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Associations of cardiorespiratory fitness and adiposity with arterial stiffness and arterial dilatation capacity in response to a bout of exercise in children

Running head: **Fitness, adiposity, and arterial dilatation**

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Abstract

Purpose: To investigate the associations of directly measured peak oxygen uptake ($\dot{V}O_{2\text{peak}}$) and body fat percentage (BF%) with arterial stiffness and arterial dilatation capacity in children.

Methods: Findings are based on 329 children (177 boys, 152 girls) aged 8–11 years. $\dot{V}O_{2\text{peak}}$ was assessed during a maximal cardiopulmonary exercise test on a cycle ergometer and scaled by lean body mass (LM). BF% and LM were measured by bioelectrical impedance. Stiffness index (SI, measure of arterial stiffness) and change in reflection index (ΔRI , measure of arterial dilatation capacity) were assessed by pulse contour analysis. Data were analysed by linear regression models. **Results:** $\dot{V}O_{2\text{peak}}/\text{LM}$ was positively associated with ΔRI in boys adjusted for age and BF% ($\beta=0.169$, $p=0.031$). Further adjustments for systolic blood pressure, heart rate, and the study group had no effect on this association, but additional adjustment for clinical puberty attenuated it ($\beta=0.171$, $p=0.073$). BF% was inversely related to ΔRI in boys adjusted for age and $\dot{V}O_{2\text{peak}}/\text{LM}$ ($\beta=-0.171$, $p=0.029$). $\dot{V}O_{2\text{peak}}$ or BF% was not associated with ΔRI in girls or with SI in either boys or girls. **Conclusion:** Increasing cardiorespiratory fitness and decreasing adiposity may improve arterial health in childhood, especially among boys.

Keywords: aerobic fitness, body composition, paediatrics, endothelial function, maximal exercise test

INTRODUCTION

Arteriosclerosis has its origin in paediatric years and several traditional risk factors, such as increased fasting low-density lipoprotein cholesterol, triglyceride, and insulin concentrations, have been associated with the development of arteriosclerosis throughout the lifespan (10,13,22,53). Increased arterial stiffness and reduced arterial dilatation capacity refer to a poor arterial response to an elevation in pulse pressure, and these are essential features of an arteriosclerotic pathophysiological process (3,53). While several studies have found that cardiorespiratory fitness (CRF) is inversely and adiposity is directly associated with traditional cardiometabolic risk factors (1,7,21), the evidence on the associations of CRF and obesity with arterial stiffness and arterial dilatation capacity in children is limited and controversial (28,48).

CRF has been inversely associated with arterial stiffness in children and adolescents in cross-sectional studies (35,37,48). However, these studies have utilised indirect measures of CRF, such as maximal workload achieved in an exercise test (48) or a 20-metre endurance shuttle run test (35), instead of peak oxygen uptake ($\dot{V}O_{2peak}$), which is considered the gold standard measure of CRF (12). Some evidence suggests an inverse association between $\dot{V}O_{2peak}$ and arterial stiffness in adolescents (18,19) and that improved $\dot{V}O_{2peak}$ from adolescence to adulthood is associated with more compliant arteries at 36 years of age (14). Previous studies on the association between CRF and arterial stiffness have scaled CRF by body mass (BM) (6,37). Such scaling procedure may have partly obscured the role of true CRF in the associations of CRF with arterial stiffness and therefore dividing CRF by lean body mass (LM) or fat-free mass has been recommended (25).

Previous reviews have described a moderate relationship between obesity and increased arterial stiffness in children (10,20). However, most studies on the association between adiposity and arterial stiffness have used body mass index (BMI) or BMI-based weight status as a measure of adiposity instead of a more direct measure of body fat content (10). This relationship may be

influenced by sex and pubertal status through changes in vasoactive hormone concentrations (10). Nonetheless, earlier studies have not accounted for sex and puberty in their analyses (10,39). Moreover, studies on the association between adiposity and arterial dilatation capacity in response to a bout of exercise in children are sparse (48).

We investigated the associations of directly measured $\dot{V}O_{2\text{peak}}$ scaled by LM and body fat content with arterial stiffness and arterial dilatation capacity in response to a bout of exercise in children aged 8–11 years.

METHODS

Study design and study population

The Physical Activity and Nutrition in Children (PANIC) Study is a long-term physical activity and dietary intervention study (ClinicalTrials.gov NCT01803776) in a population sample of primary school children living in the city of Kuopio, Finland. Altogether 736 children 6–9 years of age who had been registered for the first grade in one of the 16 public schools of the city of Kuopio were invited for baseline examinations conducted between 2007 and 2009.

Altogether 512 children (248 girls, 264 boys), who accounted for 70% of those invited, participated in the baseline examinations. The participants did not differ in sex distribution, age, or body mass index standard deviation score (BMI-SDS) from all children who started the first grade in the city of Kuopio in 2007–2009 based on data from the standard school health examinations performed for all Finnish children before the first grade. The present analyses are based on the 2-year follow-up data. We had complete 2-year data on variables needed in the analyses for 329 children (177 boys, 152 girls) 8–11 years of age. Of these children, 99.1% are Caucasian.

The PANIC Study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. A written informed consent was acquired from the parent or caregiver of each child and every child provided assent to participation.

Experimental protocol

Children and their parents or caregivers arrived at our exercise and health laboratory at the Institute of Biomedicine 0800 am or 0915 am after fasting for at least 12 hours. They were pre-informed to abstain from anti-inflammatory drugs, such as ibuprofen, aspirin, and paracetamol, and caffeinated drinks for at least 12 hours, and avoid strenuous physical activity for at least 24 hours before the visit. The visit was rescheduled for children who had suffered from an illness or a condition that could hamper biochemical analyses performed using blood samples, cause a risk during the exercise test, or make it difficult to perform the exercise test. An experienced research nurse assessed body height, mass, and composition, measured blood pressure, and took blood samples. The children were offered a breakfast by the PANIC study and asked to rest to standardise the conditions before the exercise test that was performed about an hour after having the breakfast. The research nurse and a research physician gave the children instructions on how to perform the exercise test. The children were reminded of the exercise test they had performed two years earlier at baseline. They were also allowed to practice cycling with the ergometer, using the paediatric mask, 10 minutes before lying in supine position. The children rested in this position for 15 minutes prior to commencing the exercise test protocol. The research physician assessed arterial indices at least three times during the last five minutes of this rest period. As soon as the exercise test protocol was completed, arterial indices were measured again at least three times during the supine rest of five minutes. The parents or caregivers were allowed to be with their children during the assessments, including the exercise test.

Assessment of arterial stiffness, tone, and dilatation capacity

A research physician assessed stiffness index (SI) and reflection index (RI) by pulse contour analysis (PCA) based on non-invasive finger photoplethysmography using the PulseTrace PCA2[®] device (Micro Medical, Gillingham, Kent, UK) as explained in detail earlier (49,50). Another research physician confirmed and recorded correct digital volume pulse contours using the manufacturer's guide. SI and RI were assessed in a supine position before and after a maximal exercise test in an exercise test laboratory at a stable room temperature (20–22 °C). SI was calculated as the ratio of body height to time between the first (systolic) peak and the second (diastolic) peak of the pulse contour and was expressed in meters per second. A raised SI indicated stiffer or less compliant arteries. RI was estimated as the proportion of the height of the second peak from the height of the first peak and was expressed in percentage. An elevated RI indicated an increased arterial tone. We calculated a change in RI (Δ RI) as the difference between RI before the exercise test and RI after the exercise test. A larger difference in Δ RI indicated a better arterial dilatation capacity (32). We have earlier reported the evaluation of PCA quality and have shown relatively good reliability for these measures earlier (50). Δ RI measured in response to vasoactive agents has been found to have a relatively good agreement with flow-mediated arterial dilatation with high sensitivity and specificity (36).

Assessment of cardiorespiratory fitness

We assessed CRF by a maximal exercise test using an electromagnetically braked Ergoselect 200 K[®] cycle ergometer coupled with a paediatric saddle module (Ergoline, Bitz, Germany). The children were fully familiarized and habituated with the exercise test before commencement. The exercise test protocol included a 2.5-minute anticipatory period with the child sitting on the ergometer, a 3-minute warm-up period with a workload of five watts, a 1-minute steady-state period with a workload of 20 watts, an exercise period with an increase in

the workload of one watt per six seconds until exhaustion, and a 4-minute recovery period with a workload of five watts.

The children were asked to keep the cadence stable within 70–80 revolutions per minute. The children were verbally encouraged to exercise until voluntary exhaustion. As previously described in detail (24), the exercise test was considered maximal if the peak heart rate was at least 185 beats per minute and respiratory exchange ratio was at least 1.0 in addition to a drop in cadence below 65 revolutions per minute despite motivation to continue the test. We did not perform a formal verification of maximal oxygen uptake via the use of a supra-maximal verification test, but previous research has shown that true maximal oxygen uptake is recorded in approximately 90% of cases during an incremental cycle exercise test to exhaustion in children (4). The peak workload was defined as the workload at the end of the exercise test. Heart rate was measured continuously during the last five minutes of the supine rest prior to commencing the exercise test protocol right through to the 5-minute supine post-exercise rest period using a 12-lead electrocardiogram registered by the Cardiosoft® V6.5 Diagnostic System (GE Healthcare Medical Systems, Freiburg, Germany).

Respiratory gas analysis was performed from the beginning of the 2.5-minute anticipatory period sitting on the ergometer before the exercise test to the end of the 4-minute recovery period after the exercise test using the Oxycon Pro® respiratory gas analyser (Jaeger, Hoechberg, Germany) and the Hans-Rudolph® paediatric mask (Shawnee, Kansas, USA). $\dot{V}O_{2peak}$ was measured using the breath-by-breath method and was averaged over consecutive 15-second periods. CRF was expressed as absolute $\dot{V}O_{2peak}$ ($L \cdot min^{-1}$), $\dot{V}O_{2peak}$ scaled by BM ($mL \cdot kg \cdot BM^{-1} \cdot min^{-1}$), and $\dot{V}O_{2peak}$ scaled by LM ($mL \cdot kg \cdot LM^{-1} \cdot min^{-1}$).

Assessment of resting blood pressure

Systolic and diastolic blood pressure (BP) were measured from the right arm using the Heine Gamma[®] G7 aneroid sphygmomanometer (Heine Optotechnik, Herrsching, Germany) to accuracy of 2 mmHg (24). The measurement protocol included a rest of 5 minutes and thereafter 3 measurements in the sitting position at 2-minute intervals. The mean of all 3 values was used as the systolic and diastolic BP.

Assessment of body size and composition

Body height was measured three times, the child standing in the Frankfurt plane without shoes, by a wall-mounted stadiometer to an accuracy of 0.1 cm. The mean of the two nearest values was used in the analyses. Body mass was measured twice, the children having fasted for 12 hours; emptied the bladder, and standing in light underwear, using a weight scale incorporated in the InBody[®] 720 bioelectrical impedance (BIA) device (Biospace, Seoul, South Korea) to an accuracy of 0.1 kg. The mean of the two values was used in the analyses. BMI was calculated as the ratio of mass in kilograms to height in meters squared. BMI-SDS was calculated based on Finnish reference values (41). We defined overweight and obesity based on the age and sex-specific BMI cut-points of the International Task Force criteria (8). We combined overweight and obese children in the analyses because the prevalence of obesity at 2-year follow up was only 3.6%. Waist circumference was measured three times after expiration at mid-distance between the bottom of the rib cage and the top of the iliac crest using a non-stretchable measuring tape to an accuracy of 0.1 cm. The mean of the two nearest values was used in the analyses. Total body fat mass, body fat percentage (BF%) and LM were assessed by BIA using standardized protocols (52). We also assessed BF% and LM by the Lunar[®] dual-energy X-ray absorptiometry (DXA) device (Lunar Prodigy Advance; GE-Medical Systems, Madison, WI, USA) and the enCore 2006 software, Version. 10.51.006 (GE-Medical Systems, Madison, WI, USA). We have shown strong correlations of BF% and LM assessed by BIA with those assessed

by DXA (46). We primarily used BF% and LM assessed by BIA in the analyses because we had more children with BF% and LM measures from BIA than DXA.

Assessment of maturation

A research physician assessed pubertal status using a 5-stage scale described by Tanner (26,27). Boys were defined as having entered clinical puberty if their testicular volume assessed by an orchidometer was ≥ 4 mL (Tanner stage ≥ 2) (26). Girls were defined as having entered clinical puberty if their breast development had started (Tanner stage ≥ 2) (27).

Statistical Analysis

Statistical analyses were performed using the SPSS statistics software, Version 25.0 (IBM Corp, Armonk, NY, USA). Differences in the variables between boys and girls were tested using Student's t-test for normally distributed continuous variables, Mann–Whitney U test for skewed continuous variables, and Chi-square test for dichotomous variables. The associations of measures of CRF and adiposity with SI, RI, and Δ RI were studied using linear regression analyses. Absolute $\dot{V}O_{2peak}$, $\dot{V}O_{2peak}$ scaled by BM, or $\dot{V}O_{2peak}$ scaled by LM, were entered into the linear regression model one by one 1) without adjustments, 2) adjusted for age, 3) adjusted for age and BF%, 4) adjusted for age, BF%, systolic BP, heart rate, and the study group, and 5) adjusted for age, BF%, systolic BP, heart rate, the study group, and clinical puberty. We added the study group as a covariate to control for the possible effect of lifestyle intervention that some participants underwent during the 2-year follow up period (51). We also investigated the associations of waist circumference, BMI-SDS, and BF% with SI, RI, and Δ RI by the linear regression analysis using the same adjustment strategy except that we replaced BF% with $\dot{V}O_{2peak}$ scaled by LM. The associations of $\dot{V}O_{2peak}$ scaled by LM and BF% remained similar when LM and BF% were assessed by DXA instead of BIA, and therefore the associations of $\dot{V}O_{2peak}$ scaled by LM and BF% assessed by DXA are not presented in the results. Differences and associations with p-values less than 0.05 were considered statistically significant.

RESULTS

Characteristics of children

Boys had a higher LM, a lower BF%, a higher waist circumference, and a higher $\dot{V}O_{2peak}$ scaled by BM and LM compared to girls (Table 1). Girls had a higher ΔRI and a higher prevalence of clinical puberty than boys.

Associations of cardiorespiratory fitness with arterial stiffness, tone, and dilatation capacity

$\dot{V}O_{2peak}$ scaled by BM was directly associated with RI in boys and in girls after adjustment for age (Table 2, Model 2). However, this association was no longer statistically significant after further adjustment for BF% (Table 2, Model 3). $\dot{V}O_{2peak}$ scaled by BM and $\dot{V}O_{2peak}$ scaled by LM were directly associated with ΔRI in boys but not in girls after adjustment for age (Table 2, Model 2). Further adjustment for BF% had little or no effect on these associations (Table 2, Model 3). Additional adjustments for systolic BP, heart rate, and the study group had no effect on these associations, either (Table 2, Model 4). The association between $\dot{V}O_{2peak}$ scaled by BM and ΔRI in boys remained statistically significant even after further adjustment for clinical puberty, whereas the association between $\dot{V}O_{2peak}$ scaled by LM and ΔRI became statistically non-significant after this adjustment (Table 2, Model 5). $\dot{V}O_{2peak}$ scaled by BM or LM was not associated with SI in either boys or girls.

Associations of adiposity with arterial stiffness, tone, and dilatation capacity

Table 3, Model 2 shows the inverse associations of waist circumference, BMI-SDS, and BF% with RI in both boys and girls after adjustment for age. These relationships remained statistically significant after further adjustment for $\dot{V}O_{2peak}$ scaled by LM (Table 3, Model 3), systolic BP, heart rate, study group (Table 3, Model 4), and clinical puberty (Table 3, Model 5).

Waist circumference, BMI-SDS, and BF% were inversely associated with Δ RI in boys but not in girls after adjustment for age (Table 3, Model 2). These relationships in boys remained statistically significant after further adjustment for $\dot{V}O_{2\text{peak}}$ scaled by LM (Table 3, Model 3). The associations of waist circumference and BMI-SDS with Δ RI in boys remained statistically significant and that of BF% was close to statistical significance after additional adjustment for systolic BP, heart rate, and the study group (Table 3, Model 4). The associations of waist circumference and BMI-SDS with Δ RI in boys were no longer statistically significant after further adjustment for clinical puberty (Table 3, Model 5). None of the measures of adiposity was associated with SI in either boys or girls.

DISCUSSION

We found that higher CRF and lower body fat content were independently associated with higher arterial dilatation capacity in response to a bout of exercise in boys. However, CRF or body fat content had no association with arterial stiffness in either boys or girls. Body fat content had a strong inverse relationship with arterial tone at rest in both boys and girls.

Our results on the direct association between CRF and arterial dilatation capacity in response to a bout of exercise in boys are in consonance with the findings of a previous study in which an 8-week aerobic exercise training improved endothelium-dependent arterial dilatation among children 10-11 years of age (23). Another study showed that increasing bicycling assessed by a questionnaire was associated with improved arterial distensibility in boys aged 15-16 years (38). However, CRF was not measured, which makes it difficult to compare the results with our observations (38). Poorer CRF has earlier been linked to reduced arterial dilatation capacity in children aged 6-8 years (48). Some evidence also suggests an inverse association between CRF and arterial stiffness in adolescents (19). However, a recent study reported a contrary result that better CRF was associated with higher arterial stiffness in children, indicating that children with good fitness level are at risk of developing arterial stiffness (28). Our findings suggest that CRF

may be an important determinant of arterial health in children, especially arterial dilatation capacity in boys.

Increased CRF may increase arterial dilatation capacity through exercise as supported by the observation of an exercise-induced reduction in late systolic and early diastolic pressure augmentation, which may enhance ventricular ejection and decrease muscular artery tone especially when elastin content is increased and collagen content is reduced in the arterial wall (16,32,33). However, controlling for heart rate and systolic BP in our study had no effect on the direct relationship of CRF with arterial dilatation capacity among boys. One of the explanations for the direct association between CRF and arterial dilatation capacity in boys but not in girls could be that boys had a higher proportion of LM than girls. Increased LM and muscular arterial networks in boys, especially in the lower limb, may enhance increased pulse wave reflection that may improve arterial dilatation during exercise (33).

CRF scaled by BM or LM lacked any association with arterial dilatation capacity in response to a bout of exercise in girls. Our result that girls had better arterial dilatation capacity during exercise than boys contrasts a previous finding that pre-pubertal girls had poorer arterial dilatation capacity than boys (39). Moreover, another study reported an increase in endothelial-dependent flow-mediated arterial dilatation in boys and in girls as they advance in pubertal development (5). Since more girls in our study had already attained puberty, the sex difference in our results may be partly explained by sex hormones, such as oestrogen, and maturation status. Oestrogen has anti-atherogenic effects that could reduce arterial stiffness by inhibiting smooth muscle cell proliferation (2). A significantly larger increase in body fat content during maturation in girls than in boys could also contribute to the sex disparity in the relationship of CRF with arterial dilatation capacity in response to a bout of exercise (28). However, controlling for puberty conferred little or no alteration in the association between CRF and arterial dilatation capacity during exercise. It is therefore possible that explanations for the lack

of association between CRF and arterial dilatation capacity in response to a bout of exercise in girls may be that they have lower muscle mass, higher body fat content, and larger hormonal changes caused by earlier sexual maturation than in boys.

We observed no associations of CRF with arterial stiffness in boys or in girls. These results are in contrast to the results of a recent study in which improved CRF was associated with increased arterial stiffness among adolescents (28). Another study among adolescents found no association between CRF and carotid intima-media thickness but reported an inverse relationship between CRF and aortic intima-media thickness (35). Nonetheless, some studies have reported an inverse association between CRF and arterial stiffness in children and adolescents (18,19,48), with few of them utilizing a direct measurement of $\dot{V}O_{2peak}$ (18,19). Previous findings on the associations of CRF with arterial stiffness in children and adolescents have been inconsistent partly due to differences in age ranges, ethnicity, sample sizes, measures of CRF and arterial stiffness, and the segments of arterial network investigated between the studies (28,35,48).

We have earlier found an inverse association between CRF and arterial stiffness in children aged 6-9 years (48). The contrast between our previous observations and the present results in children 8-11 years of age might be explained by regression towards the mean phenomenon that reflects the natural improvement of cardiovascular structure and function with age and maturation among those who had poorer cardiovascular health at the baseline of the study. The beneficial effects of CRF on the arterial wall may mainly occur in later life (35). Furthermore, we did not have complete data on CRF measured directly by respiratory gas analysis at baseline among children 6-8 years of age and we utilized maximal workload scaled by LM instead of $\dot{V}O_{2peak}$ scaled by LM in the baseline analyses (48). CRF expressed as maximal workload reflects both cardiorespiratory and neuromuscular performance (31,34) and is therefore not identical with CRF expressed as $\dot{V}O_{2peak}$ (45). Nevertheless, in the current study sample, we

found no relationship between maximal workload scaled by LM with arterial stiffness, either. This disparity in the same study population at two different time points may be clarified in analyses dealing with follow-up from childhood to adolescence.

We found that higher body fat content was related to poorer arterial dilatation capacity in response to a bout of exercise in boys but this relationship attenuated after controlling for clinical puberty. This observation suggests that changes during puberty in boys, such as increased muscle mass, are beneficial for their arterial function especially when physically active. Obesity-induced insulin resistance in adults has been associated with impaired function of endothelial cells and decreased endothelium-dependent vasodilatation (44). This is in consonance with the result of a review that adiposity is a strong determinant of arterial health in children (10). Higher body fat content has been found to increase cardiac pre-load, heart rate, and insulin resistance in children and adults (17,53). In addition, excess fat within arterial walls has been observed to cause arterial wall remodelling, which could result in increased arterial tone and decreased arterial dilatation capacity (10). Obesity has also been reported to exacerbate the effect of systemic inflammation on endothelial function in adults (15). Moreover, increased serum levels of leptin that is secreted by adipocytes, have been linked to reduced arterial dilatation capacity via the proliferation of smooth muscle cells and angiogenesis (9,43).

There was no relationship between body fat content and arterial stiffness or arterial dilatation capacity in girls in the present study. A plausible explanation for this is that higher body fat content has been associated with larger blood volume which could result in chronic vasodilatation due to adiposity-induced arterial adaptation (11). In our study, girls with more body fat content had a significantly higher arterial dilatation capacity than boys. Nonetheless, a single bout of aerobic exercise may not be sufficient to elicit a significant relationship between adiposity and arterial response in girls probably due to an adiposity-induced arterial adaptation. Furthermore, despite the similarity in the prevalence of overweight and obesity among girls and

boys in our study, almost forty percent of girls had attained puberty which is more than twice the number of boys who had matured sexually. Although evidence has suggested that increased body fat content in childhood may cause a premature peak in arterial compliance as a result of an adiposity-induced pubertal development (47), it remains unclear how pubertal status may interact with the relationship of body fat content with arterial measures in girls aged 8-11 years.

Our study had some strengths including a large population sample of children aged 8-11 years and a direct measure of $\dot{V}O_{2\text{peak}}$ scaled by LM, considered the “gold standard” of physiological aerobic power (12). LM is a functional measure of the skeletal muscles that are responsible for body movements and augment venous return from peripheral tissues to the heart by their contractions and therefore increases stroke volume and cardiac output (40,42). Moreover, using valid and reproducible measurement we controlled for maturation and body composition in our statistical analyses (28,46). Our study participants were entirely Caucasian children; therefore, these results may not be generalised to children of different ethnicity. One of the limitations of our study is that we used SI as a measure of arterial stiffness instead of pulse wave velocity between carotid and femoral arteries. However, SI has been found to be strongly correlated with pulse wave velocity (29). Furthermore, the main outcome measure in the present report, ΔRI in response to a bout of exercise, reflects arterial dilatation capacity well (32). It does not however specifically measure endothelial function but may be used as a surrogate marker of endothelium-dependent arterial dilatation (32,48). Our finding on the association between higher CRF and higher ΔRI in response to a bout of exercise among boys is congruent with the hypothesis that higher CRF levels, through improved endothelial function, increases exercise-induced arterial dilatation (30). Higher arterial dilatation capacity may also result in improved CRF; nonetheless, from cross-sectional analyses, it is impossible to arrive at a conclusion regarding the direction of the association.

In conclusion, increased CRF and decreased body fat content were independently associated with increased arterial dilatation capacity in response to a bout of exercise in boys but not in girls. Neither CRF nor adiposity had any association with arterial stiffness in either boys or girls. Our findings emphasise that increasing CRF and decreasing adiposity in childhood, particularly among boys, are important in improving arterial health in childhood. Higher CRF and lower adiposity in childhood are likely important in reducing the risk of atherosclerotic cardiovascular diseases in adulthood.

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Role of the sponsor

340 The funding sources had no role in the design and conduct of the study; in the collection,
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505 Table 1 Characteristics of 329 children (177 boys, 152 girls)

	Children	Boys	Girls	<i>P</i> for difference
Age (y)	9.8 (0.4)	9.8 (0.5)	9.8 (0.4)	0.696
Clinical Puberty (%) [†]	23.3	15.1	37.7	<0.001
Body height (cm)	141.0 (6.3)	141.5 (6.0)	140.4 (6.6)	0.127
Body mass (kg)	34.9 (7.5)	35.3 (7.4)	34.5 (7.5)	0.316
BMI-SDS	-0.07 (1.1)	-0.09 (1.1)	-0.05 (1.0)	0.754
Prevalence of overweight and obesity (%)	19.3	19.9	18.7	0.917
Waist circumference (cm)	61.8 (7.4)	62.8 (7.6)	60.8 (7.1)	0.012
Body fat mass (kg)	7.1 (4.5)	6.7 (4.6)	7.6 (4.5)	0.070
Body fat percentage (%)	19.1 (8.0)	17.6 (8.1)	20.8 (7.6)	<0.001
Lean body mass (kg)	26.2 (3.6)	27.0 (3.6)	25.3 (3.5)	<0.001
$\dot{V}O_{2peak}$ (L·min ⁻¹)	1.7 (0.3)	1.8 (0.3)	1.6 (0.2)	<0.001
$\dot{V}O_{2peak}$ (mL·kg BM ⁻¹ ·min ⁻¹)	49.3 (8.1)	52.0 (8.1)	46.3 (6.9)	<0.001
$\dot{V}O_{2peak}$ (mL·kg LM ⁻¹ ·min ⁻¹)	64.4 (6.9)	66.7 (6.5)	61.9 (6.5)	<0.001
Systolic BP before exercise (mmHg)	105.9 (10.3)	106.1 (9.7)	106.1 (10.3)	0.979
Diastolic BP before exercise (mmHg)	47.2 (27.7)	47.6 (27.5)	49.9 (26.0)	0.438
Heart rate before exercise (beats/min)*	66.0 (8.1)	64.9 (7.7)	66.7 (8.3)	0.048
Peak heart rate during exercise (beats/min)	199.4 (8.6)	198.8 (8.7)	200.2 (8.6)	0.167
Heart rate 5-min after exercise (beats/min)*	101.6 (10.6)	100.0 (10.3)	103.0 (10.4)	0.009
Peak respiratory exchange ratio	1.06 (0.1)	1.05 (0.1)	1.08 (0.1)	<0.001
Stiffness index before exercise (m/s)	5.0 (0.4)	5.0 (0.4)	4.9 (0.4)	0.777
Reflection index before exercise (%)	50.2 (12.2)	49.8 (12.0)	51.1 (12.4)	0.311

Change in reflection index in response to exercise (%)	27.0 (14.9)	23.9 (14.6)	30.8 (14.4)	<0.001
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506 The values are means (standard deviations) except that those for the prevalence of overweight and obesity, and
 507 clinical puberty are percentages.

508 Differences between girls and boys were tested using Student's t-test for normally distributed continuous
 509 variables, Mann–Whitney U test for skewed continuous variables, and Chi-square test for dichotomous variables.

510 BMI-SDS, body mass index standard deviation score calculated using Finnish reference values (41); $\dot{V}O_{2peak}$,
 511 peak oxygen uptake; BM, body mass; LM, lean mass; BP, blood pressure; min, minute.

512 †Boys were defined having entered clinical puberty if their testicular volume assessed by an orchidometer was
 513 ≥ 4 mL (Tanner stage ≥ 2) (26). Girls were defined having entered clinical puberty if their breast development had
 514 started (Tanner stage ≥ 2) (27). *Supine heart rate. Bold values indicate statistical significance at $P < 0.05$.

515 Table 2 Associations of cardiorespiratory fitness with arterial stiffness, tone, and dilatation capacity

	SI						RI						ΔRI					
	Boys (n= 177)			Girls (n= 152)			Boys (n= 177)			Girls (n= 152)			Boys (n= 177)			Girls (n= 152)		
Model 1	<i>B</i>	β	<i>P</i>	<i>B</i>	β	<i>P</i>	<i>B</i>	β	<i>P</i>	<i>B</i>	β	<i>P</i>	<i>B</i>	β	<i>P</i>	<i>B</i>	β	<i>P</i>
$\dot{V}O_{2peak}$ (L·min ⁻¹)	0.001	0.144	0.056	0.001	0.095	0.247	-0.003	-0.058	0.441	-0.014	-0.266	0.001	0.001	-0.006	0.934	0.001	0.019	0.827
$\dot{V}O_{2peak}$ (mL·kg BM ⁻¹ ·min ⁻¹)	0.006	0.112	0.138	-0.002	-0.030	0.710	0.375	0.253	0.001	0.362	0.203	0.012	0.468	0.253	0.001	-0.097	-0.048	0.577
$\dot{V}O_{2peak}$ (mL·kg LM ⁻¹ ·min ⁻¹)	0.007	0.113	0.149	-0.001	-0.001	0.995	0.025	0.013	0.863	-0.184	-0.097	0.236	0.346	0.156	0.049	-0.052	-0.024	0.782
Model 2																		
$\dot{V}O_{2peak}$ (L·min ⁻¹)	0.001	0.109	0.163	0.001	0.070	0.428	-0.002	-0.054	0.497	-0.013	-0.248	0.004	0.001	0.018	0.822	-0.003	-0.051	0.578
$\dot{V}O_{2peak}$ (mL·kg BM ⁻¹ ·min ⁻¹)	0.005	0.107	0.152	-0.001	-0.020	0.809	0.377	0.254	0.001	0.337	0.189	0.020	0.470	0.254	0.001	-0.057	-0.028	0.743
$\dot{V}O_{2peak}$ (mL·kg LM ⁻¹ ·min ⁻¹)	0.006	0.099	0.202	-0.001	-0.015	0.861	0.031	0.017	0.831	-0.147	-0.078	0.347	0.355	0.160	0.044	-0.112	-0.051	0.556
Model 3																		
$\dot{V}O_{2peak}$ (L·min ⁻¹)	0.001	0.132	0.102	0.001	0.079	0.392	0.001	0.018	0.814	-0.009	-0.180	0.040	0.003	0.059	0.466	-0.003	-0.053	0.581

$\dot{V}O_{2peak}$ (mL·kg BM ⁻¹ ·min ⁻¹)	0.008	0.155	0.212	-0.004	-0.058	0.628	0.076	0.051	0.672	-0.048	-0.027	0.815	0.641	0.346	0.005	-0.145	-0.071	0.569
$\dot{V}O_{2peak}$ (mL·kg LM ⁻¹ ·min ⁻¹)	0.006	0.102	0.192	-0.001	-0.014	0.867	0.051	0.027	0.718	-0.126	-0.067	0.404	0.376	0.169	0.031	-0.112	-0.051	0.557
Model 4																		
$\dot{V}O_{2peak}$ (mL·kg BM ⁻¹ ·min ⁻¹)	–	–	–	–	–	–	–	–	–	–	–	–	0.683	0.368	0.004	-0.171	-0.084	0.500
$\dot{V}O_{2peak}$ (mL·kg LM ⁻¹ ·min ⁻¹)	–	–	–	–	–	–	–	–	–	–	–	–	0.375	0.168	0.038	-0.122	-0.055	0.519
Model 5																		
$\dot{V}O_{2peak}$ (mL·kg BM ⁻¹ ·min ⁻¹)	–	–	–	–	–	–	–	–	–	–	–	–	0.628	0.334	0.011	-0.115	-0.057	0.655
$\dot{V}O_{2peak}$ (mL·kg LM ⁻¹ ·min ⁻¹)	–	–	–	–	–	–	–	–	–	–	–	–	0.345	0.150	0.073	-0.080	-0.036	0.680

516 Values are unstandardized regression coefficients (*B*), standardized regression coefficients (β), and *P*-values from linear regression analyses. Bold values indicate statistical significance at
517 *P* < 0.05. SI, stiffness index before exercise; RI, reflection index before exercise; Δ RI, change in reflection index in response to exercise; $\dot{V}O_{2peak}$, peak oxygen uptake; BM, body mass;
518 LM, lean mass. Model 1: unadjusted data. Model 2: data were adjusted for age. Model 3: data were adjusted for age and body fat percentage. Model 4: Further adjustment of variables in
519 Model 3 for systolic blood pressure, study group, and heart rate. Model 5: Additional adjustment of Model 4 for clinical puberty. Hyphens (–) indicate statistically non-significant regression
520 coefficients.

521 Table 3 Associations of adiposity with arterial stiffness, tone, and dilatation capacity.

[illegible]

Waist circumference	0.001	-0.008	0.917	0.001	-0.005	0.957	-0.376	-0.241	0.002	-0.569	-0.331	<0.001	-0.354	-0.187	0.018	-0.151	-0.077	0.380
BMI-SDS	-0.032	-0.087	0.266	-0.052	-0.120	0.150	-3.005	-0.279	<0.001	-3.813	-0.315	<0.001	-2.413	-0.185	0.020	-0.621	-0.044	0.613
Body fat percentage	-0.003	-0.064	0.408	-0.001	-0.013	0.880	-0.415	-0.279	<0.001	-0.445	-0.274	0.001	-0.307	-0.171	0.029	0.002	0.001	0.991
Model 4																		
Waist circumference	–	–	–	–	–	–	-0.361	-0.231	0.002	-0.634	-0.371	<0.001	-0.331	-0.174	0.032	-0.189	-0.096	0.268
BMI-SDS	–	–	–	–	–	–	-2.750	-0.256	0.001	-4.810	-0.395	<0.001	-2.202	-0.169	0.038	-1.030	-0.073	0.398
Body fat percentage	–	–	–	–	–	–	-0.364	-0.244	0.001	-0.451	-0.279	<0.001	-0.280	-0.155	0.052	0.002	0.001	0.989
Model 5																		
Waist circumference	–	–	–	–	–	–	-0.304	-0.192	0.015	-0.610	-0.358	<0.001	-0.265	-0.138	0.106	-0.312	-0.159	0.106
BMI-SDS	–	–	–	–	–	–	-2.298	-0.207	0.008	-4.654	-0.382	<0.001	-1.630	-0.120	0.155	-1.709	-0.121	0.203
Body fat percentage	–	–	–	–	–	–	-0.325	-0.216	0.005	-0.404	-0.249	0.001	-0.599	-0.122	0.143	-0.054	-0.028	0.765

522 Values are unstandardized regression coefficients (*B*), standardized regression coefficients (β) and *P*-values from linear regression analyses. Bold values indicate statistical
523 significance at *P* < 0.05. SI, stiffness index before exercise; RI, reflection index before exercise; ΔRI, change in RI in response to exercise, BMI-SDS, body mass index standard

524 deviation score, calculated from Finnish reference values (41). Model 1: unadjusted data. Model 2: data were adjusted for age. Model 3: data were adjusted for age and $\dot{V}O_{2\text{peak}}$ scaled
525 by lean mass. Model 4: Further adjustment of variables in Model 3 for systolic blood pressure, study group, and heart rate. Model 5: Additional adjustment of Model 4 for clinical
526 puberty. Hyphens (–) indicate statistically non-significant regression coefficients.