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# Associations of Objectively Measured Physical Activity and Sedentary Time With Arterial Stiffness in Pre-pubertal Children

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## **Associations of objectively measured physical activity and sedentary time with arterial stiffness in pre-pubertal children**

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## Abstract

**Purpose:** To investigate the relationships of objectively measured physical activity (PA) and sedentary time (ST) to arterial stiffness in pre-pubertal children. **Method:** Altogether 136 children (57 boys, 79 girls) aged 6–8-years participated in the study. Stiffness index (SI) was assessed by pulse contour analysis based on photoplethysmography. ST, light PA, moderate PA, and vigorous PA were assessed using combined acceleration and heart rate monitoring. We investigated the associations of ST (<1.5METs) and time spent in intensity level of PA above 2–7METs in min/d with SI using linear regression analysis. We studied the optimal duration and intensity of PA to identify children being in the highest quarter of SI using Receiver Operating Characteristics curves. **Results:** Moderate PA, vigorous PA, and cumulative time spent in PA above 3 ( $\beta=-0.279$ ,  $P=0.002$ ), 4 ( $\beta=-0.341$ ,  $P<0.001$ ), 5 ( $\beta=-0.349$ ,  $P<0.001$ ), 6 ( $\beta=-0.312$ ,  $P<0.001$ ), and 7 ( $\beta=-0.254$ ,  $P=0.005$ ) METs were inversely associated with SI after adjustment for age, sex, and monitor wear time. The cutoffs for identifying children being in the highest quarter of SI <68 min/d for PA exceeding 5 METs and <26 min/d for PA exceeding 6 METs. **Conclusion:** Lower levels of PA exceeding 3–6 METs were related to higher arterial stiffness in children.

**Key words:** physical activity, sedentary behavior, children, vascular stiffness, arteries

## INTRODUCTION

The process leading to arteriosclerosis begins early in life (28, 52) and autopsy studies have documented that fatty streaks in the arterial wall exist already in young children (7) suggesting that the prevention of cardiovascular diseases should begin as early as possible. Increased arterial stiffness is one of the first signs of arteriosclerosis in children (18). Arterial stiffness refers to decreased compliance and distensibility of arteries in response to pressure changes (5, 57). Arterial stiffness develops through age-associated fractures in elastin caused by repeated cyclic stress and complicated cellular and molecular factors such as systemic inflammation and free radicals leading to smooth muscle media calcification, changes in cell signaling, and endothelial dysfunction (5, 57). Arterial stiffness has been associated with increased cardiovascular morbidity and mortality in adults (33, 53). Exercise training has been shown to improve artery compliance in middle-aged and older adults (4). However, evidence on the associations of physical activity (PA) with arterial stiffness, structure, and function in pre-pubertal children is limited (12, 18).

PA may improve arterial stiffness by reducing adiposity and other traditional cardiometabolic risk factors, systemic low-grade inflammation, sympathetic drive, and oxidative stress and by increasing nitric oxide bioavailability and improving endothelial vasodilatation (19). The evidence on the mechanisms explaining possible adverse effects of sedentary time (ST) on arterial stiffness is limited, but may include increased arterial vasoconstriction and cardiometabolic risk factors related to higher ST (24, 45, 48, 54).

Lower levels of self-reported PA have been related to higher artery stiffness and aortic intima-media thickness (IMT) in children and adolescents (31, 32, 42). Lower levels of PA during childhood have also been found to predict higher artery stiffness in adulthood (35). Nevertheless, the results of some studies suggest that there is no relationship between PA and arterial stiffness among children (36, 55). The main limitation of most of the previous studies

is that they have used measures based on self-reported PA that are prone to recall bias and have limited ability to correctly identify the level and intensity of PA (14, 16). Furthermore, most previous investigations have been cross-sectional in design and the few longitudinal studies have included overweight or obese children (12).

Studies using objective methods to assess PA have observed either inverse (30, 37) or no association (38, 41) between PA and arterial stiffness in children and adolescents. There is also some evidence that higher levels of objectively measured PA are related to improved flow-mediated dilation, a measure of arterial endothelial function, in children and adolescents (1, 23). Although PA of higher intensity may have a stronger association with arterial stiffness than lower intensity PA among children (23), there are no studies on objectively measured and individually calibrated PA intensities in relation to arterial stiffness in mid-childhood. Previous studies have found no association between self-reported or objectively measured ST and arterial stiffness or IMT in children (29, 47). However, higher levels of ST have been linked to increased arterial stiffness in young adults aged 30 years (24).

Exercise training at higher intensity has been found to improve arterial stiffness more than exercise training at lower intensity (4). However, the evidence of the associations of PA at different intensities and ST with arterial stiffness in children is limited (12, 18). The aim of the present study was to investigate the relationships of objectively measured ST and PA at different intensities to arterial stiffness in pre-pubertal children.

## **METHODS**

### **Study design and study population**

Data for the present analyses were obtained from the Physical Activity and Nutrition in Children (PANIC) Study (50). The PANIC Study is an ongoing controlled physical activity and dietary intervention study in a population sample of primary-school children from the city

of Kuopio. In total, 736 children 6–8 years of age who were in Grade 1 in 2007–2009 were invited to participate, and 512 (70%) participated. At baseline, arterial stiffness was assessed in a subsample of 230 children and complete data on variables used in the present analyses were available for 136 children (57 boys, 79 girls). Children who were included in the study sample were more likely to be girls ( $P=0.007$ ), had higher levels of moderate PA ( $P<0.001$ ), and had better cardiorespiratory fitness ( $P=0.003$ ) than children who were excluded. The included children did not differ in any other characteristic from the excluded children. The PANIC Study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio, Finland. All participating children and their parents provided written informed consent.

### **Assessment of physical activity and sedentary time**

PA and ST were assessed using a combined heart rate and movement monitor (Actiheart®, CamNtech Ltd., Papworth, UK) (9) worn continuously for a minimum of four consecutive days without interruption. The monitor was attached to the chest with two standard electrocardiogram electrodes (Bio Protech Inc, South Korea) and recorded data in 60-second epochs. Upon retrieving the monitoring device, heart rate data were cleaned (43), individually calibrated with parameters from the exercise test (27), and combined with trunk acceleration (10) using branched equation modeling to produce intensity time-series (8). Whilst minimizing diurnal bias caused by any potential non-wear episodes (11), PA energy expenditure (PAEE) was calculated by the integration of the intensity time-series, and the time distribution of activity intensity was generated by using standard metabolic equivalents (METs). For these analyses, the equivalent of 3.5ml O<sub>2</sub>/min/kg (71 J/min/kg) was used to define one MET and data summarized as ST (<1.5 METs), light PA (1.5-4 METs), moderate PA (4-7 METs), and vigorous PA (>7 METs). Awake-time ST was calculated by subtracting sleep time from total

time <1.5METs. PA records were included in the analysis if they contained  $\geq 48$ h of total wear data, distributed as  $\geq 32$ h during weekdays and  $\geq 16$ h during weekend days, and  $\geq 12$ h of morning, noon, afternoon, and evening wear data. These time-distribution criteria shielded against potential bias from the over-representation of specific parts of days, and optimized the diurnal bias minimization procedure.

### **Assessment of arterial stiffness**

Stiffness index (SI) was assessed by pulse contour analysis (PCA) based on noninvasive photoplethysmography using the PulseTrace PCA2® device (Micro Medical, Gillingham, Kent, UK), as described in more detail previously (48, 49). SI was assessed in a supine position prior to the exercise test in a test laboratory at stable room temperature (20–22 °C) after a 15-minute rest. SI was calculated by dividing body height by time between the first (systolic) peak and the second (diastolic) peak of the pulse contour and was expressed in meters per second. All pulse contours were visually examined afterwards to control for quality. Data were excluded from analysis if there was an extra peak between the systolic and diastolic peak that was incorrectly defined as the diastolic peak; if the PCA indexes were incorrectly measured by the device; or if the pulse contours of the same child markedly differed visually (48). A higher SI indicates stiffer (less compliant) arteries. The within-subject coefficient of variation is 7.4% for SI and the intraclass-correlation=0.44 (95% Confidence Interval (CI)=0.13 to 0.67) (49).

### **Assessment of body composition and size**

Body fat mass, body fat percentage, and lean body mass were measured by the Lunar Prodigy Advance® dual-energy x-ray absorptiometry device (GE Medical Systems, Madison, WI, USA) using standardized protocols (46). Body weight was measured twice to accuracy of 0.1kg after overnight fasting with empty bladder, and whilst standing in light underwear using the InBody® 720 bioelectrical impedance device (Biospace, Seoul, Korea). The mean of the



two values was used for analyses. Body height was measured three times to accuracy of 0.1 cm using a wall-mounted stadiometer whilst barefoot and with the head positioned in the Frankfurt plane. The mean of the nearest two values was used for analyses. 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 with an unstretchable measuring tape to an accuracy of 0.1 cm. Body mass index - standard deviation score was calculated using Finnish references (40), and the prevalence of overweight and obesity was defined using cut-offs published by Cole and co-workers (13).

### **Other assessments**

Cardiorespiratory fitness was assessed by a maximal exercise test with an electromagnetically-braked Ergoselect 200K® cycle ergometer (Ergoline, Bitz, Germany) (26). Cardiorespiratory fitness was defined as maximal workload attained during the exercise test and expressed per kilograms of lean body mass. Blood pressure was measured manually in a sitting position from the right arm using a calibrated Heine 130 Gamma G7® aneroid sphygmomanometer (Heine, Munich, Germany). Blood pressure was also assessed in a supine position before the exercise test to obtain blood pressure values in a standardized hemodynamic state for the analyses (48). Venous blood samples for the measurement of fasting serum insulin, leptin, and 25-hydroxyvitamin D (25(OH)D) and fasting plasma glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol were taken after 12-h overnight fasting (51). We calculated a cardiometabolic risk score using population-specific z-scores as waist circumference + fasting serum insulin concentration + fasting plasma glucose concentration + fasting plasma triglyceride concentration – fasting plasma HDL cholesterol concentration + mean of systolic and diastolic blood pressure with a higher score indicating higher cardiometabolic risk (51). The number of meals and snacks per day, food consumption, total

energy intake, nutrient intakes as percentages of total energy intake, and sodium intake were assessed using food records on four consecutive days including two weekdays and two weekend days (17). A clinical nutritionist instructed the parents how to fill in the food record. A clinical nutritionist also reviewed the food records at return. Data on birth weight were acquired from the records of Kuopio University Hospital, Kuopio, Finland. A research physician assessed pubertal status using the five-stage scale described by Tanner (44).

### **Statistical methods**

Basic characteristics between boys and girls were compared using the Student's t-test, the Mann-Whitney U-test, or the Chi Square-test. The associations of ST and PA as independent variables with SI as the dependent variable were studied using linear regression analysis. First, ST, light PA, moderate PA, or vigorous PA were entered into the linear regression model one by one and the data were adjusted for age, sex, and monitor wear time. The associations were then mutually adjusted for ST, light PA, moderate PA, and vigorous PA to investigate the independent associations of these measures with SI. We also investigated the associations of the cumulative time (min/d) spent above one MET, two METs, three METs, four METs, five METs, six METs, and seven METs with SI using sequential linear regression analyses. We studied combined associations of PA and ST with SI using general linear models adjusted for age, sex, and monitor wear time. We dichotomized PA using cut-offs determined in the ROC curve analyses and ST was dichotomized at the median. All data were additionally adjusted for cardiorespiratory fitness, body fat percentage, systolic blood pressure, the cardiometabolic risk score, serum leptin, serum 25(OH)D, saturated fat, sucrose, sodium intake, eating all three main meals daily, or birth weight.

Receiver operating characteristics (ROC) curves were used to investigate the optimal cutoff for the duration and intensity of PA to identify children being in the highest quarter of

SI. The area under the curve (AUC) is considered a measure of the utility of the predictor variable and represents the trade-off between the correct identification of children in the highest quarter of SI (sensitivity) and the correct identification of other children (specificity). An AUC of 1 represents the ability to perfectly identify children in the highest quarter of SI from other children, whereas an AUC of 0.5 indicates no greater predictive ability than chance alone. We determined the optimal cutoff using the Youden index (34), which is the maximum value of  $J$  that is computed as sensitivity + specificity – 1.

We performed statistical analyses using the SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA), except that the ROC curve analyses were performed using MedCalc Statistical Software, Version 16.1 (MedCalc Software bvba, Ostend, Belgium).

## **RESULTS**

### **Characteristics**

All 136 children were pre-pubertal. Boys were slightly older and taller and had a lower body fat percentage and a better cardiorespiratory fitness than girls (Table 1). Boys also accumulated more moderate PA and vigorous PA and had a higher PAEE than girls.

### **Associations of light, moderate, and vigorous physical activity and sedentary time with arterial stiffness**

Higher levels of moderate PA and vigorous PA were associated with lower SI after adjustment for age, sex, and monitor wear time (Table 2). Mutual adjustment for ST, light PA, moderate PA, or vigorous PA had no effect on these associations. Further adjustment for cardiorespiratory fitness, body fat percentage, systolic blood pressure, the cardiometabolic risk score, serum leptin, serum 25(OH)D, saturated fat, sucrose, sodium intake, eating all three main meals daily, or birth weight had no effect on these associations (data not shown). ST or light PA were not associated with SI.

## **Associations of cumulative times spent in physical activity above different intensities with arterial stiffness**

Higher cumulative time spent in PA above three METs ( $\beta=-0.279$ , 95% confidence interval (CI)=-0.453 to -0.106,  $P=0.002$ ), four METs ( $\beta=-0.341$ , 95% CI=-0.515 to -0.167,  $P<0.001$ ), five METs ( $\beta=-0.349$ , 95% CI=-0.524 to -0.174,  $P<0.001$ ), six METs ( $\beta=-0.312$ , 95% CI=-0.220 to -0.064,  $P<0.001$ ), and seven METs ( $\beta=-0.254$ , 95% CI=-0.428 to -0.080,  $P=0.005$ ) were associated with lower SI after adjustment for age, sex, and monitor wear time. Further adjustments had no effect on these relationships (data not shown).

The ROC curve analyses revealed that the optimal cutoff for time spent in PA above five METs to identify children being in the highest quarter of SI was  $<68$  min/d (95% CI = 63 to 105) with a sensitivity of 79%, a specificity of 49%, an AUC of 0.609 (95% CI = 0.522 to 0.691,  $P=0.047$ ), and the Youden index of 0.2843. The optimal cutoff for time spent PA above six METs was  $<26$  min/d (95% CI 23 to 60) with a sensitivity of 71%, a specificity of 64%, an AUC of 0.625 (95% CI 0.538 to 0.707,  $P=0.027$ ), and the Youden index of 0.3431. There were no other statistically significant associations of ST or PA with SI in the ROC curve analyses.

Age, sex, or monitor wear time were not related to SI. Of the other variables in the model, a lower cardiorespiratory fitness ( $\beta=-0.250$ , 95% CI=-0.437 to -0.064,  $P=0.009$ ) and higher systolic blood pressure ( $\beta=0.235$ , 95% CI=0.064 to 0.406,  $P=0.008$ ) were associated with a higher SI after controlling for age, sex, and monitor wear time. The association between systolic blood pressure and SI remained significant when PA was in the same model ( $P<0.05$ ), but the association with cardiorespiratory fitness was no longer statistically significant when PA exceeding intensity of four METs was included in the model ( $P>0.05$ ). Cardiorespiratory fitness remained statistically significantly related to SI when the data were adjusted for time spent at PA exceeding three METs ( $P<0.05$ ).

### **Combined associations of physical activity and sedentary time with stiffness index**

Children with lower levels of ST and higher levels of PA above 5 METs had a lower SI than children with lower levels of both ST and PA above 5 METs (mean difference -0.383, 95% CI for difference=-0.511 to -0.128,  $P<0.001$ ) (Figure 1). Those with lower levels of ST and higher levels of PA above 5 METs also had a lower SI than children with higher levels of ST along with lower levels of PA above 5 METs (mean difference -0.320, 95% CI for difference=-0.612 to -0.155,  $P<0.001$ ). Further adjustment had no effect on these differences (data not shown).

Children with lower levels of ST and higher levels of PA above 6 METs had a lower SI than children with lower levels of ST accompanied by lower levels of PA above 6 METs (mean difference -0.292, 95% CI for difference=-0.558 to -0.026,  $P=0.024$ ) and children with higher levels of ST along with lower levels of PA above 6 METs (mean difference -0.295, 95% CI for difference=-0.481 to -0.109,  $P<0.001$ ) (Figure 1).

Children with a combination of higher levels of ST and higher levels of PA above 6 METs had a lower SI than children with lower levels of both ST and PA above 6 METs (mean difference -0.325, 95% CI for difference=-0.633 to -0.018,  $P<0.001$ ) (Figure 1). They also had a lower SI than children with higher levels of ST and lower levels of PA above 6 METs (mean difference -0.329, 95% CI for difference=-0.568 to -0.089,  $P=0.002$ ). Further adjustment had no effect on these differences.

### **DISCUSSION**

We found that lower levels of objectively measured PA exceeding the intensity of three METs were associated with a higher SI in pre-pubertal children after adjustment for confounding factors. Lower levels of PA exceeding the intensity of 4–6 METs had the strongest associations with SI. The optimal thresholds for PA above five and six METs to identify

children being in the highest quarter of SI were less than 68 and 26 min/d, respectively. These results dealing with arterial stiffness agree relatively well with the recommendation that children should have at least 60 minutes of moderate-to-vigorous PA daily (56).

The findings of a previous study suggest that adolescents doing more than 67 minutes of moderate-to-vigorous PA per day have more compliant carotid arteries than adolescents doing less than 17 minutes of moderate-to-vigorous PA per day (37). Furthermore, higher levels of light PA and moderate-to-vigorous PA have been linked to lower stiffness of small, but not large, arteries (30). Some other studies have found no association between objectively measured PA and arterial stiffness among children and adolescents (38, 41). We observed that lower levels of PA were associated with a higher SI and that PA above 4–6 METs had the strongest relationship with SI among pre-pubertal children. These findings suggest that PA intensity may be an important determinant of arterial stiffness in children. One reason for the weaker association between PA and arterial stiffness among children in some other studies (41) than in our study may be that the number of steps taken daily, which is unable to capture the intensity distribution of PA, has been used as a measure of PA. Other plausible explanations for the partly inconsistent findings include differences in the measures of arterial stiffness used and the segments of the arteries studied in earlier studies.

We found that PAs exceeding the intensity of 5–6 METs were optimal to differentiate children in the highest quarter of SI with the optimal cutoff of <68 minutes for PA exceeding 5 METs and <26 minutes for PA exceeding 6 METs. These findings are supported by the observation from the European Young Heart Study that adolescent boys exceeding 67 minutes of moderate-to-vigorous PA daily had more compliant arteries than those with less than 17 minutes of moderate-to-vigorous PA daily (37). Furthermore, the results of some studies have suggested that 30–75 minutes of moderate-to-vigorous PA for at least three times a week improve endothelial function in overweight or obese children and adolescents (15). These

findings together suggest that achieving a minimum of 60 minutes of moderate-to-vigorous PA is linked to reduced arterial stiffness already in childhood.

The reasons for the inverse association between moderate-to-vigorous PA and SI could be simplified to increased arterial remodeling and compliance due to repeated shear stress in response to PA (22). Increased shear stress has been found to activate various signaling pathways leading to increased bioavailability of nitric oxide and thereby improved arterial compliance (19). However, the mechanisms for the inverse relationship between PA and arterial stiffness are likely complex and are associated with various beneficial effects of increased PA such as decreased adiposity, blood pressure, oxidative stress, and systemic inflammation and improved insulin sensitivity and lipid metabolism (20).

We observed that the association between time spent in PA above 7 METs and SI were weaker than the associations of time spent in PA above 4, 5, and 6 METs. An explanation for this observation may be that children had relatively low levels of vigorous PA which reduced variance and statistical power. Another explanation is that very strenuous PA may impair nitric oxide bioavailability and increase oxidative stress which may increase arterial stiffness (21).

We found no relationship of ST or light PA to SI among children. Although sedentary behavior has been directly associated with cardiometabolic risk in children even independent of PA (2, 54), it has not been related to carotid artery IMT, arterial stiffness, or brachial artery flow-mediated dilation, in other studies among children (23, 29, 47) or in adults (45). We also showed that lower levels of PA were related to a higher SI among children regardless of ST. These observations suggest that sedentary behavior is not strongly associated with arterial structure or function during childhood. A reason for these weak associations between ST and arterial stiffness in children may be that children are highly adaptive and can compensate the negative effects of increased ST on arterial stiffness. Children in our study also had relatively low levels of ST which may weaken the associations of ST with SI. Arteriosclerosis is a

progressive but slow process and is partly due to a long-term exposure to a sedentary lifestyle. Furthermore, one explanation on the null association between light PA and SI may be due to insufficient shear stress and nitric oxide synthesis in response to light PA (3, 4). Therefore, longitudinal studies from childhood to adolescence and young adulthood are needed to investigate whether higher levels of ST and low levels of light PA are independently associated with increased arterial stiffness in the long term.

The strengths of the present study are the objective measures of PA and ST and the possibility to adjust the data for a number of confounding factors. The main limitation of the study is that we were unable to use the gold-standard measures of arterial stiffness, such as pulse wave velocity between the carotid and femoral arteries. In ROC-curve analyses, we defined children having increased arterial stiffness as those being in the highest quarter of SI. Our study sample included mainly healthy children for whom there is no widely accepted threshold for a clinically meaningful increase in SI. However, a previous study reported a strong association between SI and pulse wave velocity in adults suggesting that SI is a good surrogate measure for pulse wave velocity (39). There are no data on the tracking of arterial stiffness from childhood to adulthood and whether it predicts cardiovascular diseases in adulthood. Nevertheless, arterial stiffness has been found to be a strong independent predictor of cardiovascular diseases and premature mortality in adults (53) and increased cardiometabolic risk in childhood has been related to increased arterial stiffness in adulthood (25).

Another limitation of the study is that the relatively small sample size decreased power to detect statistically significant associations. Therefore, the cutoffs observed in the ROC curve analyses should be interpreted cautiously and treated as interim thresholds for PA needed to decrease arterial stiffness. These cutoffs should be confirmed in larger population based samples of children. We had to exclude a relatively high number of children because of



insufficient PA monitor wear time and errors in the pulse contour analyses. Children included in the present study population also had higher levels of moderate PA and cardiorespiratory fitness than the excluded children. It is therefore possible that the association between PA and arterial stiffness would have been even stronger if the study sample had included more children with lower levels of PA and cardiorespiratory fitness that would have increased variation in these measures. A small sample size and the exclusion of children with higher levels of PA and cardiorespiratory fitness also decreases generalizability of the results. Furthermore, we did not assess diet quality or fluid balance before the assessment of arterial stiffness, although both of them could have an effect on the assessment. We used only objectively measured ST in our study although some specific types of sedentary behavior may have a stronger association with arterial stiffness than others. However, we have previously found no association between screen-based sedentary behavior and SI in children (47). We also set the combined heart rate and movement monitor to record data at 60-second resolution. This may have some minor effects on the results as typically, children’s activity includes short vigorous activity periods followed by ST or light PA (6). Finally, the cross-sectional study design does not allow us to draw a conclusion on the causality of the relationship between PA and arterial stiffness and their implications for the prevention of cardiovascular disease later in life. Therefore, longitudinal studies are needed to investigate whether certain levels of PA in childhood are associated with increased arterial stiffness in adulthood and whether they predict the risk of cardiovascular disease in adulthood (18).

In conclusion, we found that lower levels of PA exceeding the intensity of 4–6 METs had the strongest association with higher artery stiffness in children. We also observed that accumulating at least 68 minutes of PA exceeding the intensity of five METs and 26 minutes of PA exceeding six METs per day were optimal to reduce the risk of having increased arterial stiffness among children. Furthermore, our findings suggested that higher levels of physical

activity were related to lower SI regardless of sedentary time. These findings emphasize the role of at least moderate-intensity PA in maintaining normal arterial function since childhood.

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## REFERENCES

1. Abbott RA, Harkness MA, Davies PSW. Correlation of habitual physical activity levels with flow-mediated dilation of the brachial artery in 5-10 year old children. *Atherosclerosis*. 2002;160:233–239.
2. Andersen LB, Sardinha LB, Froberg K, Riddoch CJ, Page AS, Anderssen SA. Fitness, fatness and clustering of cardiovascular risk factors in children from Denmark, Estonia and Portugal: the European Youth Heart Study. *Int J Pediatr Obes*. 2008;3(Suppl 1):58–66.
3. Ashor AW, Lara J, Siervo M, Celis-Morales C, Mathers JC. Effects of exercise modalities on arterial stiffness and wave reflection: a systematic review and meta-analysis of randomized controlled trials. *PLoS One*. 2014;9(10):e110034.
4. Ashor AW, Lara J, Siervo M, et al. Exercise modalities and endothelial function: A systematic review and dose–response meta-analysis of randomized controlled trials. *Sport Med*. 2015;45(2):279–296.
5. Avolio A. Arterial stiffness. *Pulse*. 2013;1(1):14–28.
6. Bailey R, Olson J, Pepper S, Porszasz J, Barstow T, Cooper D. The level and tempo of children’s physical activities: an observational study. *Med Sci Sport Exerc*. 1995;27(7):1033–1041.
7. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. *NEJM* 1998;338(23):1650–1656.
8. Brage S, Brage N, Franks PW, Ekelund U, Wareham NJ. Reliability and validity of the combined heart rate and movement sensor Actiheart. *Eur J Clin Nutr*. 2005;59:561–570.
9. Brage S, Brage N, Franks PW, et al. Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure. *J Appl Physiol*. 2004;96(1):343–351.
10. Brage S, Ekelund U, Brage N, et al. Hierarchy of individual calibration levels for heart rate and accelerometry to measure physical activity. *J Appl Physiol* 2007;103(2):682–92.
11. Brage S, Westgate K, Wijndaele K, Godinho J, Griffin S, Wareham N. Evaluation of a method for minimising diurnal information bias in objective sensor data. *Int Conf Amb Mon Phys Act Mov*. 2013 (Conference Proceeding).
12. Cayres SU, Agostinete RR, de Moura Mello Antunes B, Lira FS, Fernandes RA. Impact of physical exercise/activity on vascular structure and inflammation in pediatric populations: A literature review. *J Spec Pediatr Nurs*. 2016;21(3):99–108.
13. Cole T, Bellizzi M, Flegal K, Dietz W. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;320(7244):1240–1243.

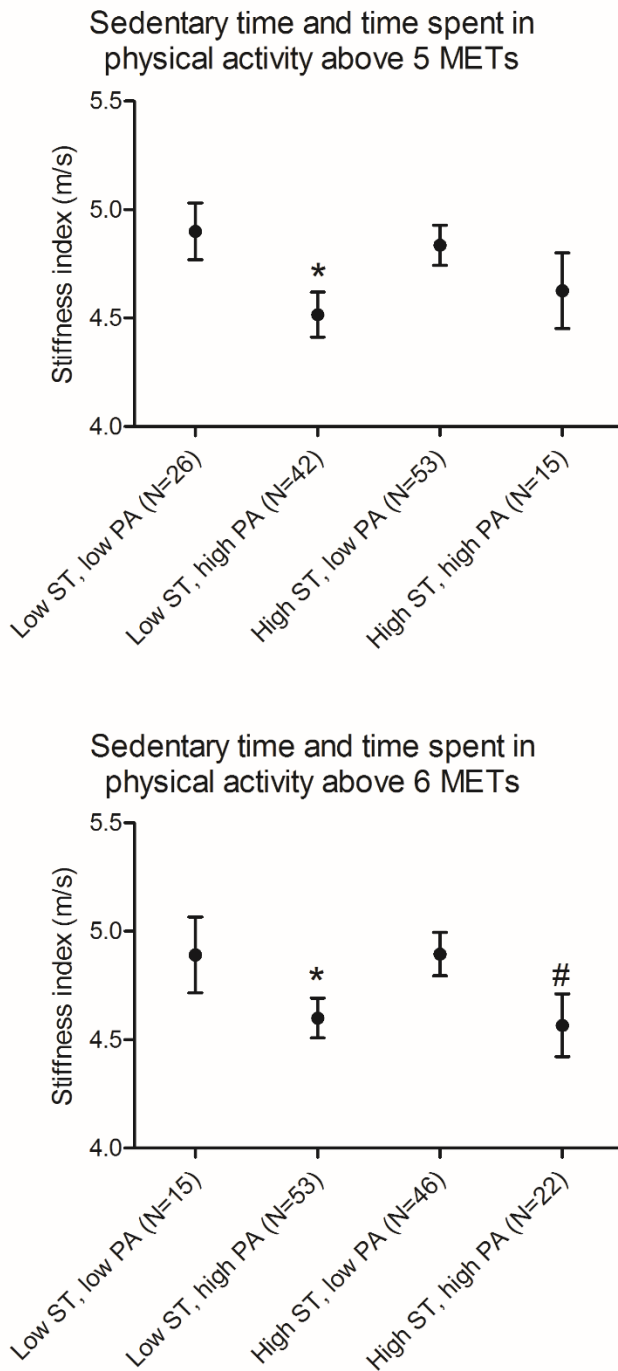
14. Corder K, van Sluijs E, Wright A, Whincup P, Wareham NJ, Ekelund U. Is it possible to assess free-living physical activity and energy expenditure in young people by self-report? *Am J Clin Nutr.* 2009;89:862–870.
15. Dias KA, Green DJ, Ingul CB, Pavey TG, Coombes JS. Exercise and vascular function in child obesity: A meta-analysis. *Pediatrics.* 2015;136(3):648–659.
16. Ekelund U, Tomkinson G, Armstrong N. What proportion of youth are physically active? Measurement issues, levels and recent time trends. *Br J Sports Med.* 2011;45(11):859–865.
17. Eloranta A-M, Lindi V, Schwab U, et al. Dietary factors associated with overweight and body adiposity in Finnish children aged 6-8 years: the PANIC Study. *Int J Obes.* 2012;36(7):950–955.
18. Fernhall B, Agiovlasitis S, Rowland T, Saltin B. Arterial function in youth : window into cardiovascular risk. *J Appl Physiol.* 2008;105:325–333.
19. Gielen S, Schuler G, Adams V. Cardiovascular effects of exercise training: Molecular mechanisms. *Circulation.* 2010;122(12):1221–1238.
20. Gill J, Malkova D. Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. *Clin Sci* 2006;110:409–425.
21. Goto C, Higashi Y, Kimura M, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: Role of endothelium-dependent nitric oxide and oxidative stress. *Circulation.* 2003;108(5):530–535.
22. Green DJ, Spence A, Halliwill JR, Cable NT, Thijssen DHJ. Exercise and vascular adaptation in asymptomatic humans. *Exp Physiol.* 2011;96(2):57–70.
23. Hopkins ND, Stratton G, Tinken TM, et al. Relationships between measures of fitness, physical activity, body composition and vascular function in children. *Atherosclerosis.* 2009;204(1):244–249.
24. Horta BL, Schaan BD, Bielemann RM, et al. Objectively measured physical activity and sedentary-time are associated with arterial stiffness in Brazilian young adults. *Atherosclerosis.* 2015;243(1):148–154.
25. Juonala M, Jarvisalo MJ, Mäki-Torkko N, Kähönen M, Viikari JSA, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: The cardiovascular risk in young finns study. *Circulation.* 2005;112(10):1486–1493.
26. Lintu N, Tompuri T, Viitasalo A, et al. Cardiovascular fitness and haemodynamic responses to maximal cycle ergometer exercise test in children 6–8 years of age. *J Sports Sci.* 2014;32:652–659.
27. Lintu N, Viitasalo A, Tompuri T, et al. Cardiorespiratory fitness, respiratory function and hemodynamic responses to maximal cycle ergometer exercise test in girls and boys aged 9–11 years: the PANIC Study. *Eur J Appl Physiol.* 2014;115:235–243.

28. McGill HC, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of atherosclerosis in childhood and adolescence. *Am J Clin Nutr.* 2000;72(5 Suppl):1307–1315.
29. Melo X, Santa-Clara H, Pimenta NM, et al. Intima-media thickness in 11-13 years-old children: variation attributed to sedentary behavior, physical activity, cardiorespiratory fitness and waist circumference. *J Phys Act Health.* 2014;610–617.
30. Nettlefold L, McKay HA, Naylor P-J, Breding S, Warburton DER. The relationship between objectively measured physical activity, sedentary time, and vascular health in children. *Am J Hypertens.* 2012;25(8):914–919.
31. Pahkala K, Heinonen OJ, Lagström H, et al. Vascular endothelial function and leisure-time physical activity in adolescents. *Circulation* 2008;118(23):2353–9.
32. Pahkala K, Heinonen OJ, Simell O, et al. Association of physical activity with vascular endothelial function and intima-media thickness. *Circulation.* 2011;124(18):1956–1963.
33. Palombo C, Kozakova M. Arterial stiffness, atherosclerosis and cardiovascular risk: Pathophysiologic mechanisms and emerging clinical indications. *Vascul Pharmacol.* e-pub ahead of print 2015 Nov 28. doi: 10.1016/j.vph.2015.11.083.
34. Perkins NJ, Schisterman EF. The inconsistency of “optimal” cutpoints obtained using two criteria based on the receiver operating characteristic curve. *Am J Epidemiol.* 2006;163(7):670–675.
35. Pälve KS, Pahkala K, Magnussen CG, et al. Association of physical activity in childhood and early adulthood with carotid artery elasticity 21 years later: the cardiovascular risk in Young Finns Study. *J Am Heart Assoc.* 2014;3(2):e000594.
36. Reed KE, Warburton DER, Lewanczuk RZ, et al. Arterial compliance in young children: the role of aerobic fitness. *Eur J Cardiovasc Prev Rehabil.* 2005;12(5):492–497.
37. Ried-Larsen M, Grøntved A, Froberg K, Ekelund U, Andersen LB. Physical activity intensity and subclinical atherosclerosis in Danish adolescents: The European Youth Heart Study. *Scand J Med Sci Sport.* 2013;23(3):168–177.
38. Ried-Larsen M, Grøntved A, Kristensen PL, Froberg K, Andersen LB. Moderate-and-vigorous physical activity from adolescence to adulthood and subclinical atherosclerosis in adulthood: prospective observations from the European Youth Heart Study. *Br J Sports Med.* 2015;49(2):107-112.
39. Millasseau SC, Kelly RP, Ritter JM, Chowienczyk PJ. Determination of age-related increases in large artery stiffness by digital pulse contour analysis. *J Chem Inf Model.* 2002;103(4):371–377.
40. Saari A, Sankilampi U, Hannila M-L, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med.* 2011;43(3):235–248.

41. Sakuragi S, Abhayaratna K, Gravenmaker KJ, et al. Influence of adiposity and physical activity on arterial stiffness in healthy children the lifestyle of our kids study. *Hypertension*. 2009;53(4):611–616.
42. Schack-Nielsen L, Molgaard C, Larsen D, Martyn C, Michaelsen KF. Arterial stiffness in 10-year-old children: current and early determinants. *Br J Nutr*. 2005;94(6):1004–1011.
43. Stegle O, Fallert S, DJC M, Brage S. Gaussian process robust regression for noisy heart rate data. *IEEE Trans Biomed Eng*. 2008;55(9):2143–2151.
44. Tanner J. *Growth at adolescence*. Oxford: Blackwell; 1962.
45. Thijssen DHJ, Maiorana AJ, O’Driscoll G, Cable NT, Hopman MTE, Green DJ. Impact of inactivity and exercise on the vasculature in humans. *Eur J Appl Physiol*. 2010;108(5):845–875.
46. Tompuri TT, Lakka TA, Hakulinen M, et al. Assessment of body composition by dual-energy X-ray absorptiometry, bioimpedance analysis and anthropometrics in children: the Physical Activity and Nutrition in Children study. *Clin Physiol Funct Imaging*. 2015;35:21–33.
47. Veijalainen A, Tompuri T, Haapala EA, et al. Associations of cardiorespiratory fitness, physical activity, and adiposity with arterial stiffness in children. *Scand J Med Sci Sports*. 2016;26(8):943–950.
48. Veijalainen A, Tompuri T, Laitinen T, et al. Metabolic risk factors are associated with stiffness index, reflection index and finger skin temperature in children. *Circ J*. 2013;77(5):1281–1288.
49. Veijalainen A, Tompuri T, Lakka H-M, Laitinen T, Lakka TA. Reproducibility of pulse contour analysis in children before and after maximal exercise stress test: the Physical Activity and Nutrition in Children (PANIC) study. *Clin Physiol Funct Imaging*. 2011;31(2):132–138.
50. Viitasalo A, Eloranta A-M, Lintu N, et al. The effects of a 2-year individualized and family-based lifestyle intervention on physical activity, sedentary behavior and diet in children. *Prev Med*. 2016;87:81–88.
51. Viitasalo A, Lakka TA, Laaksonen DE, 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(5):940–949.
52. Visentin S, Grumolato F, Nardelli GB, Di Camillo B, Grisan E, Cosmi E. Early origins of adult disease: low birth weight and vascular remodeling. *Atherosclerosis*. 2014;237(2):391–399.
53. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness. A systematic review and meta-analysis. *J Am Coll Cardiol*. 2010;55(13):1318–1327.

54. Väistö J, Eloranta A-M, Viitasalo A, et al. Physical activity and sedentary behaviour in relation to cardiometabolic risk in children: cross-sectional findings from the Physical Activity and Nutrition in Children (PANIC) Study. *Int J Behav Nutr Phys Act.* 2014;11(1):55.
55. Walker DJ, MacIntosh A, Kozyrskyj A, Becker A, McGavock J. The associations between cardiovascular risk factors, physical activity, and arterial stiffness in youth. *J Phys Act Health.* 2013;10(2):198–204.
56. World Health Organization. *Global Recommendations on Physical Activity for Health.* Geneva: 2010.57
57. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol.* 2005;25(5):932–943.





**Figure 1.** Combined associations of sedentary time (ST) and physical activity (PA) with stiffness index. MET=metabolic equivalent. \*children with lower levels of ST and higher levels of PA had a lower stiffness index than children with lower or higher levels of ST along with lower levels of PA ( $P<0.03$ ). #children with higher levels of both ST and PA had a lower stiffness index than children with lower or higher levels of ST along with lower levels of PA ( $P<0.01$ ).

**Table 1.** Characteristics of the children.

|  | All (n=136)     | Boys<br>(n=57)  | Girls<br>(n=79) | <i>P</i> for<br>difference |
|--|-----------------|-----------------|-----------------|----------------------------|
| Age (years)  | 7.6 (0.4)       | 7.7 (0.4)       | 7.6 (0.4)       | 0.017                      |
| Body height (cm)                                   | 129.0 (5.1)     | 130.5 (4.2)     | 128.0 (5.4)     | 0.002                      |
| Body weight (kg)                                   | 26.7 (4.0)      | 27.4 (3.8)      | 26.2 (4.1)      | 0.075                      |
| Body mass index standard deviation score           | -0.2 (1.0)      | -0.2 (1.0)      | -0.2 (1.0)      | 0.891                      |
| Prevalence of overweight, n (%)                    | 12 (8.8)        | 4 (7.0)         | 8 (10.1)        | 0.528                      |
| Body fat percentage                                | 19.6 (7.1)      | 16.5 (6.5)      | 21.7 (6.8)      | <0.001                     |
| Cardiorespiratory fitness (w/kg of lean body mass) | 3.8 (0.5)       | 4.0 (0.5)       | 3.6 (0.5)       | <0.001                     |
| Monitor wear time weekday (hours)                  | 62.5 (15.2)     | 63.1 (17.4)     | 62.2 (13.6)     | 0.821                      |
| Monitor wear time weekend day (hours)              | 38.6 (6.7)      | 38.1 (5.3)      | 38.8 (7.1)      | 0.573                      |
| Sedentary time (min/d)*                            | 190 (124)       | 175 (114)       | 210 (165)       | 0.166                      |
| Light PA (min/d)*                                  | 520 (124)       | 510 (100)       | 529 (141)       | 0.369                      |
| Moderate PA (min/d)*                               | 94.7 (78.0)     | 117 (95.4)      | 91.2 (68.4)     | 0.002                      |
| Vigorous PA (min/d)*                               | 19.2 (28.3)     | 23.6 (34.1)     | 14.7 (19.5)     | 0.001                      |
| Physical activity energy expenditure (kJ/kg/d)     | 103.7<br>(31.8) | 115.1<br>(32.0) | 95.5 (29.2)     | <0.001                     |
| Stiffness index (m/s)                              | 4.7 (0.4)       | 4.7 (0.4)       | 4.7 (0.4)       | 0.253                      |

The data are means (standard deviations) or \*medians (interquartile ranges) unless otherwise specified. The *P*-values are from the *t*-test for independent samples for continuous variables with normal distribution, the Mann-Whitney U-test for continuous variables with skewed distribution, or Chi-square test for the prevalence of overweight.

**Table 2.** Associations of sedentary time and light, moderate, and vigorous physical activity with stiffness index.

|                                   | All     |                    |              | Boys    |                    |          | Girls   |                    |              |
|-----------------------------------|---------|--------------------|--------------|---------|--------------------|----------|---------|--------------------|--------------|
|                                   | $\beta$ | 95% CI for $\beta$ | <i>P</i>     | $\beta$ | 95% CI for $\beta$ | <i>P</i> | $\beta$ | 95% CI for $\beta$ | <i>P</i>     |
| Sedentary time, min/d             | 0.144   | -0.030 to 0.318    | 0.105        | 0.043   | -0.243 to 0.329    | 0.764    | 0.201   | -0.022 to 0.424    | 0.077        |
| Light physical activity, min/d    | 0.048   | -0.126 to 0.223    | 0.583        | 0.216   | -0.060 to 0.493    | 0.122    | -0.049  | -0.277 to 0.180    | 0.673        |
| Moderate physical activity, min/d | -0.273  | -0.448 to -0.097   | <b>0.003</b> | -0.262  | -0.537 to 0.013    | 0.061    | -0.281  | -0.500 to -0.062   | <b>0.013</b> |
| Vigorous physical activity, min/d | -0.254  | -0.428 to -0.080   | <b>0.005</b> | -0.252  | -0.521 to 0.017    | 0.066    | -0.237  | -0.460 to -0.013   | <b>0.038</b> |

The data are standardized regression coefficients ( $\beta$ ) and their 95% confidence intervals (CI) adjusted for age, sex, and monitor wear time.