Longitudinal associations of physical activity, sedentary time, and cardiorespiratory fitness with arterial health in children – The PANIC Study

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ABSTRACT

Background: The evidence on the longitudinal associations of physical activity (PA), sedentary time (ST), and cardiorespiratory fitness (CRF) with arterial health is limited. Therefore, we investigated the longitudinal associations of PA, ST, and CRF with arterial health among children.

Methods: In our primary analyses, we investigated 245 children (girls 51.8%) aged 6-9 years participating in the baseline examinations of a 2-year PA and dietary intervention study. We also utilized a subsample of 90 children who had a complete arterial health data at baseline and 2-year follow-up. ST (≤1.5 METs), light PA (>1.5-4 METs), moderate PA (>4-7 METs), vigorous PA (>7METs), and moderate-to-vigorous PA (>4 METs) were assessed by combined body movement and heart rate monitoring and CRF (maximal power output) by maximal exercise testing on a cycle ergometer at baseline and 2-year follow-up. Stiffness index (SI) as a measure of arterial stiffness and change in reflection index during exercise test (ΔRI) as a measure of arterial dilation capacity were assessed by pulse contour analysis at 2-year follow-up. Data were analyzed by linear regression models adjusted for age and sex.

Results: 2-year change in vigorous PA was directly associated with ΔRI at 2-year follow-up (β=0.137, 95% CI=0.013 to 0.260). However, 2-year change in vigorous PA was associated with ΔRI in boys (β=0.208, 95% CI=0.027 to 0.388) but not in girls (β=0.042, 95% CI=-0.134 to 0.217; p=0.021 for interaction). In a subsample analyses, 2-year changes in MPA (β=-0.283, 95% CI=-0.484 to -0.082), VPA (β=-0.214, 95% CI=-0.421 to -0.007), and MVPA (β=-0.313, 95% CI=-0.512 to -0.114) were inversely associated with 2-year change in SI.

Conclusion: Increasing MPA and VPA during mid-childhood may be important in maintaining arterial heath in children. Therefore, promoting PA at higher intensities may confer larger benefits on arterial health than reducing ST and increasing LPA.

Keywords: Children, arterial stiffness, physical activity, sedentary time, cardiorespiratory fitness

INTRODUCTION

Cardiovascular diseases induce a substantial health problem causing premature morbidity and mortality and major economic burden worldwide.[1] The development of atherosclerosis is a slow process beginning already in childhood.[2] Autopsy studies have found atherosclerotic lesions of arterial walls in children, and cardiometabolic risk factors, such as increased body fat content, elevated blood pressure, and increased serum cholesterol, in childhood have been associated with these atherosclerotic changes in adulthood.[3] In addition, increased arterial stiffness and endothelial dysfunction have been associated with obesity, hypertension, and hypercholesterolemia already in childhood.[4] Increased arterial stiffness and impaired endothelial function are among the first measurable signs of cardiovascular disease progression reflecting pathological changes in the structure and function of the arteries[5] and they predict future cardiovascular events in adults.[6, 7] The beginning of the development of cardiovascular diseases in childhood emphasizes the early prevention of clinical cardiovascular changes.[2]

Exercise training, especially at higher intensities, has been found to reduce arterial stiffness and improve endothelial function in adults.[8,9] The results of some cross-sectional studies also suggest an inverse association of total physical activity (PA)[10] or moderate-to-vigorous PA (MVPA) [11] with arterial stiffness in children. Furthermore, total PA[12] and vigorous PA (VPA)[13] have been directly associated with endothelial function. In addition, a decrease in VPA was related to an impairment in endothelial function over 4-6 months in children aged 10-11 years.[14] However, some cross-sectional studies have reported statistically insignificant associations of total PA[15-17] or MVPA[18] with different measures of arterial stiffness in children. Because of these contradictory observations, especially longitudinal studies about the associations of PA at different intensities with arterial function in children are warranted. Furthermore, little is known about the relationship of sedentary time (ST) with arterial stiffness and endothelial

function among children. The results of few cross-sectional studies suggest weak if any associations of ST with measures of arterial health in pediatric populations.[11,18-20]

Higher cardiorespiratory fitness (CRF), assessed either by field tests[15,16] or by exercise tests indirectly using maximal power output[20] or directly using peak oxygen uptake (VO2peak)[13,21], has been related to lower arterial stiffness or better endothelial function in previous cross-sectional studies among children. Nevertheless, only few of these studies have used CRF scaled by lean body mass (LM)[20,21] which is recommended to minimize the influence of body size and composition on CRF.[22] We have earlier reported that higher maximal power output scaled by LM was associated with lower arterial stiffness and better arterial dilatation capacity in a cross-sectional study among children aged 6-8 years.[20] However, in our previous cross-sectional study, VO2peak per LM was associated with arterial dilatation capacity but not with arterial stiffness in children aged 8-11 years.[21] Because of these mixed results from studies using varying study designs and methodologies, more research dealing with the association between CRF and arterial health is warranted.

There are limited number of longitudinal studies examining the associations of intensity-specific PA, ST, and CRF with early signs of cardiovascular diseases in children. Therefore, we first investigated the associations of PA at different intensities, ST, CRF at baseline with arterial stiffness and arterial dilation capacity two years later among school-aged children. Second, we studied whether changes in PA at different intensities, ST, and CRF during 2-year follow-up are related to arterial stiffness and arterial dilation capacity at 2-year follow-up assessment. Finally, we conducted the analyses of changes in PA, ST, and CRF with changes in arterial stiffness and arterial dilation capacity over 2-years in a sub-sample of children.

METHODS

Study design and participants

The present longitudinal analyses are based on the baseline and 2-year follow-up data of the Physical Activity and Nutrition in Children (PANIC) study, that is a long-term PA and dietary intervention and follow-up study in a population sample of children from the city of Kuopio, Finland. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio. The parents or caregivers of the children gave their written informed consent, and the children provided their assent to participation. The PANIC study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2008.

Altogether 736 children aged 6–9 years from primary schools of Kuopio were invited to participate in the baseline examinations in 2007–2009, and a total of 512 children (70% of those invited) participated. The participants did not differ in sex distribution, age, or body mass index standard deviation score from all children who started the first grade in Kuopio in 2007–2009 based on data from the standard school health examinations (data not shown). 2-year follow-up examinations were conducted in 2009-2011, and a total of 440 children (87 % of invited children) participated.

Arterial stiffness and arterial dilatation capacity were assessed in a subsample of 230 children at baseline and from 400 children at 2-year follow-up. For the present main analyses dealing with the prospective associations of PA, ST, and CRF with arterial stiffness and arterial dilatation capacity, we only used these measures of arterial health assessed at 2-year follow-up to maintain a sufficient sample size. Valid data on variables used for the present analyses were available for 245 children (girls 51.8%). We also performed analyses in a subsample of 90

children (girls 54.4%) with complete data on measures of PA, ST, CRF, arterial stiffness, and arterial dilatation capacity at baseline and 2-year follow-up.

Assessment of physical activity and sedentary time

PA and ST were assessed using a combined heart rate and body movement sensor (Actiheart®, CamNtech Ltd., Papworth, UK) for a minimum of four consecutive days without interruption, including two weekdays and two weekend days, analyzed in 60 second epochs.[23] The combined heart rate and movement sensor was attached to the child´s chest with two standard eletrocardiogram electrodes (Bio Protech Inc, Wonju, South Korea). The children were instructed to wear the monitor continuously, including sleep and water-based activities, and not to change their usual behavior during the monitoring period.

We pre-processed heart rate[24] and estimated PA intensity time-series using individual calibration of heart rate combined with movement in a branched equation modelling framework, as explained in detail earlier.[25,26] We classified non-wear as >90min periods of non-movement if accompanied by non-physiological heart rate, and accounted for this when summarizing the time-series.[27] PA was summarized as daily PA volume (kJ/day/kg) and time spent at specific intensity levels in standard metabolic equivalents of task (METs) in minutes per day. For the present analyses, we re-categorized these intensity categories into a broader groups of sedentary time (≤1.5 METs), LPA (>1.5 -4 METs), MPA (>4-7 METs), VPA (>7METs), and MVPA (>4 METs), which have been commonly applied in investigations of PA among children and youth. In order to estimate the time spent sedentary whilst awake, we subtracted average daily sleep duration from total ST. We only included children who had sufficient valid data, i.e. a recording period of at least 48 hours of wear data. Furthermore, at least 12 hours of wear data from all four quadrants of a 24-hour-day (morning (3 am – 9 am),

noon (9 am – 3 pm), afternoon / evening (3 pm – 9 pm), and night (9 pm – 3 am)) was required to avoid bias from over-representation of specific times of the day.

Assessment of cardiorespiratory fitness

We assessed CRF by a maximal exercise test using an electromagnetically braked Ergoselect 200 K® cycle ergometer coupled with a pediatric saddle module (Ergoline, Bitz, Germany), as explained in more detail earlier.[28] 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 5 watts; a 1-minute steady-state period with a workload of 20 watts; an exercise period with an increase in the workload of 1 watt per 6 seconds until exhaustion, and a 4-minute recovery period with a workload of 5 watts. The children were asked to keep the cadence stable and within 70–80 revolutions per minute. The exercise test was considered maximal, if the reason for terminating the test indicated maximal effort and maximal cardiorespiratory capacity. Maximal power output measured at the end of the exercise test divided by LM was used as a measure of CRF. Maximal power output per LM has been found to be a good surrogate measure of CRF in children.[29]

Assessment of arterial stiffness and dilatation capacity

A research physician assessed arterial stiffness with stiffness index (SI) and arterial dilation capacity with reflection index (RI) by pulse contour analysis based on noninvasive finger photoplethysmography using the PulseTrace PCA2® device (Micro Medical, Gillingham, Kent, United Kingdom) as explained in detail earlier.[30] Another research physician confirmed and recorded the digital volume pulse contours using the manufacturer's instructions. 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°C–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 larger SI indicated stiffer, 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. A larger RI indicated a higher arterial tone. We calculated the acute change in RI (ΔRI) in response to exercise as the difference between RI before and after the exercise test. A larger difference in ΔRI indicated a better arterial dilatation capacity. We have earlier reported the evaluation of pulse contour analysis quality and have shown relatively good reliability for these measures.[30,31] ΔRI 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.[32]

Assessment of body size, body composition, blood pressure, and maturity

Body weight was measured twice with the children having fasted for 12 hours, emptied the bladder, and standing in light underwear using a weight scale integrated into a calibrated InBody® 720 bioelectrical impedance device (Biospace, Seoul, South Korea) to an accuracy of 0.1 kg. The mean of these two values was used in the analyses. Stature was measured three times with the children standing in the Frankfurt plane without shoes using a wall-mounted stadiometer to an accuracy of 0.1 cm. The mean of the nearest two values was used in the analyses. Body fat percentage (BF%), and LM were measured by the Lunar® dual-energy X-ray absorptiometry device (GE Medical Systems, Madison, WI, USA) using standardized protocols. Systolic and diastolic blood pressure (BP) was measured from the right arm using the Heine Gamma® G7 aneroid sphygmomanometer (Heine Optotechnik, Herrsching, Germany) to an accuracy of 2 mmHg. 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 in the analysis. Maturity was estimated with maturity offset which was calculated for boys and girls from sex-specified prediction models using estimated years from peak height velocity.[33]

Statistical methods

The statistical analyses were performed using IBM SPSS Statistics software, version 25.0 (IBM Corp. Armonk, NY, USA). We estimated statistical power using G*Power software (version 3.1.9.7.). One hundred and ninety three observations was needed to observe the correlation of 0.2 at the power of 0.80 when statistical significance level was set at alpha level of 0.05. Moreover, a correlation coefficient needed to reveal statistical significance at the alpha level of 0.05 was 0.30 in a subsample of 90 children.

Differences in baseline characteristics between sexes were tested using the independent samples T-test for variables with normal distributions and the Mann-Whitney U-test for variables with skewed distributions. The longitudinal associations of PA at different intensities, ST and CRF at baseline, and changes in these variables during 2- year follow-up as independent variables with arterial stiffness and arterial dilatation capacity at 2-year follow-up as dependent variables were analyzed using linear regression models adjusted for age and sex. First, PA at different intensities, ST, and CRF at baseline were entered into the linear regression models one by one with age at baseline (or alternatively maturity offset) and sex. If a statistically significant association was observed, the data were further adjusted for baseline BF% and systolic BP, change in BF% and systolic BP during 2-year follow-up, or study group (intervention/control) and the corresponding explanatory variable at baseline. The study group was used as a confounding factor to adjust for the residual effect of the lifestyle intervention. However, there were no statistically significant differences in PA at different intensities, ST, CRF, SI, or ΔRI between children in the intervention and the control group ($p>0.070$). Nevertheless, we also performed sensitivity analyses separately for intervention and control

groups. The analyses were adjusted for the explanatory variable at baseline to control for their variation at baseline. Furthermore, changes in VPA and CRF over 2 years were entered into the same model with age and sex to study their independent associations with SI and ΔRI at 2-year follow-up. Changes in the measures of PA, CRF, and arterial health were computed by subtracting 2-year value from the baseline value.

We investigated the modifying effects of sex on the associations of PA at different intensities, ST, and CRF with SI, or ΔRI using general linear models. If a statistically significant interaction was observed, the analyses were performed separately for boys and girls. These models for boys and girls were further adjusted for baseline or 2-year BF% and systolic BP, changes in BF% and systolic BP during 2-year follow-up or study group, if statistically significant associations were observed.

We investigated the associations of changes in PA and CRF with changes in arterial stiffness and dilatation capacity over 2 years adjusted for age and sex in a subsample of 90 children. These data were further adjusted for PA at corresponding intensity or CRF and arterial stiffness or arterial dilatation capacity at baseline. These models were further adjusted for changes in BF% or systolic BP during 2-year follow-up or study group, if statistically significant association was observed. These analyses were performed only for the whole study sample due to the small sample size for sex-specific analyses.

RESULTS

Descriptive characteristics

Girls were younger, shorter, and lighter and had a higher body fat percentage and maturity offset compared with boys (Table 1). Boys accumulated more MPA, VPA, and MVPA, and had higher CRF, and lower ΔRI than girls.

Associations of PA, ST and CRF at baseline with arterial stiffness and arterial dilatation capacity at 2-year follow-up

LPA, MPA, VPA, MVPA, ST, or CRF at baseline were not associated with SI or ΔRI at 2-year follow-up after adjustment for age and sex (Table 2). These results remained similar when data were adjusted for maturity offset instead of age.

Associations of changes in PA, ST and CRF over 2 years with arterial stiffness and dilatation capacity at 2-year follow-up

A change in VPA over 2 years was directly associated with ΔRI at 2-year follow-up adjusted for age and sex (Table 2). This association remained statistically significant after further adjustments for VPA, BF%, and systolic BP at baseline and study group (β=0.174, 95% CI=0.038 to 0.309). The association also remained statistically significant with further adjustment for 2-year changes in BF% and systolic BP (β=0.164, 95% CI=0.026 to 0.302). Changes in LPA, MPA, MVPA, ST, or CRF were not associated ΔRI at 2-year follow-up adjusted for age and sex. A change in CRF over 2-year follow-up had a borderline statistically significant positive association with ΔRI at 2-year follow-up after adjustment for age and sex. This relationship was further attenuated when a change in VPA was entered in the same model (β=0.108, 95% CI=-0.016 to 0.231). In this model, the association between change in VPA over 2 years and ΔRI at 2-year follow-up was slightly attenuated but remained statistically significant (β=0.127, 95% CI=0.003 to 0.251). Changes in LPA, MPA, MVPA, VPA, ST, and

CRF were not related to SI at 2- year follow-up adjusted for age and sex. These results remained similar when the data were adjusted for maturity offset instead of age.

A change in VPA over 2-year follow-up was positively associated with ΔRI at 2-year follow-up in boys (β=0.208, 95% CI=0.027 to 0.388) but not in girls (β=0.042, 95% CI=-0.134 to 0.217; p=0.021 for interaction). The association in boys remained statistically significant after further adjustment for VPA, maturity offset, BF% and systolic BP at baseline, changes in BF% and systolic BP over 2 years, and study group. In girls, age (β=0.217, 95% CI=0.044 to 0.390) and maturity offset at baseline (β=0.275, 95% CI=0.105 to 0.445) were positively related to ΔRI at 2-year follow-up.

The sensitivity analyses revealed that the magnitude of the positive association between a change in VPA over 2 years and ΔRI at 2-year follow-up was relatively similar (p=0.517 for interaction) 141 children from the intervention group (β=0.184, 95% CI=0.018 to 0.350) and for 104 children from the control group (β=0.99, 95% CI=-0.056 to 0.173). The magnitude of this association was also relatively similar for 69 boys from the intervention group (β=0.215, 95% CI=-0.025 to 0.455) and for 49 boys from the control group (β=0.218, 95% CI=-0.077 to 0.512, p=0.224 for interaction 0.824) and for 72 girls from the intervention group (β=0.163, 95% CI=-0.075 to 0.401) and for 55 girls from the control group (β=-0.037, 95% CI=-0.307 to 0.232, p=0.224 for interaction).

Associations of changes in PA, ST and CRF with changes in arterial stiffness and dilatation capacity over 2 years follow-up in a subsample of children

Changes in MPA, VPA, and MVPA were inversely associated with changes in SI after adjustment for age and sex (Table 3). The inverse association of a change in MPA (β=-0.327, 95% CI=-0.592 to -0.062), VPA (β=-0.224, 95% CI=-0.445 to -0.003), and MVPA (β=-0.276, 95% CI=-0.551 to -0.002) with change in SI remained statistically significant after further adjustment for corresponding PA intensity and SI at baseline. Adjustment for change in SBP had no effect on the association. Nevertheless, the inverse associations of changes in VPA (β=-0.152, 95% CI=-0.400 to 0.096) and MVPA (β=-0.202, 95% CI=-0.490 to 0.086) with changes in SI were weakened after additional adjustment for change in BF%.

Change in CRF was directly associated with a change in ΔRI after adjustment for age and sex (Table 3), but the respective relationship weakened after further adjustment for CRF and ΔRI at baseline (β=0.136, 95% CI=-0.080 to 0.352). Furthermore, a change in LPA was inversely associated with change in ΔRI after adjustment for age, sex, and LPA and ΔRI at baseline (β=-0.287, 95% CI=-0.562 to -0.012). Further adjustments had no effect on the magnitude of the association.

DISCUSSION

In the present longitudinal study, a larger increase in VPA over 2 years was independently associated with better arterial dilatation capacity in response to a single bout of exercise at 2-year follow-up among school-aged children, particularly among boys. We observed no other associations of PA intensities, ST, or CRF with arterial dilatation capacity or any of the explanatory variables and arterial stiffness at 2-year follow-up. Furthermore, a change in

MVPA was inversely associated with change in SI over 2 years in a subsample of children. However, this relationship was partly explained by a change in BF%.

Our finding on the positive association between change in VPA over 2 years and arterial dilatation capacity at 2-year follow-up is in accordance with the results of earlier studies in children.[13,14] These observations suggest that PA at higher intensities may be an important determinant of arterial function in children. These findings in children support the evidence from intervention studies in adults that high-intensity exercise enhances arterial function more than PA at lower intensities.[9] The positive relationship between VPA and arterial dilatation capacity could be explained by improvements in nitric oxide-dependent vasodilatation through increased endothelial shear stress as a response to exercise.[34] However, exercise may induce a larger increase in nitric oxide-dependent vasodilatation in individuals with impaired arterial function whereas younger and health individuals may need higher exercise volumes or intensities to obtain such a beneficial effect on arterial function. [34] Therefore, high intensity PA may be needed to activate sufficient nitric oxide production among healthy children, which may explain our observation that only VPA was associated with arterial function.

We observed that the positive association between changes in VPA and ΔRI was mainly due to the stronger positive association in boys. This is a similar finding to that of another longitudinal study among school-aged children in which arterial dilatation capacity was assessed by flow-mediated dilation.[14] In girls, we observed that a change in maturity was positively related to ΔRI at 2-year follow-up which supports a result from a previous study in children.[35] In the present study sample, girls had a higher maturity level at baseline than boys. Because sex hormones may affect on the arterial structure and function[36], the sex disparities found in our study could be partly explained by earlier puberty in girls. However, it should be considered that girls had lower levels of daily VPA than boys. Therefore, girls might have not

engaged enough VPA in order to improve arterial function, which may be one plausible explanation for the different results between sexes in the present study.

In contrast to our previous cross-sectional study in children aged 6-8 years showing an inverse association between MVPA and SI,[11] we found no statistically significant association of PA at different intensities at baseline or changes in PA during the 2-year follow-up with arterial stiffness at 2-year follow-up in the present study. However, we observed that a change in MVPA was inversely related to a change in arterial stiffness in a subsample of children suggesting that increasing MVPA during childhood could slow-down the age-related increase in arterial stiffness. These results from our study agree with those inconsistent findings from previous studies. For example, a cross-sectional study showed that higher levels of MVPA were associated only with higher small artery compliance but not with large arterial compliance in children 8-11 years of age.[18] MVPA has neither been associated with arterial stiffness measured by PWV in a cross-sectional study among adolescents aged 15-16 years.[37] The inconsistent observations in children and adolescents of different ages could be partly explained by the development of the changes in the size and compliance of arteries during normal growth[36] which may compensate for the development of arterial stiffness. This normal variability in arterial stiffness may also explain why PA at baseline or change in PA was not associated with arterial stiffness when baseline arterial stiffness was not accounted for. Nevertheless, we also found that the longitudinal association between changes in MVPA and changes in arterial stiffness was weakened after adjustment for BF%. Therefore, these results together indicate that PA may improve arterial compliance since childhood but that this effect may be partly mediated by its beneficial effects on body fat content.

Our findings on the lack of association of ST with SI or ΔRI are in line with previous observations in children[11,18-20], suggesting that ST may not have a notable influence on arterial

health among school-aged children. However, higher levels of ST have been linked to increased arterial stiffness in adults.[38-40] Thus, it is possible that the adverse effects of ST on arteries occur in adulthood when the accumulated exposure is more severe. Children may also naturally break ST more often than adults. Breaking ST has been suggested to preserve normal endothelial function[41] that may be one explanation for the weak association between total ST and arterial health in children. Moreover, the development of arteries during childhood[36] may partly compensate for the adverse effects of ST on arteries among children and thus explain the different findings in children and adults.

Our result suggesting no association between CRF and arterial measures contrasts with the findings of previous studies in children.[13,15,16,20,21] However, most previous studies have used different methods for assessing CRF and arterial health. Therefore, it is difficult to directly compare these results with our observations. In the present study, we defined CRF as maximal power output per LM and found that a change in CRF had a modest positive association with ΔRI at 2- year follow-up that was largely explained by a change in VPA. Nonetheless, in our earlier cross-sectional study, maximal power output per LM was favorably associated with SI and ΔRI in children aged 6-8 years.[20] In our previous cross-sectional study among 9-11- years old children, we also found a direct association between VO2peak per LM and ΔRI only in boys. In that study, however, no association was found between VO2peak per LM and SI[21] that is consistent with the present observations. The development of arteries during children's normal growth[36] may explain the lack of association in the present study.

The strengths of our study include the longitudinal study design and relatively large population sample of children, the device-based assessment of PA and ST by individually calibrated combined heart rate and movement sensing, the directly measured maximal power output scaled by DXA-measured LM, and the comprehensive adjustment for confounding factors. The

main limitation of the study is the use of SI and ΔRI that are only surrogate measures of arterial stiffness and endothelial function and that the RI was not assessed in response to a standardized bout of exercise. Nevertheless, SI has correlated strongly with direct carotid-femoral PWV among adults.[42] Moreover, ΔRI reflects arterial dilatation capacity as a response to singe bout of exercise that may be related to an activation of endothelium-derived nitric-oxide bioavailability.[43] In the present study, we were able to use baseline SI and ΔRI measurements only among a subsample of children because remarkably reduced the study sample. Moreover, we collected PA in 60-second epochs and as children accumulate MPA and VPA in short bouts, it is possible that our results underestimate the true magnitude of the associations of PA with arterial stiffness and dilatation capacity. We did not use directly measured VO2peak which is considered as the gold standard method for assessing CRF in children.[44] Although maximal power output has been shown to be a good surrogate measure for directly measured CRF in children[45], it not only reflect cardiorespiratory performance but also neuromuscular performance.[46] While we were able to adjust the data for potential confounding factors, we cannot exclude the possibility that the results are influenced by residual confounding. Furthermore, the relatively large number of analyses increases the likelihood that some associations were observed by chance. Finally, the longitudinal study design does not allow drawing firm conclusions about the causality of the observed association.

In conclusion, the results of our longitudinal study suggest that VPA may improve arterial dilatation capacity among children, particularly among boys. Our findings thus emphasize the role of increasing VPA to improve arterial health since childhood. Our study also provides some evidence that MVPA may attenuate the increase in arterial stiffness in children. Therefore, increasing MPA and VPA during mid-childhood may be important in maintaining arterial heath in children and promoting PA at higher intensities may confer larger benefits on arterial health than reducing ST and increasing LPA. More research on the longitudinal

associations of PA at different intensities, ST, and CRF with arterial health during childhood and adolescence is warranted to inform future guidelines to prevent cardiovascular disease since childhood.

DISCLOSURE OF INTERESTS

The Authors report no conflict of interests.

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Table 1. Baseline characteristics

	All (n=245)	Girls (n=127)	Boys (n=118)	P
Age (years)	7.7 (0.4)	7.6 (0.4)	7.8 (0.4)	**0.022**
Stature (cm)	129.1 (5.1)	127.9 (5.4)	130.6 (4.2)	**0.001**
Weight (kg)	26.8 (4.0)	26.4 (4.2)	27.4 (3.7)	**0.007**
BMI-SDS	-0.18 (1.0)	-0.17 (1.0)	-0.19 (1.0)	0.372
Maturity offset (years)	- 4.0 (0.5)	-3.6 (0.4)	-4 .4 (0.3)	**<0.001**
Body fat percentage (%)	19.6 (7.2)	22.0 (6.9)	16.7 (6.5)	**<0.001**
Systolic blood pressure (mmHg)	100 (7.0)	100 (7.1)	101 (6.9)	0.268
Sedentary time (min/d)	218 (119)	233 (120)	199 (116)	0.173
Light physical activity (min/d)	510 (97)	512 (100)	507 (92)	0.756
Moderate physical activity (min/d)	108 (56)	96 (51)	123 (58)	**0.006**
Vigorous physical activity (min/d)	25 (22)	18 (16)	33 (26)	**<0.001**
Moderate-to-vigorous physical activity (min/d)	133 (119)	114 (59)	156 (64)	**<0.001**
Physical activity energy expenditure (kJ/day/kg)	102 (22.6)	101 (22.6)	103 (24.5)	**0.001**
Maximal power output (Watts / kg lean mass)	3.8 (0.5	3.7 (0.5)	4.0 (0.5)	**<0.001**
Stiffness index (m/s)	5.0 (0.4)	5.0 (0.5)	5.0 (0.4)	0.844
Reflection index (%)	50.7 (12.1)	51.4 (12.0)	49.9 (12.3)	0.359
Δ Reflection index	26.4 (14.6)	29.4 (14.2)	23.1 (14.4)	**0.001**

Data are mean and standard deviation and the p-values are from the independent samples t-test. BMI-SDS, body mass index standard deviation score. Note: stiffness index (m/s), reflection index (%), and Δ Reflection index (%) were measured at 2-year follow-up.

Table 2. Associations of physical activity, sedentary time, and cardiorespiratory fitness at baseline and their changes over 2-years with arterial stiffness and dilatation capacity at 2-year follow-up in 245 children.

	Stiffness index (m/s)		Δ Reflection index (%)	
	β	95% CI	β	95% CI
Physical activity, sedentary time, and cardiorespiratory fitness at baseline				
Sedentary time (min / d)	-0.042	-0.169 to 0.086	-0.017	-0.141 to 0.108
Light physical activity (min / d)	-0.017	-0.145 to 0.111	0.050	-0.074 to 0.174
Moderate physical activity (min / d)	-0.041	-0.170 to 0.088	-0.011	-0.136 to 0.114
Vigorous physical activity (min / d)	-0.056	-0.187 to 0.075	-0.008	-0.136 to 0.119
Moderate-to-vigorous physical activity (min / d)	-0.030	-0.162 to 0.101	-0.044	-0.172 to 0.084
Maximal power output (Watts / kg lean mass)	-0.088	-0.224 to 0.048	-0.031	-0.164 to 0.102
Changes in physical activity, sedentary time, and cardiorespiratory fitness				
Δ Sedentary time	-0.026	-0.153 to 0.101	0.028	-0.096 to 0.152
Δ Light physical activity (min / d)	0.036	-0.091 to 0.163	-0.070	-0.193 to 0.053
Δ Moderate physical activity (min / d)	-0.009	-0.136 to 0.118	-0.013	-0.111 to 0.136
Δ Vigorous physical activity (min / d)	0.058	-0.070 to 0.186	**0.137**	**0.013 to 0.260**
Δ Moderate-to-vigorous physical activity (min / d)	0.014	-0.113 to 0.142	0.061	-0.062 to 0.185
Δ Maximal power output (Watts / kg lean mass)	0.045	-0.083 to 0.173	0.119	-0.005 to 0.242

Data are standardized regression coefficients with their 95% confidence intervals (CI). Data were adjusted for age and sex.

Table 3. Associations of changes in physical activity, sedentary time, and cardiorespiratory fitness with changes in arterial stiffness and dilatation capacity over 2 years in 90 children.

	Change in stiffness index (m/s)		Change in Δ Reflection index (%)	
	β	95% CI	β	95% CI
Δ Sedentary time (min / d)	0.087	-0.123 to 0.298	-0.001	-0.251 to .0253
Δ Light physical activity (min / d)	0.089	-0.120 to 0.298	-0.086	-0.324 to 0.139
Δ Moderate physical activity (min / d)	**-0.283**	**-0.484 to -0.082**	0.186	-0.027 to 0.421
Δ Vigorous physical activity (min / d)	**-0.214**	**-0.421 to -0.007**	0.068	-0.193 to 0.368
Δ Moderate-to-vigorous physical activity (min / d)	**-0.313**	**-0.512 to -0.114**	0.182	-0.033 to 0.440
Δ Maximal power output (Watts / kg lean mass)	0.006	-0.209 to 0.221	**0.263**	**0.049 to 0.476**

Data are standardized regression coefficients with their 95% confidence intervals (CI). Data were adjusted for age and sex.