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

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24 **ABSTRACT**

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

29 **Methods:** In our primary analyses, we investigated 245 children (girls 51.8%) aged 6-9 years
30 participating in the baseline examinations of a 2-year PA and dietary intervention study. We
31 also utilized a subsample of 90 children who had a complete arterial health data at baseline and
32 2-year follow-up. ST (≤ 1.5 METs), light PA ($>1.5-4$ METs), moderate PA ($>4-7$ METs),
33 vigorous PA (>7 METs), and moderate-to-vigorous PA (>4 METs) were assessed by combined
34 body movement and heart rate monitoring and CRF (maximal power output) by maximal
35 exercise testing on a cycle ergometer at baseline and 2-year follow-up. Stiffness index (SI) as
36 a measure of arterial stiffness and change in reflection index during exercise test (Δ RI) as a
37 measure of arterial dilation capacity were assessed by pulse contour analysis at 2-year follow-
38 up. Data were analyzed by linear regression models adjusted for age and sex.

39 **Results:** 2-year change in vigorous PA was directly associated with Δ RI at 2-year follow-up
40 ($\beta=0.137$, 95% CI=0.013 to 0.260). However, 2-year change in vigorous PA was associated
41 with Δ RI in boys ($\beta=0.208$, 95% CI=0.027 to 0.388) but not in girls ($\beta=0.042$, 95% CI=-0.134
42 to 0.217; $p=0.021$ for interaction). In a subsample analyses, 2-year changes in MPA ($\beta=-0.283$,
43 95% CI=-0.484 to -0.082), VPA ($\beta=-0.214$, 95% CI=-0.421 to -0.007), and MVPA ($\beta=-0.313$,
44 95% CI=-0.512 to -0.114) were inversely associated with 2-year change in SI.

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

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49 **Keywords:** Children, arterial stiffness, physical activity, sedentary time, cardiorespiratory

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69 **INTRODUCTION**

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71 Cardiovascular diseases induce a substantial health problem causing premature morbidity and
72 mortality and major economic burden worldwide.¹ The development of atherosclerosis is a
73 slow process beginning already in childhood.² Autopsy studies have found atherosclerotic
74 lesions of arterial walls in children, and cardiometabolic risk factors, such as increased body
75 fat content, elevated blood pressure, and increased serum cholesterol, in childhood have been
76 associated with these atherosclerotic changes in adulthood.³ In addition, increased arterial
77 stiffness and endothelial dysfunction have been associated with obesity, hypertension, and
78 hypercholesterolemia already in childhood.⁴ Increased arterial stiffness and impaired
79 endothelial function are among the first measurable signs of cardiovascular disease progression
80 reflecting pathological changes in the structure and function of the arteries⁵ and they predict
81 future cardiovascular events in adults.^{6, 7} The beginning of the development of cardiovascular
82 diseases in childhood emphasizes the early prevention of clinical cardiovascular changes.²

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85 Exercise training, especially at higher intensities, has been found to reduce arterial stiffness
86 and improve endothelial function in adults.^{8,9} The results of some cross-sectional studies also
87 suggest an inverse association of total physical activity (PA)¹⁰ or moderate-to-vigorous PA
88 (MVPA)¹¹ with arterial stiffness in children. Furthermore, total PA¹² and vigorous PA (VPA)¹³
89 have been directly associated with endothelial function. In addition, a decrease in VPA was
90 related to an impairment in endothelial function over 4-6 months in children aged 10-11 years.¹⁴
91 However, some cross-sectional studies have reported statistically insignificant associations of
92 total PA¹⁵⁻¹⁷ or MVPA¹⁸ with different measures of arterial stiffness in children. Because of
93 these contradictory observations, especially longitudinal studies about the associations of PA
94 at different intensities with arterial function in children are warranted. Furthermore, little is
95 known about the relationship of sedentary time (ST) with arterial stiffness and endothelial

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

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100 Higher cardiorespiratory fitness (CRF), assessed either by field tests^{15,16} or by exercise tests
101 indirectly using maximal power output²⁰ or directly using peak oxygen uptake (VO₂peak)^{13,21},
102 has been related to lower arterial stiffness or better endothelial function in previous cross-
103 sectional studies among children. Nevertheless, only few of these studies have used CRF scaled
104 by lean body mass (LM)^{20,21} which is recommended to minimize the influence of body size and
105 composition on CRF.²² We have earlier reported that higher maximal power output scaled by
106 LM was associated with lower arterial stiffness and better arterial dilatation capacity in a cross-
107 sectional study among children aged 6-8 years.²⁰ However, in our previous cross-sectional
108 study, VO₂peak per LM was associated with arterial dilatation capacity but not with arterial
109 stiffness in children aged 8-11 years.²¹ Because of these mixed results from studies using
110 varying study designs and methodologies, more research dealing with the association between
111 CRF and arterial health is warranted.

112

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

121 **METHODS**

122 **Study design and participants**

123 The present longitudinal analyses are based on the baseline and 2-year follow-up data of the
124 Physical Activity and Nutrition in Children (PANIC) study, that is a long-term PA and dietary
125 intervention and follow-up study in a population sample of children from the city of Kuopio,
126 Finland. The study protocol was approved by the Research Ethics Committee of the Hospital
127 District of Northern Savo, Kuopio. The parents or caregivers of the children gave their written
128 informed consent, and the children provided their assent to participation. The PANIC study has
129 been carried out in accordance with the principles of the Declaration of Helsinki as revised in
130 2008.

131 Altogether 736 children aged 6–9 years from primary schools of Kuopio were invited to
132 participate in the baseline examinations in 2007–2009, and a total of 512 children (70% of
133 those invited) participated. The participants did not differ in sex distribution, age, or body mass
134 index standard deviation score from all children who started the first grade in Kuopio in 2007–
135 2009 based on data from the standard school health examinations (data not shown). 2-year
136 follow-up examinations were conducted in 2009–2011, and a total of 440 children (87 % of
137 invited children) participated.

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

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

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

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

156 We pre-processed heart rate²⁴ and estimated PA intensity time-series using individual
157 calibration of heart rate combined with movement in a branched equation modelling
158 framework, as explained in detail earlier.^{25,26} We classified non-wear as >90min periods of
159 non-movement if accompanied by non-physiological heart rate, and accounted for this when
160 summarizing the time-series.²⁷ PA was summarized as daily PA volume (kJ/day/kg) and time
161 spent at specific intensity levels in standard metabolic equivalents of task (METs) in minutes
162 per day. For the present analyses, we re-categorized these intensity categories into a broader
163 groups of sedentary time (≤ 1.5 METs), LPA ($>1.5 - 4$ METs), MPA ($>4-7$ METs), VPA
164 (>7 METs), and MVPA (>4 METs), which have been commonly applied in investigations of
165 PA among children and youth. In order to estimate the time spent sedentary whilst awake, we
166 subtracted average daily sleep duration from total ST. We only included children who had
167 sufficient valid data, i.e. a recording period of at least 48 hours of wear data. Furthermore, at
168 least 12 hours of wear data from all four quadrants of a 24-hour-day (morning (3 am – 9 am),

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

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174 **Assessment of cardiorespiratory fitness**

175 We assessed CRF by a maximal exercise test using an electromagnetically braked Ergoselect
176 200 K[®] cycle ergometer coupled with a pediatric saddle module (Ergoline, Bitz, Germany), as
177 explained in more detail earlier.²⁸ The exercise test protocol included a 2.5-minute anticipatory
178 period with the child sitting on the ergometer; a 3-minute warm-up period with a workload of
179 5 watts; a 1-minute steady-state period with a workload of 20 watts; an exercise period with an
180 increase in the workload of 1 watt per 6 seconds until exhaustion, and a 4-minute recovery
181 period with a workload of 5 watts. The children were asked to keep the cadence stable and
182 within 70–80 revolutions per minute. The exercise test was considered maximal, if the reason
183 for terminating the test indicated maximal effort and maximal cardiorespiratory capacity.
184 Maximal power output measured at the end of the exercise test divided by LM was used as a
185 measure of CRF. Maximal power output per LM has been found to be a good surrogate measure
186 of CRF in children.²⁹

187

188 **Assessment of arterial stiffness and dilatation capacity**

189 A research physician assessed arterial stiffness with stiffness index (SI) and arterial dilation
190 capacity with reflection index (RI) by pulse contour analysis based on noninvasive finger
191 photoplethysmography using the PulseTrace PCA2[®] device (Micro Medical, Gillingham,
192 Kent, United Kingdom) as explained in detail earlier.³⁰ Another research physician confirmed
193 and recorded the digital volume pulse contours using the manufacturer's instructions. SI and
194 RI were assessed in a supine position before and after a maximal exercise test in an exercise

195 test laboratory at a stable room temperature (20°C–22°C). SI was calculated as the ratio of
196 body height to time between the first (systolic) peak and the second (diastolic) peak of the pulse
197 contour and was expressed in meters per second. A larger SI indicated stiffer, less compliant
198 arteries. RI was estimated as the proportion of the height of the second peak from the height of
199 the first peak and was expressed in percentage. A larger RI indicated a higher arterial tone. We
200 calculated the acute change in RI (Δ RI) in response to exercise as the difference between RI
201 before and after the exercise test. A larger difference in Δ RI indicated a better arterial dilatation
202 capacity. We have earlier reported the evaluation of pulse contour analysis quality and have
203 shown relatively good reliability for these measures.^{30,31} Δ RI measured in response to
204 vasoactive agents has been found to have a relatively good agreement with flow-mediated
205 arterial dilatation with high sensitivity and specificity.³²

206

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

208 Body weight was measured twice with the children having fasted for 12 hours, emptied the
209 bladder, and standing in light underwear using a weight scale integrated into a calibrated
210 InBody® 720 bioelectrical impedance device (Biospace, Seoul, South Korea) to an accuracy
211 of 0.1 kg. The mean of these two values was used in the analyses. Stature was measured three
212 times with the children standing in the Frankfurt plane without shoes using a wall-mounted
213 stadiometer to an accuracy of 0.1 cm. The mean of the nearest two values was used in the
214 analyses. Body fat percentage (BF%), and LM were measured by the Lunar® dual-energy X-
215 ray absorptiometry device (GE Medical Systems, Madison, WI, USA) using standardized
216 protocols. Systolic and diastolic blood pressure (BP) was measured from the right arm using
217 the Heine Gamma® G7 aneroid sphygmomanometer (Heine Optotechnik, Herrsching,
218 Germany) to an accuracy of 2 mmHg. The measurement protocol included a rest of 5 minutes
219 and thereafter 3 measurements in the sitting position at 2-minute intervals. The mean of all 3

220 values was used in the analysis. Maturity was estimated with maturity offset which was
221 calculated for boys and girls from sex-specified prediction models using estimated years from
222 peak height velocity.³³

223 **Statistical methods**

224 The statistical analyses were performed using IBM SPSS Statistics software, version 25.0 (IBM
225 Corp. Armonk, NY, USA). We estimated statistical power using G*Power software (version
226 3.1.9.7.). One hundred and ninety three observations was needed to observe the correlation of
227 0.2 at the power of 0.80 when statistical significance level was set at alpha level of 0.05.
228 Moreover, a correlation coefficient needed to reveal statistical significance at the alpha level
229 of 0.05 was 0.30 in a subsample of 90 children.

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

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

250

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

257

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

265

266

267 **RESULTS**

268 **Descriptive characteristics**

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

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

274

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

278

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

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

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

295

296

297 A change in VPA over 2-year follow-up was positively associated with Δ RI at 2-year follow-
298 up in boys ($\beta=0.208$, 95% CI=0.027 to 0.388) but not in girls ($\beta=0.042$, 95% CI=-0.134 to
299 0.217; $p=0.021$ for interaction). The association in boys remained statistically significant after
300 further adjustment for VPA, maturity offset, BF% and systolic BP at baseline, changes in BF%
301 and systolic BP over 2 years, and study group. In girls, age ($\beta=0.217$, 95% CI=0.044 to 0.390)
302 and maturity offset at baseline ($\beta=0.275$, 95% CI=0.105 to 0.445) were positively related to
303 Δ RI at 2-year follow-up.

304

305 The sensitivity analyses revealed that the magnitude of the positive association between a
306 change in VPA over 2 years and Δ RI at 2-year follow-up was relatively similar ($p=0.517$ for
307 interaction) 141 children from the intervention group ($\beta=0.184$, 95% CI=0.018 to 0.350) and
308 for 104 children from the control group ($\beta=0.99$, 95% CI=-0.056 to 0.173). The magnitude of
309 this association was also relatively similar for 69 boys from the intervention group ($\beta=0.215$,
310 95% CI=-0.025 to 0.455) and for 49 boys from the control group ($\beta=0.218$, 95% CI=-0.077 to
311 0.512, $p=0.224$ for interaction 0.824) and for 72 girls from the intervention group ($\beta=0.163$,
312 95% CI=-0.075 to 0.401) and for 55 girls from the control group ($\beta=-0.037$, 95% CI=-0.307 to
313 0.232, $p=0.224$ for interaction).

314

315

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

318 Changes in MPA, VPA, and MVPA were inversely associated with changes in SI after
319 adjustment for age and sex (Table 3). The inverse association of a change in MPA ($\beta=-0.327$,
320 95% CI=-0.592 to -0.062), VPA ($\beta=-0.224$, 95% CI=-0.445 to -0.003), and MVPA ($\beta=-0.276$,
321 95% CI=-0.551 to -0.002) with change in SI remained statistically significant after further
322 adjustment for corresponding PA intensity and SI at baseline. Adjustment for change in SBP
323 had no effect on the association. Nevertheless, the inverse associations of changes in VPA ($\beta=-$
324 0.152, 95% CI=-0.400 to 0.096) and MVPA ($\beta=-0.202$, 95% CI=-0.490 to 0.086) with changes
325 in SI were weakened after additional adjustment for change in BF%.

326

327 Change in CRF was directly associated with a change in Δ RI after adjustment for age and sex
328 (Table 3), but the respective relationship weakened after further adjustment for CRF and Δ RI
329 at baseline ($\beta=0.136$, 95% CI=-0.080 to 0.352). Furthermore, a change in LPA was inversely
330 associated with change in Δ RI after adjustment for age, sex, and LPA and Δ RI at baseline ($\beta=-$
331 0.287, 95% CI=-0.562 to -0.012). Further adjustments had no effect on the magnitude of the
332 association.

333

334

335 **DISCUSSION**

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

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

343

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

357

358 We observed that the positive association between changes in VPA and Δ RI was mainly due
359 to the stronger positive association in boys. This is a similar finding to that of another
360 longitudinal study among school-aged children in which arterial dilatation capacity was
361 assessed by flow-mediated dilation.¹⁴ In girls, we observed that a change in maturity was
362 positively related to Δ RI at 2-year follow-up which supports a result from a previous study in
363 children.³⁵ In the present study sample, girls had a higher maturity level at baseline than boys.
364 Because sex hormones may affect on the arterial structure and function³⁶, the sex disparities
365 found in our study could be partly explained by earlier puberty in girls. However, it should be
366 considered that girls had lower levels of daily VPA than boys. Therefore, girls might have not

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

369

370 In contrast to our previous cross-sectional study in children aged 6-8 years showing an inverse
371 association between MVPA and SI,¹¹ we found no statistically significant association of PA at
372 different intensities at baseline or changes in PA during the 2-year follow-up with arterial
373 stiffness at 2-year follow-up in the present study. However, we observed that a change in
374 MVPA was inversely related to a change in arterial stiffness in a subsample of children
375 suggesting that increasing MVPA during childhood could slow-down the age-related increase
376 in arterial stiffness. These results from our study agree with those inconsistent findings from
377 previous studies. For example, a cross-sectional study showed that higher levels of MVPA
378 were associated only with higher small artery compliance but not with large arterial compliance
379 in children 8-11 years of age.¹⁸ MVPA has neither been associated with arterial stiffness
380 measured by PWV in a cross-sectional study among adolescents aged 15-16 years.³⁷ The
381 inconsistent observations in children and adolescents of different ages could be partly
382 explained by the development of the changes in the size and compliance of arteries during
383 normal growth³⁶ which may compensate for the development of arterial stiffness. This normal
384 variability in arterial stiffness may also explain why PA at baseline or change in PA was not
385 associated with arterial stiffness when baseline arterial stiffness was not accounted for.
386 Nevertheless, we also found that the longitudinal association between changes in MVPA and
387 changes in arterial stiffness was weakened after adjustment for BF%. Therefore, these results
388 together indicate that PA may improve arterial compliance since childhood but that this effect
389 may be partly mediated by its beneficial effects on body fat content.

390

391 Our findings on the lack of association of ST with SI or Δ RI are in line with previous
392 observations in children^{11,18-20}, suggesting that ST may not have a notable influence on arterial

393 health among school-aged children. However, higher levels of ST have been linked to increased
394 arterial stiffness in adults.³⁸⁻⁴⁰ Thus, it is possible that the adverse effects of ST on arteries
395 occur in adulthood when the accumulated exposure is more severe. Children may also naturally
396 break ST more often than adults. Breaking ST has been suggested to preserve normal
397 endothelial function⁴¹ that may be one explanation for the weak association between total ST
398 and arterial health in children. Moreover, the development of arteries during childhood³⁶ may
399 partly compensate for the adverse effects of ST on arteries among children and thus explain
400 the different findings in children and adults.

401
402 Our result suggesting no association between CRF and arterial measures contrasts with the
403 findings of previous studies in children.^{13,15,16,20,21} However, most previous studies have used
404 different methods for assessing CRF and arterial health. Therefore, it is difficult to directly
405 compare these results with our observations. In the present study, we defined CRF as maximal
406 power output per LM and found that a change in CRF had a modest positive association with
407 Δ RI at 2- year follow-up that was largely explained by a change in VPA. Nonetheless, in our
408 earlier cross-sectional study, maximal power output per LM was favorably associated with SI
409 and Δ RI in children aged 6-8 years.²⁰ In our previous cross-sectional study among 9-11- years
410 old children, we also found a direct association between VO₂peak per LM and Δ RI only in
411 boys. In that study, however, no association was found between VO₂peak per LM and SI²¹ that
412 is consistent with the present observations. The development of arteries during children's
413 normal growth³⁶ may explain the lack of association in the present study.

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415
416 The strengths of our study include the longitudinal study design and relatively large population
417 sample of children, the device-based assessment of PA and ST by individually calibrated
418 combined heart rate and movement sensing, the directly measured maximal power output
419 scaled by DXA-measured LM, and the comprehensive adjustment for confounding factors. The

420 main limitation of the study is the use of SI and Δ RI that are only surrogate measures of arterial
421 stiffness and endothelial function and that the RI was not assessed in response to a standardized
422 bout of exercise. Nevertheless, SI has correlated strongly with direct carotid-femoral PWV
423 among adults.⁴² Moreover, Δ RI reflects arterial dilatation capacity as a response to single bout
424 of exercise that may be related to an activation of endothelium-derived nitric-oxide
425 bioavailability.⁴³ In the present study, we were able to use baseline SI and Δ RI measurements
426 only among a subsample of children because remarkably reduced the study sample. Moreover,
427 we collected PA in 60-second epochs and as children accumulate MPA and VPA in short bouts,
428 it is possible that our results underestimate the true magnitude of the associations of PA with
429 arterial stiffness and dilatation capacity. We did not use directly measured VO₂peak which is
430 considered as the gold standard method for assessing CRF in children.⁴⁴ Although maximal
431 power output has been shown to be a good surrogate measure for directly measured CRF in
432 children⁴⁵, it not only reflect cardiorespiratory performance but also neuromuscular
433 performance.⁴⁶ While we were able to adjust the data for potential confounding factors, we
434 cannot exclude the possibility that the results are influenced by residual confounding.
435 Furthermore, the relatively large number of analyses increases the likelihood that some
436 associations were observed by chance. Finally, the longitudinal study design does not allow
437 drawing firm conclusions about the causality of the observed association.

438
439 In conclusion, the results of our longitudinal study suggest that VPA may improve arterial
440 dilatation capacity among children, particularly among boys. Our findings thus emphasize the
441 role of increasing VPA to improve arterial health since childhood. Our study also provides
442 some evidence that MVPA may attenuate the increase in arterial stiffness in children.
443 Therefore, increasing MPA and VPA during mid-childhood may be important in maintaining
444 arterial health in children and promoting PA at higher intensities may confer larger benefits on
445 arterial health than reducing ST and increasing LPA. More research on the longitudinal

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

449 **DISCLOSURE OF INTERESTS**

450 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.