‡	-0.13‡	-0.06	-0.14‡	-0.15‡	-0.14‡	-0.14‡
CRF (HR_{STEP}) + Covariates	R^2	0.29	0.10	0.15	0.10	0.34	0.15	0.34

	β (CRF)	-0.45‡	0.18‡	0.11†	0.15‡	0.49‡	0.22‡	0.48‡
MVPA + Covariates	R^2	0.13	0.08	0.14	0.11	0.18	0.13	0.19
	β (MVPA)	-0.10‡	0.02	0.05	0.18‡	0.22‡	0.18‡	0.21‡
Fat% + Covariates	R^2	0.12	0.08	0.14	0.09	0.17	0.12	0.19
	β (Fat%)	0.08†	-0.06*	-0.09*	-0.06*	-0.22‡	-0.15‡	-0.26‡
CRF, MVPA & Fat% (Initial block)	R^2	0.26	0.06	0.07	0.08	0.32	0.13	0.33
	β (CRF)	-0.53‡	0.23‡	0.15‡	0.15‡	0.48‡	0.19‡	0.45‡
	β (MVPA)	-0.03	-0.01	0.03	0.17‡	0.14‡	0.15‡	0.12‡
	β (Fat%)	-0.07†	-0.04	-0.14‡	-0.07*	-0.06*	-0.14‡	-0.12‡
CRF, MVPA & Fat% + Covariates	R^2	0.30	0.10	0.15	0.12	0.35	0.16	0.35
	β (CRF)	**-0.51‡**	**0.20‡**	**0.09***	**0.13‡**	**0.46‡**	**0.17‡**	**0.44‡**
	β (MVPA)	-0.01	-0.02	0.02	**0.15‡**	**0.13‡**	**0.13‡**	**0.11‡**
	β (Fat%)	**-0.15‡**	0.03	-0.05	0.03	0.02	-0.05	-0.04

The values are R^2 and standardized coefficients β (per percentile). *HbA1c* glycated hemoglobin, *HDL* high-density lipoprotein, *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R intervals, *BRS* baroreflex sensitivity, *HRR* heart rate recovery. *p<0.05, †p<0.01 and ‡ p<0.001.

Figure 1.

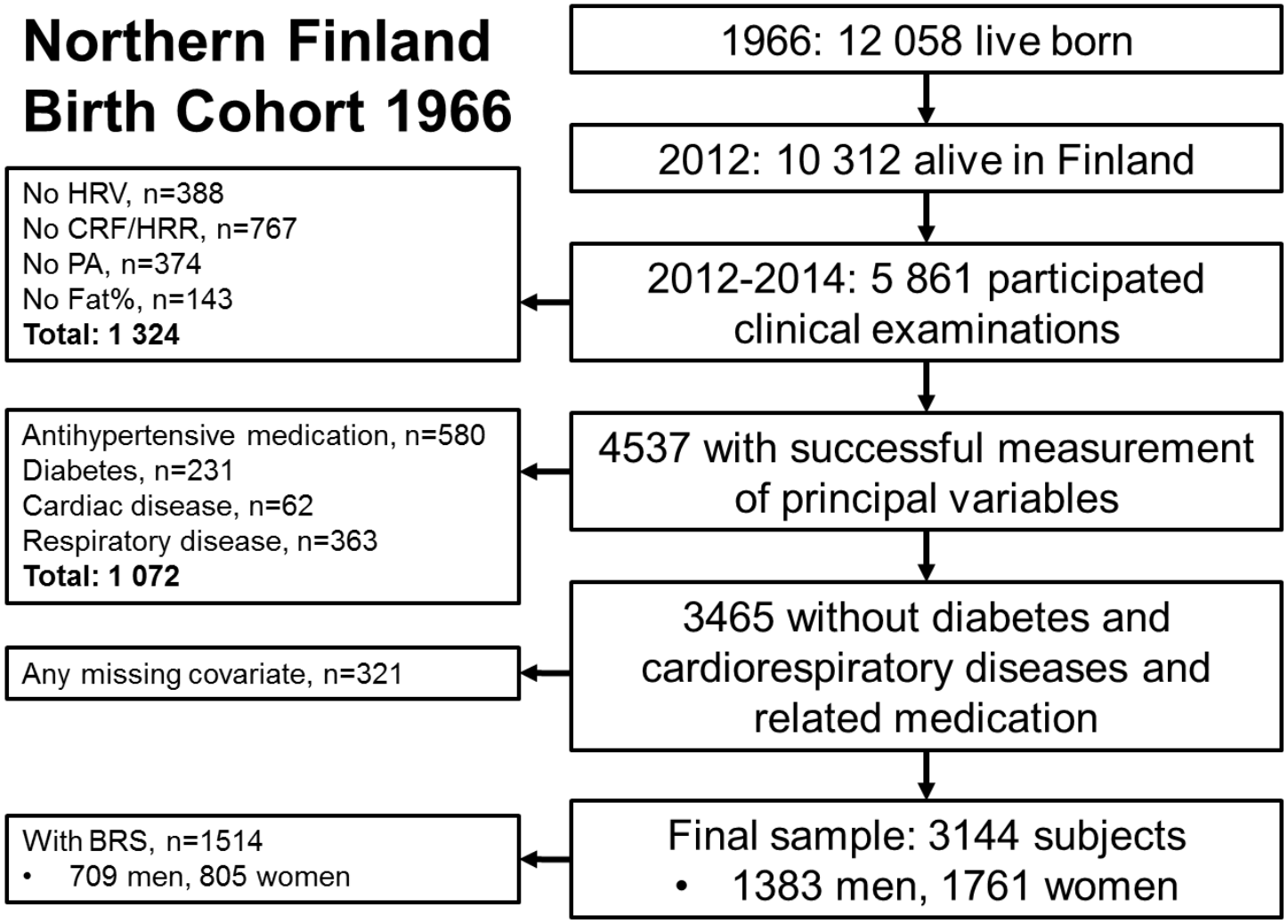

Northern Finland Birth Cohort 1966
1966: 12 058 live born
2012: 10 312 alive in Finland
No HRV, n=388
No CRF/HRR, n=767
No PA, n=374
No Fat%, n=143
Total: 1 324
2012-2014: 5 861 participated clinical examinations
Antihypertensive medication, n=580
Diabetes, n=231
Cardiac disease, n=62
Respiratory disease, n=363
Total: 1 072
4537 with successful measurement of principal variables
Any missing covariate, n=321
3465 without diabetes and cardiorespiratory diseases and related medication
With BRS, n=1514
• 709 men, 805 women
Final sample: 3144 subjects
• 1383 men, 1761 women

Figure 2.

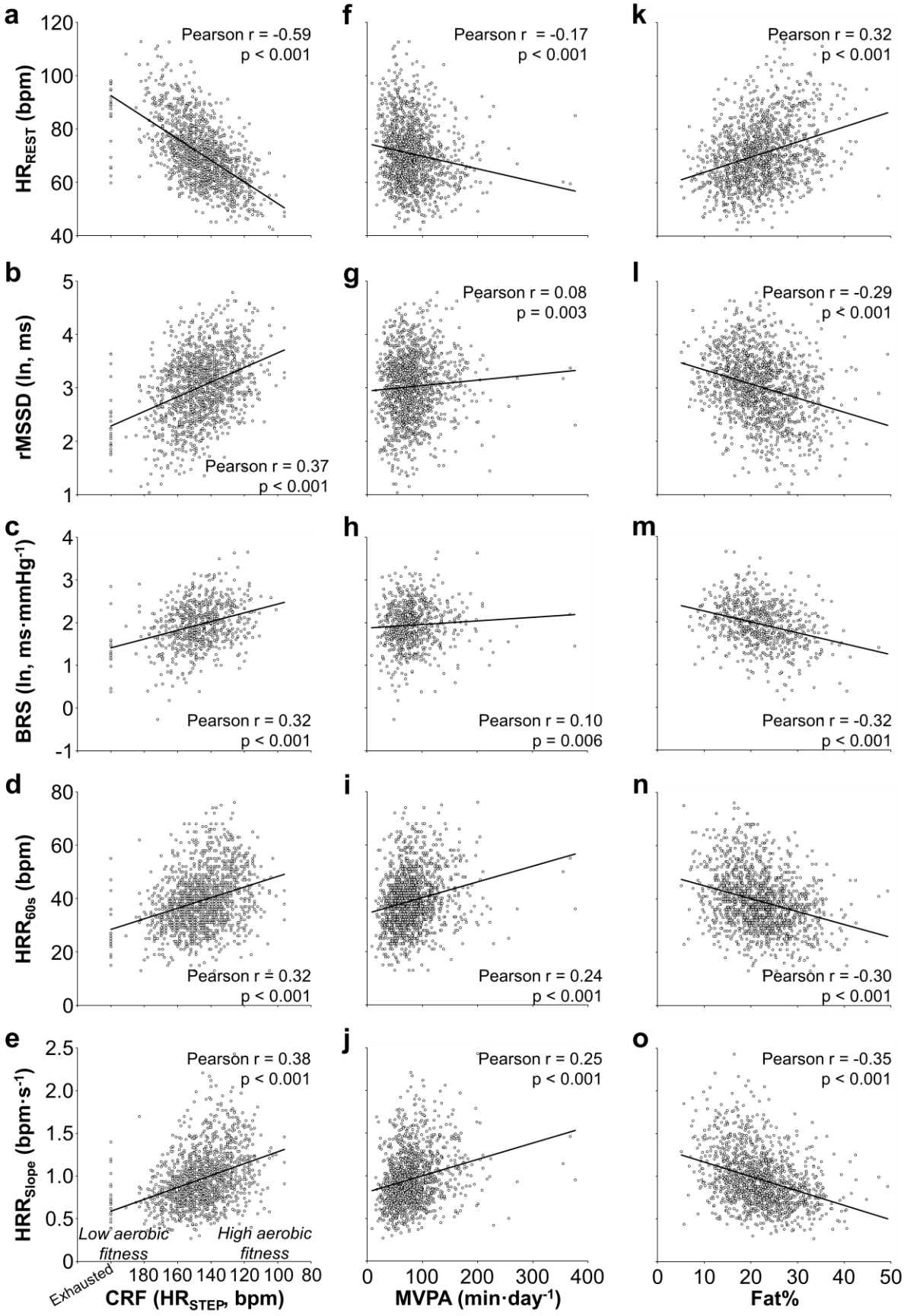
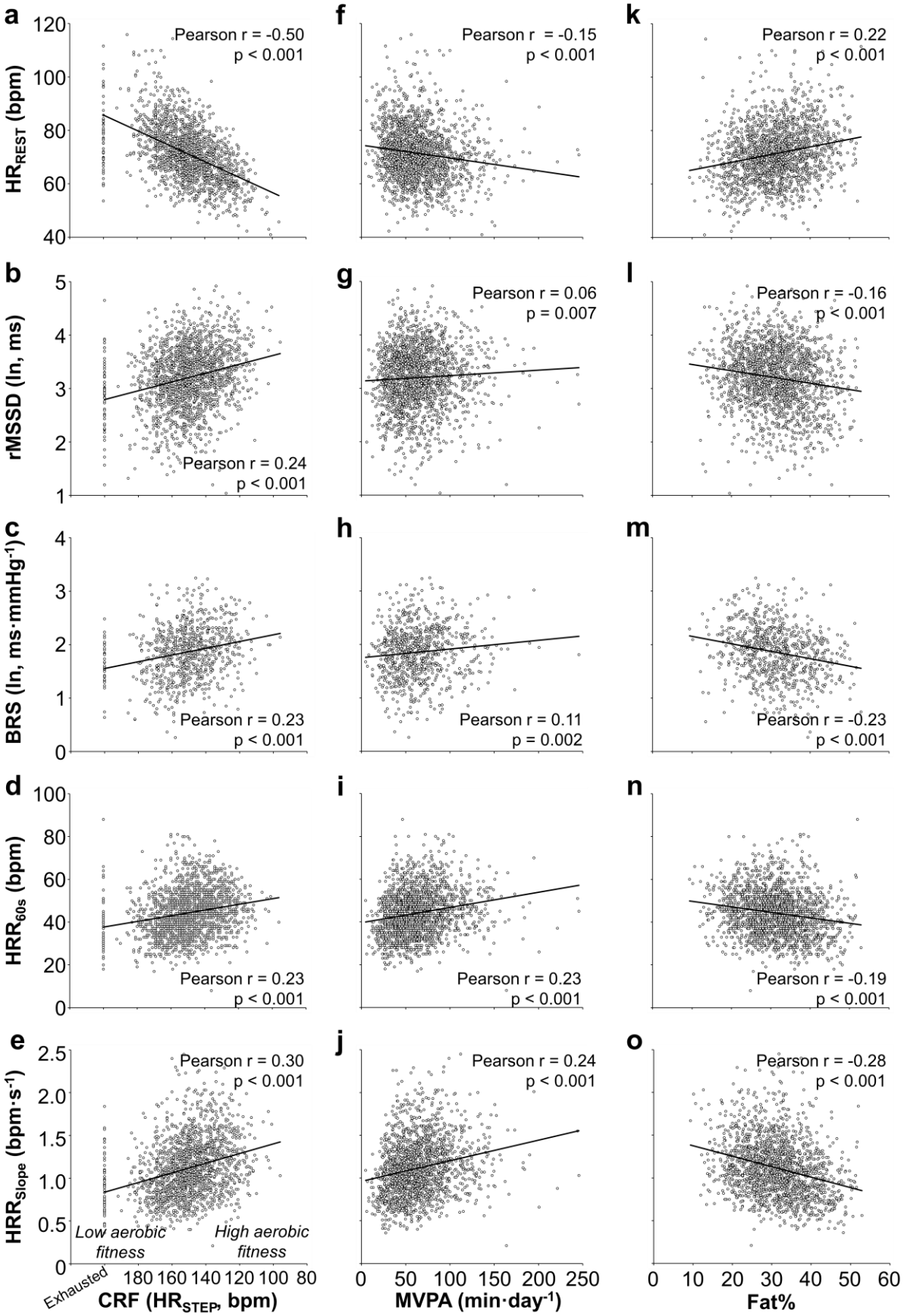

a
HR$_{REST}$ (bpm)
Pearson r = -0.59
p < 0.001
b
rMSSD (ln, ms)
Pearson r = 0.37
p < 0.001
c
BRS (ln, ms·mmHg^{-1})
Pearson r = 0.32
p < 0.001
d
HRR$_{60s}$ (bpm)
Pearson r = 0.32
p < 0.001
e
HRR$_{Slope}$ (bpm·s^{-1})
Pearson r = 0.38
p < 0.001
Low aerobic fitness
High aerobic fitness
Exhausted
CRF (HR$_{STEP}$, bpm)
f
Pearson r = -0.17
p < 0.001
g
Pearson r = 0.08
p = 0.003
h
Pearson r = 0.10
p = 0.006
i
Pearson r = 0.24
p < 0.001
j
Pearson r = 0.25
p < 0.001
MVPA (min·day^{-1})
k
Pearson r = 0.32
p < 0.001
l
Pearson r = -0.29
p < 0.001
m
Pearson r = -0.32
p < 0.001
n
Pearson r = -0.30
p < 0.001
o
Pearson r = -0.35
p < 0.001
Fat%

Figure 3.


a
Pearson r = -0.50
p < 0.001
HR_REST (bpm)
f
Pearson r = -0.15
p < 0.001
k
Pearson r = 0.22
p < 0.001
b
Pearson r = 0.24
p < 0.001
rMSSD (ln, ms)
g
Pearson r = 0.06
p = 0.007
l
Pearson r = -0.16
p < 0.001
c
Pearson r = 0.23
p < 0.001
BRS (ln, ms·mmHg⁻¹)
h
Pearson r = 0.11
p = 0.002
m
Pearson r = -0.23
p < 0.001
d
Pearson r = 0.23
p < 0.001
HRR_60s (bpm)
i
Pearson r = 0.23
p < 0.001
n
Pearson r = -0.19
p < 0.001
e
Pearson r = 0.30
p < 0.001
HRR_Slope (bpm·s⁻¹)
Low aerobic fitness
High aerobic fitness
Exhausted
CRF (HR_STEP, bpm)
j
Pearson r = 0.24
p < 0.001
MVPA (min·day⁻¹)
o
Pearson r = -0.28
p < 0.001
Fat%

Supplementary table 1. Correlations between cardiovascular autonomic function, cardiorespiratory fitness (CRF) by peak heart rate during submaximal stepping-test (HR_{STEP}), moderate-to-vigorous physical activity (MVPA) and relative amount of body fat (Fat%).

	Men (n=1383)			Women (n=1761)		
	CRF (HR_{STEP})	MVPA	Fat%	CRF (HR_{STEP})	MVPA	Fat%
	r	r	r	r	r	r
CRF (HR_{STEP})	-	0.29‡	-0.46‡	-	0.28‡	-0.52‡
MVPA	0.29‡	-	-0.26‡	0.28‡	-	-0.26‡
Fat%	-0.46‡	-0.26‡	-	-0.52‡	-0.26‡	-
HR_{REST}	-0.59‡	-0.17‡	0.32‡	-0.50‡	-0.15‡	0.22‡
rMSSD	0.37‡	0.08‡	-0.29‡	0.24‡	0.06‡	-0.16‡
BRS	0.32‡	0.10‡	-0.32‡	0.23‡	0.11‡	-0.23‡
HRR_{60s}	0.32‡	0.24‡	-0.30‡	0.23‡	0.23‡	-0.19‡
HRR_{60s}, %	0.59‡	0.29‡	-0.40‡	0.55‡	0.29‡	-0.34‡
HRR_{SLOPE}	0.38‡	0.25‡	-0.35‡	0.30‡	0.24‡	-0.28‡
HRR_{SLOPE}, %	0.59‡	0.29‡	-0.42‡	0.55‡	0.28‡	-0.39‡

HR heart rate, *rMSSD* root mean square of the successive differences in R-R intervals, *BRS* baroreflex sensitivity, *HRR* heart rate recovery.

‡$p<0.001$ for Pearson correlation.

Supplementary table 2. Cardiovascular autonomic function according to tertiles of cardiorespiratory fitness (CRF) by peak heart rate during submaximal stepping-test (HR_{STEP}), moderate-to-vigorous physical activity (MVPA) and relative amount of body fat (Fat%) in men.

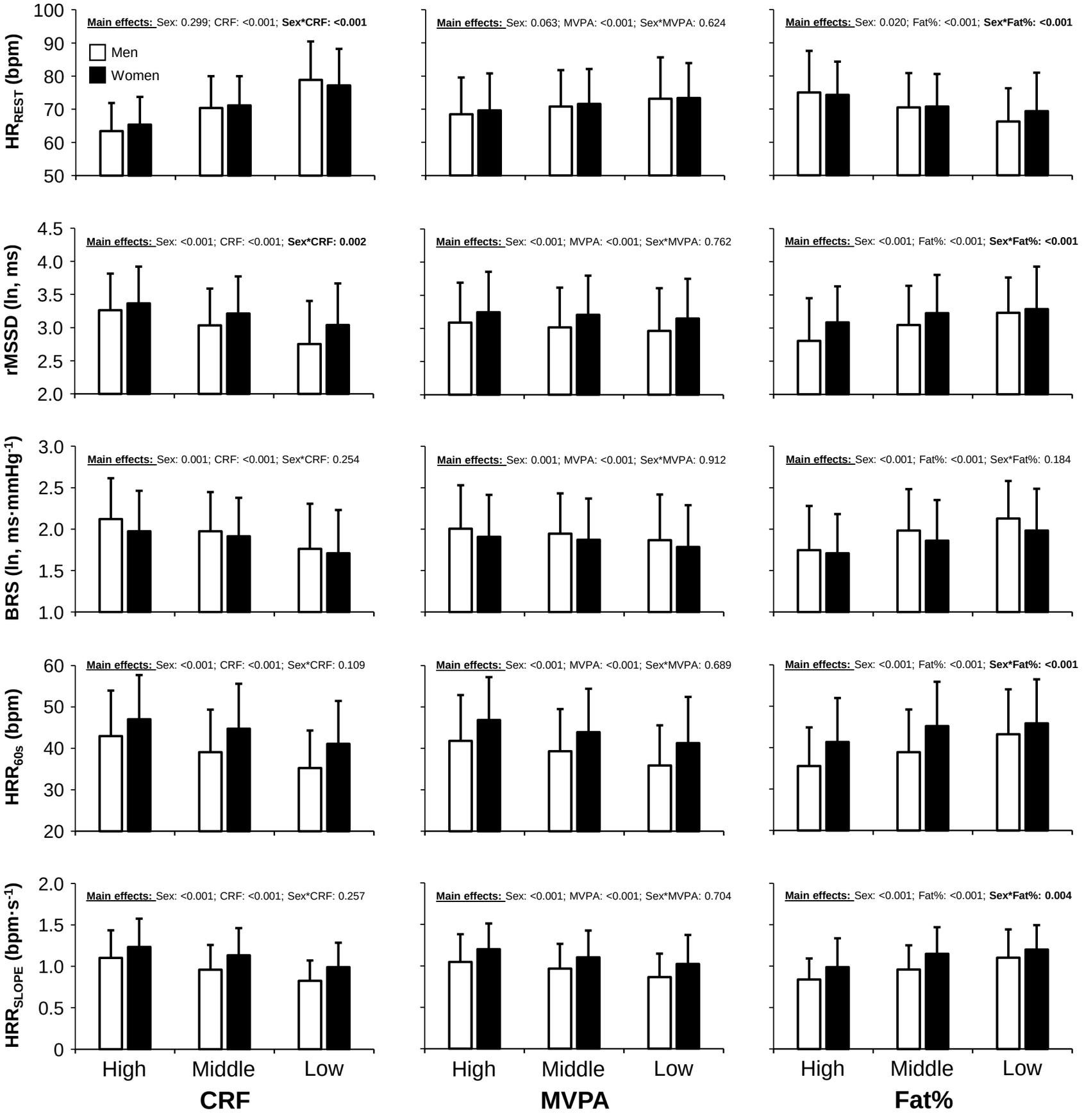
	CRF (HR_{STEP})			MVPA			Fat%		
	High n=446	Middle n=525	Low n=412	High n=482	Middle n=504	Low n=397	High n=474	Middle n=455	Low n=454
HR_{REST}, bpm	63 (8)	70* (10)	79*† (12)	68 (11)	71* (11)	73*† (13)	75 (13)	71* (10)	66*† (10)
rMSSD, ms	25 (18-38)	21 (14-30)	16 (10-26)	22 (15-33)	21 (13-30)	20 (14-20)	17 (11-26)	21 (14-30)	25 (18-37)
ln ms	3.26 (0.55)	3.03* (0.55)	2.75*† (0.65)	3.09 (0.60)	3.01 (0.60)	2.96* (0.64)	2.81 (0.64)	3.04* (0.59)	3.23*† (0.53)
BRS, $ms \cdot mmHg^{-1}$	8.2 (5.9-11.5)	7.2 (5.1-9.8)	5.9 (4.2-8.8)	7.6 (5.-10.5)	7.3 (5.0-9.6)	6.4 (4.9-9.2)	5.7 (4.2-8.5)	7.3 (5.3-9.9)	8.2 (6.1-11.2)
ln $ms \cdot mmHg^{-1}$	2.12 (0.49)	1.97* (0.47)	1.76*† (0.54)	2.00 (0.52)	1.94 (0.49)	1.87* (0.55)	1.75 (0.53)	1.98* (0.50)	2.13*† (0.45)
HRR_{60s}, bpm	43 (11)	39* (10)	35*† (9)	36 (10)	39* (10)	42*† (11)	36 (9)	39* (10)	43*† (11)
HRR_{60s}, %	34 (9)	27* (7)	22*† (6)	30 (9)	27* (8)	24*† (8)	24 (7)	27* (8)	32*† (9)
HRR_{SLOPE}, $bpm \cdot s^{-1}$	1.10 (0.33)	0.96* (0.30)	0.82*† (0.25)	1.05 (0.33)	0.97* (0.30)	0.86*† (0.28)	0.84 (0.25)	0.96* (0.29)	1.10*† (0.34)
HRR_{SLOPE}, $\%\cdot s^{-1}$	0.87 (0.28)	0.66* (0.21)	0.51*† (0.16)	0.76 (0.29)	0.68* (0.24)	0.58*† (0.22)	0.56 (0.19)	0.67* (0.23)	0.82*† (0.29)

The values are means (SD), median (1^{st}-3^{rd} quartile), *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R intervals, *BRS* baroreflex sensitivity, *HRR* heart rate recovery. *$p<0.05$ compared to High, †$p<0.05$ compared to Middle.

Supplementary table 3. Cardiovascular autonomic function according to tertiles of cardiorespiratory fitness (CRF) by peak heart rate during submaximal stepping-test (HR_{STEP}), moderate-to-vigorous physical activity (MVPA) and relative amount of body fat (Fat%) in women.

	CRF (HR_{STEP})			MVPA			Fat%		
	High	Middle	Low	High	Middle	Low	High	Middle	Low
	n=543	n=634	n=584	n=679	n=575	n=507	n=615	n=599	n=547
HR_{REST}, bpm	65	71*	77*†	70	72*	73*†	74	71*	69*†
	(8)	(8)	(10)	(10)	(10)	(10)	(11)	(9)	(10)
rMSSD, ms	30	25	22	26	25	24	23	25	28
	(21-41)	(18-37)	(15-32)	(18-39)	(18-36)	(16-35)	(14-34)	(18-38)	(19-38)
ln ms	3.36	3.21*	3.04*†	3.24	3.21	3.15*	3.08	3.22*	3.29*
	(0.55)	(0.55)	(0.59)	(0.59)	(0.57)	(0.57)	(0.61)	(0.55)	(0.55)
BRS, $ms \cdot mmHg^{-1}$	7.1	6.8	5.8	6.9	6.6	6.1	5.6	6.7	7.3
	(5.3-9.9)	(4-8-9.0)	(3.9-7.9)	(4.7-9.4)	(4.8-8.5)	(4.2-7.9)	(4.0-7.6)	(4.6-9.0)	(5.3-9.8)
ln $ms \cdot mmHg^{-1}$	1.97	1.91	1.71*†	1.91	1.87	1.78*	1.71	1.86*	1.98*†
	(0.48)	(0.45)	(0.50)	(0.49)	(0.51)	(0.47)	(0.47)	(0.48)	(0.48)
HRR_{60s}, bpm	47	45*	41*†	47	44*	41*†	41	45*	46*
	(10)	(11)	(11)	(11)	(10)	(10)	(11)	(10)	(10)
HRR_{60s}, %	36	30*	25*†	33	30*	27*†	26	31*	33*†
	(8)	(7)	(7)	(9)	(8)	(8)	(8)	(8)	(9)
HRR_{SLOPE}, $bpm \cdot s^{-1}$	1.23	1.13*	0.99*†	1.20	1.10*	1.03*†	0.99	1.15*	1.20*†
	(0.34)	(0.33)	(0.31)	(0.35)	(0.33)	(0.31)	(0.31)	(0.32)	(0.35)
HRR_{SLOPE}, $\%\cdot s^{-1}$	0.94	0.76*	0.60*†	0.84	0.75*	0.68*†	0.63	0.78*	0.87*†
	(0.27)	(0.22)	(0.19)	(0.27)	(0.26)	(0.24)	(0.21)	(0.24)	(0.28)

The values are means (SD), median (1^{st}-3^{rd} quartile), *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R intervals, *BRS* baroreflex sensitivity, *HRR* heart rate recovery. *$p<0.05$ compared to High, †$p<0.05$ compared to Middle.



Supplementary figure. Sex-interactions in the associations between autonomic function and the tertiles of cardiorespiratory fitness (CRF), daily amount of moderate-to-vigorous physical activity (MVPA) and body fat percentage (Fat%). *HR* heart rate, *rMSSD* root mean square of the successive differences in R-R interval, *BRS* baroreflex sensitivity, *HRR* heart rate recovery.