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Association between cardiorespiratory fitness and metabolic health in overweight and obese adults

Cardiorespiratory fitness and metabolic health

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BACKGROUND: Cardiorespiratory fitness (CRF) has been inversely associated with insulin resistance and clustering of cardiometabolic risk factors among overweight and obese individuals. However, most previous studies have scaled CRF by body mass (BM) possibly inflating the association between CRF and cardiometabolic health. We investigated the associations of peak oxygen uptake ($\dot{V}O_{2peak}$) and peak power output (W_{peak}) scaled either by BM^{-1}, fat free mass (FFM^{-1}), or by allometric methods with individual cardiometabolic risk factors and clustering of cardiometabolic risk factors in 55 overweight or obese adults with metabolic syndrome.

METHODS: $\dot{V}O_{2peak}$ and W_{peak} were assessed by a maximal cycle ergometer exercise test. FFM was measured by air displacement plethysmograph and glucose, insulin, HbA1c, triglycerides, and total, LDL, and HDL cholesterol from fasting blood samples. HOMA-IR and metabolic syndrome score (MetS) were computed.

RESULTS: $\dot{V}O_{2peak}$ and W_{peak} scaled by BM^{-1} were inversely associated with insulin (β=-0.404 to -0.372, 95% CI=-0.704 to -0.048), HOMA-IR (β=-0.442 to -0.440, 95% CI=-0.762 to -0.117), and MetS (β=-0.474 to -0.463, 95% CI's=-0.798 to -0.127). Other measures of CRF were not associated with cardiometabolic risk factors.

CONCLUSIONS: Our results suggest that using BM^{-1} as a scaling factor confounds the associations between CRF and cardiometabolic risk in overweight/obese adults with the metabolic syndrome.

Key words: aerobic fitness, metabolic syndrome, insulin resistance, allometry

Introduction

Insulin resistance and the metabolic syndrome form a significant burden of disease increasing the risk of type 2 diabetes, cardiovascular disease, dementia, and all-cause mortality[1,2]. Overweight and obesity, especially abdominal obesity, are strong determinants of insulin resistance and the metabolic syndrome[1]. Furthermore, low cardiorespiratory fitness (CRF) has been suggested to be an integral feature of cardiometabolic diseases and their risk factors in children and adults[3,4] while high CRF has been suggested to reduce cardiometabolic risk also in obese individuals[5,6]. However, most evidence of the latter is based on studies utilizing either a direct measure of respiratory gas exchange as maximal oxygen uptake ($\dot{V}O_{2max}$) scaled by total body mass (BM^{-1}) or indirect field tests to estimate endurance capacity.

$\dot{V}O_{2max}$ is considered the reference method for measuring CRF [4]. $\dot{V}O_{2max}$ describes the integrated ability of cardiopulmonary and vascular systems to transport oxygen and working skeletal muscles to use oxygen in energy metabolism. Ideally, to allow interindividual comparison, absolute $\dot{V}O_{2max}$ should be considered by taking into account the physiological skeletal muscle mass of the working muscles[7–9]. Skeletal muscle tissue is mostly responsible for increased oxygen consumption during exercise and physical activity and it also contributes to increased left-ventricular end-diastolic volume, left-ventricular stroke volume and cardiac output, and thereby $\dot{V}O_{2max}$[10,11]. Nevertheless, for practical reasons, lean body mass or fat free mass (FFM) are often used instead of skeletal muscle mass, as these consists mostly of muscle mass.

Traditionally, absolute $\dot{V}O_{2max}$ has been divided by absolute body mass (BM^{-1}). However, scaling of $\dot{V}O_{2max}$ by BM^{-1} has some shortcomings. First, $\dot{V}O_{2max}$ scaled by BM^{-1} often

demonstrates a statistically significant inverse association with BM suggesting the inability of this approach to remove the effect of body size on $\dot{V}O_{2max}$[12,13]. Second, BM includes fat mass that does not contribute to $\dot{V}O_{2max}$ or cardiac output [14]. $\dot{V}O_{2max}$ scaled by BM^{-1} is thereby affected by adiposity and underestimates CRF in overweight and obese people[11,12,15]. This underestimation of CRF in overweight and obese people is mostly due to their larger fat mass rather than limitations in integrated functions of cardiopulmonary, vascular, and skeletal muscle metabolic systems[9,11]. Therefore, because higher fat mass is strongly associated with increased cardiometabolic risk[1], scaling CRF by BM^{-1} may inflate the associations between CRF and cardiometabolic risk factors in adults, especially among those with higher levels of adiposity, as previously shown in children[16,17].

In the present study, we first investigated the associations of $\dot{V}O_{2max}$ scaled either by BM^{-1} or FFM^{-1} with individual cardiometabolic risk factors and clustering of risk factors in overweight or obese adults with the metabolic syndrome. Second, we also studied the effect of log-linear allometric scaling of CRF[13,18] on these associations to provide a comprehensive picture of the role of different scaling approaches on the associations between CRF and cardiometabolic risk factors. Third, we also report the results regarding peak power output (W_{peak}), which is a common measure of CRF even though it also reflects anaerobic capacity and neuromuscular performance[4,19], because direct respiratory gas analyses are not commonly available in clinical practice.

Materials and methods

Study design and population

This cross-sectional study was conducted between April 2017 and August 2019 at Turku PET Centre (Turku, Finland) as part of a larger intervention study[20]. A total of 64 participants were included in the primary study sample at baseline. Of them, 55 participants had maximal cycle ergometer data and 54 participants had valid respiratory gas exchange data. The reasons for premature exercise test termination included knee pain (n=4), hip pain (n=1), dyspnea (n=1), abnormal rise in exercise blood pressure (n=2), and cramp of the calf muscles (n=1). Those who were excluded from the final study sample were likely to be men, taller, heavier, and have more fat mass and higher fasting insulin ($p<0.05$).

All participants gave written informed consent before enrolment in the study, and the study was conducted according to good clinical practice and the Declaration of Helsinki, and approved by the Ethics Committee of the Hospital District of Southwest Finland (TO5/026/17). The study is registered at Clinicaltrials.gov with an identifier NCT03101228.

The participants were recruited from the local community by advertisements in newspapers and bulletin leaflets. The inclusion criteria were age 40-65 years; physically inactive (< 120 min/week of moderate to vigorous physical activity according to self-report); device-measured daily sitting time ≥ 10 h or 60 % of accelerometer wear time; BMI 25-40 kg/m^2; blood pressure < 160/100 mmHg; fasting plasma glucose < 7.0 mmol/l; and fulfilment of the metabolic syndrome criteria according to Alberti et al.[21] including three of the following symptoms: central obesity (waist circumference ≥ 94 cm for men

and ≥ 80 cm for women), triglycerides ≥ 1.7 mmol/l, HDL < 1.0 mmol/l for men and < 1.3 mmol/l for women, systolic blood pressure ≥ 130 and/or diastolic blood pressure ≥ 85 mmHg, or fasting glucose > 5.6 mmol/l. The exclusion criteria were history of a cardiac event; insulin- or medically treated diabetes; abundant use of alcohol; use of narcotics; cigarette smoking or consumption of snuff tobacco; diagnosed depressive or bipolar disorder; previous PET imaging or considerable exposure to radiation; presence of ferromagnetic objects that would make MR imaging contraindicated; and any chronic disease or condition that could create a hazard to the participant safety, endanger the study procedures, or interfere with the interpretation of study results.

Assessment of body size and composition

Total BM, body volume, and body density were measured by air displacement plethysmography and an electronic scale (the Bod Pod system, COSMED, Inc., Concord, CA, USA) after at least four hours of fasting and FFM and body fat percentage (BF%) were calculated using the equation provided by Siri[22]. Body density measured by the Bod Pod has been reported to have a good agreement with body density estimated by the gold standard underwater weighing in overweight and obese adults[23]. BF% assessed by the Bod Pod has been found to have an acceptable short term repeatability as indicated by the coefficient of variation of 1.7 to 4.5%[24]. Body height was measured barefooted with a standard wall-mounted stadiometer. Body mass index (BMI) was calculated using measured weight and height (kg/m^2). Waist circumference was measured midway between the iliac crest and the lowest rib with a flexible measuring tape. Two measurements were performed, or until the same measurement was obtained twice, and all measurements were performed by the same outcome assessor.

Assessment of cardiorespiratory fitness

Peak oxygen uptake ($\dot{V}O_{2peak}$) and W_{peak} were assessed by maximal cycle ergometry test (eBike EL Ergometer + CASE v6.7, GE Medical Systems Information Technologies, Inc. Milwaukee, WI, USA) with direct respiratory gas measurements (Vyntus CPX, CareFusion, Yorba Linda, CA, USA). In this study, we used peak values because we did not perform a confirmation test to investigate whether the participants achieved their true maximal aerobic capacity[25]. $\dot{V}O_{2peak}$ was determined as the average of three consequent highest values in ml/kg/min. Exercise intensity started at 25 W and was increased by 25 W every three minutes until volitional exhaustion, and participants were instructed to maintain a pace of 65 rpm throughout the test. W_{peak} was defined as the highest power output at last completed three minute step + computed workload from the next incomplete step. Perceived exertion on Borg scale and blood pressure were measured every three minutes. The exercise test was considered maximal if the primary and secondary objectives and subjective criteria indicated maximal effort and maximal cardiorespiratory capacity (a plateau of $\dot{V}O_2$ regardless of increasing workload, peak heart rate +/- 10 beat/min of predicted, or respiratory exchange ratio > 1.0), and the research staff supervising the exercise test considered the test maximal.

$\dot{V}O_{2peak}$ was defined as $\dot{V}O_{2peak}$ (mL/kg BM^{-1}/min^{-1}) and $\dot{V}O_{2peak}$ (mL/kg FFM^{-1}/min^{-1}) and W_{peak} as W_{peak}/kg BM^{-1} and W_{peak}/kg FFM^{-1}. Because $\dot{V}O_{2peak}$ expressed mL/kg BM^{-1}/min^{-1}, mL/kg FFM^{-1}/min^{-1} and W_{peak}/kg BM^{-1} and W_{peak}/kg FFM^{-1} had statistically significant ($p<0.05$) inverse associations with their denominators suggesting the inability of ratio scaling to remove the effect of body size on $\dot{V}O_{2peak}$ and W_{peak}, we also utilized log-linear allometric modelling and equation $\ln y = \ln a + b \cdot \ln x$[13,18] to identify appropriate exponent and to create sample specific power function ratios. Using power function ratios, $\dot{V}O_{2max}$

was expressed as mL/kg $BM^{-0.39}/min^{-1}$ and mL/kg $FFM^{-0.70}/min^{-1}$ and W_{peak} as W_{peak}/kg $BM^{-0.18}$ and W_{peak}/kg $FFM^{-0.52}$. None of these power function ratios were statistically significantly associated with their denominators indicating their validity in scaling of CRF.

Assessment of cardiometabolic risk factors

Venous blood samples were drawn after at least 10 hours of fasting and samples were analyzed at the Turku University Hospital Laboratory. Plasma insulin was measured by electrochemiluminescence immunoassay (Cobas 8000 e801, Roche Diagnostics GmbH, Mannheim, Germany) and plasma glucose was determined by enzymatic reference method with hexokinase GLUC3 (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). HbA1c was measured by turbidimetric inhibition immunoassay (Cobas 6000 c501, Roche Diagnostics GmbH, Mannheim, Germany). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index was calculated from insulin and glucose values with a formula: *insulin (mU/ml) x glucose (mmol/l)/22.5*[26]. Plasma triglycerides, total, LDL and HDL cholesterol were measured by enzymatic colorimetric tests (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). Blood pressure was measured by a digital blood pressure monitor (Apteq AE701f, Rossmax International LtD, Taipei, Taiwan) in a seated position after at least 10 minutes of resting. Measure was taken 2–3 times and the mean of repeated measures was used.

We calculated continuous metabolic syndrome score (MetS) using the formula *waist circumference + insulin + glucose + atherogenic index of plasma (triacylglycerol/HDL-cholesterol ratio) + the average of systolic blood pressure and diastolic blood pressure* as

described previously[27]. A higher MetS score indicates a less favorable metabolic risk profile.

Statistical methods

Statistical analyses were performed by SPSS statistical software, version 26.0 (IBM Corp. Armonk, NY, USA). Basic characteristics between men and women were compared using the Student's t-test for normally distributed continuous variables, the Mann-Whitney U-test for continuous variables with skewed distributions, or the χ^2-test for categorical variables. Associations of the measures of CRF with glucose, insulin, HbA1c, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, systolic blood pressure, diastolic blood pressure, HOMA-IR, MetS, and BF%, were investigated using multiple regression analyses adjusted for age and sex. Furthermore, we investigated the associations of BF% with glucose, insulin, HbA1c, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, systolic blood pressure, diastolic blood pressure, HOMA-IR, and MetS using were investigated multiple regression analyses adjusted for age and sex. The data on the associations of CRF and BF% with cardiometabolic risk factors were further adjusted for blood pressure or cholesterol medication use.

Previous studies have reported correlation coefficients varying from 0.27 to 0.49 between CRF and cardiometabolic risk factors[17,28,29]. A total of 105 and 30 observations was needed to observe the correlation of 0.27 and 0.49, respectively, at the power of 0.80 when two tailed statistical significance level was set at $P<0.05$.

Results

Basic characteristics

Women were shorter, lighter, had a smaller waist circumference and higher BF%, more fat mass and less FFM than men (Table 1). Women also had higher total cholesterol and HDL cholesterol, and lower HOMA-IR than men. Furthermore, women had lower absolute $\dot{V}O_{2peak}$ (mL/min^{-1}) and $\dot{V}O_{2peak}$ (mL/kg BM^{-1}/min^{-1}), and they achieved lower W_{peak} than men.

Associations of cardiorespiratory fitness with individual cardiometabolic risk factors

$\dot{V}O_{2peak}$ expressed as mL/kg BM^{-1}/min^{-1} was inversely associated with insulin (R^2_{adj}=0.072, Table 2) and BF% (β:-0.553, 95% CI: 0.766 to -0.340, p<0.001, R^2_{adj}=0.186) after adjustment for age and sex. Higher $\dot{V}O_{2peak}$ expressed as mL/kg BM$^{-0.39}$/min^{-1} was associated with lower BF% (β:-0.416, 95% CI:0.732 to -0.100, p=0.011, R^2_{adj}=0.058). Similarly, W_{peak}/kg BM^{-1} was inversely associated with insulin (R^2_{adj}=0.102, Table 2) and BF% (β:-0.549, 95% CI:0.739 to -0.359, p<0.001, R^2_{adj}=0.210) and higher W_{peak}/BM$^{-0.18}$ was associated with lower BF% (β:-0.366, 95% CI:0.659 to -0.074, p=0.015, R_{2adj}=0.051). Further adjustment for blood pressure and cholesterol medication had no effect on the magnitude of these associations. Other measures of CRF had no statistically significant associations with individual cardiometabolic risk factors.

Associations of cardiorespiratory fitness with HOMA-IR and continuous metabolic syndrome score

Higher $\dot{V}O_{2peak}$ expressed as mL/kg BM^{-1}/min^{-1} was associated with lower HOMA-IR (β:-0.440, 95% CI:-0.762 to -0.117, p=0.009, R_{2adj}=0.106) and lower MetS (β:-0.463, 95% CI:-0.798 to -0.127, p=0.008, R_{2adj}=0.118) after adjustment for age and sex. Higher

W_{peak}/kg BM^{-1} was associated with lower HOMA-IR (β:-0.442, 95% CI:-0.733 to -0.150, p=0.004, R_{2adj}=0.128) and lower MetS (β:-0.474, 95% CI:-0.769 to -0.163, p=0.003, R_{2adj}=0.144) after adjustment for age and sex. Further adjustment for blood pressure and cholesterol medication had no effect on the magnitude of these associations. Other measures of CRF had no statistically significant associations with HOMA-IR and MetS (data not shown).

Associations of body fat percentage with individual cardiometabolic risk factors, HOMA-IR, and continuous metabolic syndrome score

BF% was positively associated with insulin (β:0.706, 95% CI:0.403 to 1.010, p<0.001, R_{2adj}=0.264), systolic blood pressure (β:0.411, 95% CI:0.058 to 0.765, p=0.023, R_{2adj}=0.075), diastolic blood pressure (β:0.558, 95% CI:0.204 to 0.912, p=0.003, R_{2adj}=0.152), HOMA-IR (β:0.777, 95% CI:0.489 to 1.066, p<0.001, R_{2adj}=0.326) and MetS (β:0.831, 95% CI:0.535 to 1.127, p<0.001, R_{2adj}=0.375). Further adjustment for blood pressure and cholesterol medication had no effect on the magnitude of these associations.

Discussion

We found that $\dot{V}O_{2peak}$ scaled by FFM^{-1}, $FFM^{-0.70}$, and $BM^{-0.18}$ were not associated with cardiometabolic risk factors in overweight or obese adults with the metabolic syndrome. However, overweight or obese adults with higher $\dot{V}O_{2peak}$ scaled by BM^{-1} had lower insulin resistance and MetS than those with lower $\dot{V}O_{2peak}$. Similarly, only W_{max} scaled by BM^{-1} was inversely associated with cardiometabolic risk factors. Furthermore, BF% had strong positive associations with blood pressure, insulin resistance, and MetS. Therefore,

our results suggest that CRF is not strongly associated with cardiometabolic risk once body size and composition are partitioned out from the measure of CRF using FFM.

Our results on the inverse association between $\dot{V}O_{2peak}$ scaled by BM^{-1} and cardiometabolic risk are in line with available evidence suggesting a beneficial role of CRF in cardiometabolic health among adults[4,30–32]. However, when we partitioned out the influence of body size and body composition on CRF using FFM and allometry, CRF had very weak and statistically non-significant associations with cardiometabolic risk factors in overweight and obese adults with the metabolic syndrome suggesting that increased body adiposity is more important determinant of cardiometabolic risk than CRF. These present findings are similar to previous observations in children showing that CRF scaled by BM^{-1} is strongly and inversely associated with insulin resistance and MetS, but the associations attenuate remarkably when other scaling approaches are applied[16,17,33].

Increased body adiposity is a strong determinant of insulin resistance, the metabolic syndrome, and type 2 diabetes[34–36]. Increased adiposity may increase insulin resistance by impairing downstream insulin signaling in the skeletal muscle and increasing systemic inflammation and free fatty acids in plasma, and negatively affecting adiponectin secretion [35,36]. Therefore, the inverse association of $\dot{V}O_{2peak}$ scaled by BM^{-1} with insulin resistance observed in our study may share the same mechanisms than increased adiposity because $\dot{V}O_{2peak}$ scaled by BM^{-1} includes body adiposity. $\dot{V}O_{2peak}$ scaled by BM^{-1} may therefore reflect a combination of genetics, body composition, caloric intake, dietary quality, and physical activity in addition to the cardiopulmonary functions. These observations plausibly explain why the associations of CRF with insulin resistance and MetS were also attenuated when the effect of adiposity on CRF was reduced by expressing $\dot{V}O_{2max}$ either

scaled by FFM or allometric approaches. Thereby, because CRF is a representation of the capacity of pulmonary and cardiovascular systems, it is not surprising that the associations of CRF with insulin resistance and MetS were weak when we used methodology reducing the effect of adiposity on CRF[37]. Accordingly, some evidence suggests that CRF, even when scaled by FFM, is more strongly related to cardiovascular diseases and cardiovascular mortality[38–40] than to type 2 diabetes mellitus[37].

The strengths of the present study include valid and reproducible measurement of $\dot{V}O_{2peak}$ using an exercise test until volitional exhaustion with respiratory gas analysis, body composition using air-displacement plethysmography, and cardiometabolic risk factors using standardised measures. Our sample also included inactive overweight or obese adults with the metabolic syndrome and therefore our study provides evidence and understanding on the role of CRF among those at the highest risk of cardiometabolic diseases. However, the sample size was relatively small, and the results may not be generalized to other populations. Because of relatively small sample size, we were only able to observe associations with moderate to high effect sizes. We also had several statistical models and therefore it is possible that some associations were observed by chance. We did not investigate whether an exposure to a long sedentary lifestyle influenced observed associations and further studies are warranted to investigate the role of physical activity level in these associations. Furthermore, in further studies it is important to consider the most crucial physiological factors limiting $\dot{V}O_{2max}$ in health and disease. Although arterial oxygen content also contributes[41,42], it is well established that the most critical factor for $\dot{V}O_{2max}$ is cardiac output[41,43], which was not measured in the present study. Especially healthy skeletal muscle is well capable of extracting oxygen from the arterial blood and its extraction is usually not a limiting factor[44]. It is of

importance also to distribute oxygen supply precisely to working skeletal muscle fibers[45,46] and further studies should focus to investigate this distribution, as especially in diseased and aging states also these peripheral factors can contribute to the limitations in $\dot{V}O_{2max}$ among humans[47]. In addition, we cannot completely rule out the possibility that the medication used by the participants influences the associations between CRF and cardiometabolic risk factors. Our study was cross-sectional, so no causal inferences can be drawn and prospective studies are warranted to assess the value of allometrically scaled CRF with respect to disease outcomes. Moreover, we used only a proxy of whole-body insulin resistance so it is possible that CRF has different associations with whole body insulin resistance determined by gold standard hyperinsulinemic euglycemic clamp method, or in different organs or organ systems. Finally, we investigated the associations of CRF with traditional cardiometabolic biomarkers and therefore more research on the associations between CRF scaled by different approaches and vascular outcomes, such as endothelial functions and sub-clinical atherosclerosis, are needed.

Conclusions

In conclusion, we found that $\dot{V}O_{2max}$ scaled by FFM or allometric modeling had weak if any associations with cardiometabolic risk factors, but that $\dot{V}O_{2max}$ scaled by BM^{-1} was inversely associated with insulin and HOMA-IR. These results suggest that using BM^{-1} as a scaling factor inflate the associations between CRF and cardiometabolic risk in overweight or obese adults with the metabolic syndrome.

REFERENCES

1. Grundy SM. Metabolic syndrome update. Trends Cardiovasc Med 2016;26:364–73.

2. Atti AR, Valente S, Iodice A, Caramella I, Ferrari B, Albert U, et al. Metabolic Syndrome, Mild Cognitive Impairment, and Dementia: A Meta-Analysis of Longitudinal Studies. Am J Geriatr Psychiatry 2019;27:625–37.

3. Raghuveer G, Hartz J, Lubans DR, Takken T, Wiltz JL, Mietus-Snyder M, et al. Cardiorespiratory Fitness in Youth: An Important Marker of Health: A Scientific Statement From the American Heart Association. Circulation 2020;142:101–18.

4. Robert R, Blair SN, Arena R, Church TS, Despres JP, Franklin BA, et al. Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. Circulation 2016;134:653–99.

5. Nyström CD, Henriksson P, Martínez-Vizcaíno V, Medrano M, Cadenas-Sanches C, Arias-Palencia N, et al. Does Cardiorespiratory Fitness Attenuate the Adverse Effects of Severe/Morbid Obesity on Cardiometabolic Risk and Insulin Resistance in Children? A Pooled Analysis. Diabetes Care 2017;40:1580–7.

6. Ortega FB, Cadenas-Sanchez C, Lee D, Ruiz JR, Blair SN, Sui X. Fitness and Fatness as Health Markers through the Lifespan: An Overview of Current Knowledge. Progr Prev Med 2018;3:0013.

7. Loftin M, Sothern M, Abe T, Bonis M. Expression of VO2peak in Children and Youth, with Special Reference to Allometric Scaling. Sports Med 2016;46:1451–60.

8. Lolli L, Batterham AM, Weston KL, Atkinson G. Size Exponents for Scaling Maximal Oxygen Uptake in Over 6500 Humans: A Systematic Review and Meta-Analysis. Sports Med 2017;47:1405–19.

9. Vanderburgh PM, Katch FI. Ratio scaling of VO2max penalizes women with larger percent body fat, not lean body mass. Med Sci Sports Exerc 1996;28:1204–8.

10. Chantler PD, Clements RE, Sharp L, George KP, Tan L-B, Goldspink DF. The influence of body size on measurements of overall cardiac function. Am J Physiol - A - Heart Circ Physiol 2005;289:2059–65.

11. Krachler B, Savonen K, Komulainen P, Hassinen M, Lakka TA, Rauramaa R. Cardiopulmonary fitness is a function of lean mass, not total body weight: The DR's EXTRA study. Eur J Prev Cardiol 2015;22:1171–9.

12. Tanner JM. Fallacy of Per-Weight and Per-Surface Area Standards, and Their Relation to Spurious Correlation. J Appl Physiol 1949;2:1–15.

13. Welsman JR, Armstrong N, Nevill AM, Winter EM, Kirby BJ. Scaling peak VO2 for differences in body size. Med Sci Sports Exerc 1996;28:259–65.

14. Goran M, Fields DA, Hunter GR, Herd SL, Weinsier RL. Total body fat does not influence maximal aerobic capacity. Int J Obes 2000;24:841–8.

15. Savonen K, Krachler B, Hassinen M, Komulainen P, Kiviniemi V, Lakka TA, et al. The current standard measure of cardiorespiratory fitness introduces confounding by body mass: the DR's EXTRA study. Int J Obes 2012;36(8):1135–40.

16. Haapala EA, Wiklund P, Lintu N, Tompuri T, Väistö J, Finni T, et al. Cardiorespiratory Fitness, Physical Activity, and Insulin Resistance in Children. Med Sci Sports Exerc 2020;52:1144–52.

17. Agbaje AO, Haapala EA, Lintu N, Viitasalo A, Barker AR, Takken T, et al. Peak oxygen uptake cut-points to identify children at increased cardiometabolic risk – The PANIC Study. Scand J Med Sci Sports 2019;29:16–24.

18. Tolfrey K, Barker A, Thom JM, Morse CI, Narici MV, Batterham AM. Scaling of maximal oxygen uptake by lower leg muscle volume in boys and men. J Appl Physiol 2006;100:1851–6.

19. Haapala EA, Gao Y, Lintu N, Väistö J, Vanhala A, Tompuri T, et al. Associations between cardiorespiratory fitness, motor competence, and adiposity in children. Transl Sports Med 2021;4:56–64.

20. Sjöros T, Vähä-Ypyä H, Laine S, Garthwaite T, Lahesmaa M, Laurila SM, et al. Both sedentary time and physical activity are associated with cardiometabolic health in overweight adults in a 1 month accelerometer measurement. Sci Rep 2020;10:20578.

21. Alberti KGMM., Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the Metabolic Syndrome. Circulation 2009;120:1640–5.

22. Siri W. Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Henschel A (editors). Techniques for Measuring Body Composition. Washington, DC: National Academy of Sciences - National Research Council; 1961. p. 223–44.

23. Ginde SR, Geliebter A, Rubiano F, Silva AM, Wang J, Heshka S, et al. Air Displacement Plethysmography: Validation in Overweight and Obese Subjects. Obes Res 2005;13:1232–7.

24. Borga M, West J, Bell JD, Harvey NC, Romu T, Heymsfield SB, et al. Advanced body composition assessment: from body mass index to body composition profiling. J Investig Med 2018;66:1–9.

25. Poole DC, Jones AM. Measurement of the maximum oxygen uptake V̇o2max: V̇o2peak is no longer acceptable. J Appl Physiol 2017;122:997–1002.

26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

27. Viitasalo A, Lakka TA, Laaksonen DE, Savonen K, Lakka H-M, Hassinen M, et al. Validation of metabolic syndrome score by confirmatory factor analysis in children and adults and prediction of cardiometabolic outcomes in adults. Diabetologia 2014;57:940–9.

28. Dagan SS, Segev S, Novikov I, Dankner R. Waist circumference vs body mass index in association with cardiorespiratory fitness in healthy men and women: a cross sectional analysis of 403 subjects. Nutr J 2013;12:12.

29. Shalev-Goldman E, Ashlee M, Ross R. Waist circumference and cardiorespiratory fitness are independently associated with glucose tolerance and insulin resistance in obese women. Appl Physiol Nutr Metab 2014;39:358–62.

30. Laukkanen JA, Kunutsor SK, Yates T, Willeit P, Kujala UM, Khan H, et al. Prognostic Relevance of Cardiorespiratory Fitness as Assessed by Submaximal Exercise Testing for All-Cause Mortality: A UK Biobank Prospective Study. Mayo Clin Proc 2020;95:867–78.

31. Kujala UM, Vaara JP, Kainulainen H, Vasankari T, Vaara E, Kyröläinen H. Associations of Aerobic Fitness and Maximal Muscular Strength With Metabolites in Young Men. JAMA Netw Open 2019;2:198265.

32. Ortega FB, Ruiz JR, Labayen I, Lavie CJ, Blair SN. The Fat but Fit paradox: what we know and don't know about it. Br J Sports Med 2018;52:151–3.

33. McMurray RG, Hosick PA, Bugge A. Importance of proper scaling of aerobic power when relating to cardiometabolic risk factors in children. Ann Hum Biol 2011;38:647–54.

34. Swerdlow DI. Mendelian Randomization and Type 2 Diabetes. Cardiovasc Drugs Ther 2016;30:51–7.

35. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. Diabetes Metab Syndr Obes 2014;7:587–91.

36. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840–6.

37. Zaccardi F, O'Donovan G, Webb DR, Yates T, Kurl S, Khunti K, et al. Cardiorespiratory fitness and risk of type 2 diabetes mellitus: A 23-year cohort study and a meta-analysis of prospective studies. Atherosclerosis 2015;243:131–7.

38. Shah RV, Murthy VL, Colangelo LA, Reis J, Venkatesh BA, Sharma R, et al. Association of Fitness in Young Adulthood With Survival and Cardiovascular Risk: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. JAMA Intern Med 2016;176:87–95.

39. Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, et al. Cardiorespiratory Fitness as a Quantitative Predictor of All-Cause Mortality and Cardiovascular Events in Healthy Men and Women: A Meta-analysis. JAMA 2009;301:2024.

40. Imboden MT, Kaminsky LA, Peterman JE, Hutzler HL, Whaley MH, Fleenor BS, et al. Cardiorespiratory Fitness Normalized to Fat-Free Mass and Mortality Risk. Medicine & Science in Sports & Exercise 2020;52:1532–7.

41. Joyner MJ, Casey DP. Regulation of Increased Blood Flow (Hyperemia) to Muscles During Exercise: A Hierarchy of Competing Physiological Needs. Phys Rev 2015;95:549–601.

42. Montero D, Lundby C. Regulation of Red Blood Cell Volume with Exercise Training. Compr Physiol 2018;9:149–64.

43. Levine BD. VO2max: what do we know, and what do we still need to know? J Physiol 2008;586:25–34.

44. Boushel R, Gnaiger E, Calbet JAL, Gonzales-Alonso J, Wright-Paradis C, Sondergaard H, et al. Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. Mitochondrion 2011;11:303–7.

45. Heinonen I, Kalliokoski KK, Hannukainen JC, Duncker DJ, Nuutila P, Knuuti J. Organ-Specific Physiological Responses to Acute Physical Exercise and Long-Term Training in Humans. Physiology 2014;29:421–36.

46. Koga S, Rossiter HB, Heinonen I, Musch TI, Poole DC. Dynamic Heterogeneity of Exercising Muscle Blood Flow and O2 Utilization. Med Sci Sports Exerc 2014;46:860–76.

47. Heinonen I, Koga S, Kalliokoski KK, Musch TI, Poole DC. Heterogeneity of Muscle Blood Flow and Metabolism: Influence of Exercise, Aging and Disease States. Exerc Sport Sci Rev 2015;43:117–24.

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TABLES

Table I. Characteristics of participants

	All (55)	Women (35)	Men (20)	p
Age†	61 (52 to 64)	61 (51 to 64)	61 (53 to 63)	0.958
Height	170.3 (7.9)	166.0 (5.7)	177.8 (5.3)	**<.0.001**
Body mass	90.7 (14.3)	87.5 (13.5)	96.5 (14.3)	**0.023**
Body mass index	31.3 (4.3)	31.7 (4.3)	30.5 (4.4)	0.355
Waist circumference (cm)	109.6 (10.6)	107.5 (9.2)	113.2 (12.4)	0.056
Normal weight (n, %)	2, (3.6)	1, (2.9)	1 (5.0)	0.614
Overweight (body mass index ≥25 <30) (n, %)	22, (40.0)	12, (34.3)	10, (50.0)	
Obese (body mass index ≥30 <35) (n, %)	19, (34.5)	13, (13)	6, (30.0)	
Obese (body mass index ≥35) (n, %)	12, (21.8)	9, (25.7)	3, (15.0)	
Fat mass (kg) †	38.1 (32.9 to 47.8)	39.4 (35.2 to 51.2)	32.7 (27.2 to 46.0)	**0.014**
Body fat percentage †	44.6 (39.2 to 49.3)	47.3 (44.1.6 to 51.5)	36.1 (31.6 to 42.1)	**<0.001**
Fat free mass (kg) †	49.5 (45.4 to 69.6)	47.1 (41.6 to 48.9)	62.3 (56.2 to 68.1)	**<0.001**
Systolic blood pressure (mmHg)	141 (15)	143 (15)	139 (15)	0.324
Diastolic blood pressure (mmHg)	88 (8)	88 (7)	87 (10)	0.638
Plasma glucose (mmol/L)†	5.7 (5.5 to 6.0)	5.7 (5.5 to 6.0)	5.9 (5.5 to 6.3)	0.098
Plasma insulin (mU/L)†	9 (7 to 14)	8 (7 to 12)	13 (7 to 24)	0.075
Plasma triglycerides (mmol/L)†	1.2 (0.8 to 1.7)	1.2 (0.8 to 1.6)	1.2 (1.0 to 1.7)	0.575
Plasma total cholesterol (mmol/L)	4.7 (0.9)	4.9 (1.0)	4.4 (0.7)	**0.038**
Plasma LDL cholesterol (mmol/L)	3.0 (0.8)	3.2 (0.8)	2.8 (0.7)	0.139
Plasma HDL cholesterol (mmol/L)	1.37 (0.34)	1.43 (0.32)	1.25 (0.36)	**0.049**
HbA1c	37 (3)	36 (3)	37 (3)	0.260
Homeostatic model assessment for insulin resistance‡	0.9 (0.6)	0.8 (0.5)	1.2 (0.7)	**0.018**
Metabolic syndrome score	0.0 (1.0)	-0.18 (0.9)	0.30 (1.2)	0.121
Peak oxygen uptake (mL/min^{-1})	2074 (468)	1817 (352)	2512 (272)	**<0.001**
Peak oxygen uptake ($mL/kg\ FFM^{-1}/min^{-1}$)	40.4 (5.6)	40.0 (6.1)	41.2 (4.7)	0.466
Peak oxygen uptake ($mL/kg\ BM^{-1}/min^{-1}$)	23.0 (4.6)	21.1 (3.7)	26.2 (4.1)	**<0.001**
Peak power output (W)	130.7 (30.7)	115.8 (26.1)	156.7 (18.4)	**<0.001**
Peak power output ($W_{max}/kg\ FFM^{-1}$)	2.5 (0.4)	2.5 (0.5)	2.6 (0.4)	0.733
Peak power output ($W_{max}/kg\ BM^{-1}$)	1.5 (0.3)	1.3 (0.3)	1.7 (0.3)	**<0.001**
Peak respiratory exchange ratio	1.12 (0.1)	1.13 (0.1)	1.11 (0.1)	0.321
Peak heart rate	156 (15.6)	157 (14.2)	156 (18.0)	0.775
% of predicted peak heart rate	88.4 (8.3)	88.6 (7.4)	88.1 (9.9)	0.841
Plateau in oxygen uptake (n, %)	23.6 (13)	31.4 (11)	10.0 (2)	0.125
Exercise test duration (min:ss)	16:36 (13:03 to 18:15)	13:41 (12:05 to 16:36)	18:15 (18:00 to 20:10)	**<0.001**
Cholesterol medication (n, %)	13 (23.6)	7 (20.0)	6 (30.0)	0.401
Blood pressure medication (n, %)	29 (52.7)	13 (37.1)	16 (80.0)	**0.002**

Values are means and standard deviations or †medians and interquartile ranges. *P*-values are from independent samples *t* test for variables with normal distributions or Mann-Whitney *U* test for variables with skewed distribution and chi-square test for categorical variables. Bolded values indicate statistically significant associations between women and men (P<0.05). FFM, fat free mass; BM, body mass. ‡ Homeostatic model assessment for insulin resistance was logarithmically transformed.

Table II. Associations of the measures of cardiorespiratory fitness with individual cardiometabolic risk factors

	Glucose (mmol/L)	Insulin (mU/L)	HbA1c	triglycerides (mmol/L)	Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
$\dot{V}O_{2peak}$ (mL/kg $FFM^{-1.0}/min^{-1}$)	0.01 (-0.31 to 0.33)	0.04 (-026 to 0.35)	0.07 (-0.24 to 0.37)	0.08 (-0.24 to 0.40)	0.09 (-0.22 to 0.39)	-0.15 (-0.45 to 0.15)	0.13 (-0.18 to 0.44)	0.22 (-0.08 to 0.52)	0.20 (-0.12 to 0.51)
$\dot{V}O_{2peak}$ (mL/kg $FFM^{-0.70}/min^{-1}$)	-0.00 (-0.37 to 0.36)	0.13 (-0.21 to 0.47)	0.08 (-0.27 to 0.42)	0.14 (-0.21 to 0.50)	0.07 (-0.27 to 0.42)	-0.26 (-0.59 to 0.07)	0.15 (-0.20 to 0.50)	0.22 (-0.12 to 0.56)	0.23 (-0.13 to 0.58)
$\dot{V}O_{2peak}$ (mL/kg BM^{-1}/min^{-1})	-0.06 (-0.42 to 0.30)	**-0.37 (-0.70 to -0.05)***	-0.11 (-0.46 to 0.24)	0.08 (-0.28 to 0.44)	0.12 (-0.22 to 0.47)	-0.10 (-0.44 to 0.24)	0.10 (-0.25 to 0.46)	-0.01 (-0.36 to 0.34)	-0.10 (-0.46 to 0.27)
$\dot{V}O_{2peak}$ (mL/kg $BM^{-0.39}/min^{-1}$)	-0.05 (-0.52 to 0.43)	-0.01 (-0.45 to 0.43)	-0.06 (-0.51 to 0.38)	0.21 (-0.25 to 0.67)	0.08 (-0.36 to 0.53)	-0.35 (-0.77 to 0.08)	0.16 (-0.29 to 0.61)	-0.07 (-0.38 to 0.51)	-0.06 (-0.41 to 0.52)
Wpeak (W/kg FFM^{-1})	-0.04 (-0.35 to 0.27)	-0.06 (-0.35 to 0.23)	0.15 (-0.14 to 0.45)	0.12 (-0.19 to 0.43)	0.09 (-0.20 to 0.39)	-0.18 (-0.46 to 0.11)	0.11 (-0.19 to 0.42)	0.10 (-0.20 to 0.40)	0.18 (-0.12 to 0.49)
Wpeak (W/kg $FFM^{-0.52}$)	-0.06 (-0.42 to 0.31)	0.01 (-0.33 to 0.35)	0.16 (-0.18 to 0.50)	0.20 (-0.16 to 0.55)	0.08 (-0.27 to 0.42)	-0.32 (-0.64 to 0.01)	0.13 (-0.23 to 0.48)	0.10 (-0.25 to 0.44)	0.21 (-0.15 to 0.56)
Wpeak (W/kg BM^{-1})	-0.06 (-0.39 to 0.27)	**-0.40 (-0.70 to -0.11)****	-0.02 (-0.34 to 0.30)	0.08 (-0.25 to 0.42)	0.12 (-0.20 to 0.44)	-0.08 (-0.40 to 0.23)	0.09 (-0.24 to 0.42)	-0.10 (-0.42 to 0.23)	-0.10 (-0.44 to 0.24)
Wpeak (W/kg $BM^{-0.18}$)	-0.09 (-0.52 to 0.34)	-0.01 (-0.42 to 0.39)	0.11 (-0.30 to 0.51)	0.25 (-0.17 to 0.67)	0.07 (0.34 to 0.48)	-0.38 (-0.77 to 0.01)	0.12 (-0.30 to 0.54)	0.03 (-0.38 to 0.44)	0.14 (-0.29 to 0.56)

Data are standardised regression coefficient and their 95% confidence intervals adjusted for age and sex. *p<0.05, **p<0.01, There were 54 participants in the analyses on $\dot{V}O_{2peak}$ and 55 participants in the analyses on Wpeak.