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Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

Movement behavior and arterial stiffness in 9- to 11-year-old children

Elin Boiy

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Environmental Health Sciences

SUPERVISOR :

Prof. dr. Tim NAWROT

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Movement behavior and arterial stiffness in 9- to 11-year-old children*

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*Running title: *Physical activity and arterial stiffness*

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ABSTRACT

BACKGROUND: Regular physical activity reduces the risk of cardiovascular diseases. Moreover, it improves arterial stiffness – an independent biomarker of cardiovascular health – in adults. However, whether this effect can already be observed in childhood and which molecular mechanisms are underlying remains uncertain. Therefore, this study aimed to investigate the association between different types of movement behavior and arterial stiffness in children.

METHODS: In total, 208 (55% girls) 9- to 11-year-old children from the ENVIR*ON*AGE birth cohort (Limburg, Belgium) were included. A parent-proxy questionnaire assessed sedentary behavior (screen time), sleep duration, and physical activity (walking, cycling, and exercise). Arterial stiffness parameters, including pulse wave velocity, augmentation index, and central pulse pressure, were non-invasively measured by the oscillometric Vicorder®. Advanced glycation end-products (AGEs), a potential mediator in the association between movement behavior and arterial stiffness, were measured through skin autofluorescence. Associations were analyzed using multivariate linear regression and traditional mediation analysis with adjustment for confounders ($\alpha < 0.05$).

RESULTS: A 1-IQR higher screen time was significantly associated with 1.767 mmHg higher central pulse pressure (95% CI: 0.448 to 3.086), indicating higher arterial stiffness. However, this finding did not remain significant after adjustment for relevant confounders (β per IQR = 0.888, 95% CI: -0.395 to 2.170). No kind of movement behavior was significantly associated with pulse wave velocity or augmentation index, and no mediation by AGEs was observed.

CONCLUSION: This study suggests that movement behavior might be associated with arterial stiffness in children. However, a higher level of AGEs could not explain this effect.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of mortality and disease burden worldwide, annually responsible for over 18 million deaths and 500 million cases (1). In addition, it has been projected that the global cost of CVDs will reach one trillion US dollars by 2025 (2). However, up to 90% of CVD events could be prevented by maintaining an optimum risk profile. This profile consists of seven modifiable cardiovascular risk factors: tobacco cessation, weight management, diet, blood cholesterol, blood glucose, blood pressure, and physical activity (PA) (3, 4).

Increased arterial stiffness has been recognized as an independent predictor of CVD morbidity and mortality (5). Arterial stiffness refers to the viscoelastic properties of the blood vessel wall of the aorta and other large arteries (6). These viscoelastic properties are determined by various structural components present in the blood vessel wall. Smooth muscle cells actively contribute to stiffening, whereas elastin and collagen passively provide elasticity and stiffness, respectively. Additionally, calcification of the arterial wall and cross-linking of elastin and collagen fibers by advanced glycation end-products (AGEs) increase arterial stiffening (7). During the biological aging process, the elastin content in the arterial wall decreases due to increased elastase activity and reduced elastin synthesis. In addition, AGEs accumulate in the body over time. Therefore, arterial stiffness progressively increases as individuals age, which is a physiological process called arteriosclerosis (6). In addition to age-related stiffening, various disease states (e.g., hypertension, atherosclerosis, and diabetes mellitus) further contribute to arterial stiffening (8). This increase can already be observed in childhood; children and adolescents with type I diabetes or congenital heart disease show elevated levels of arterial stiffness (9, 10).

Under healthy conditions, the large arteries are elastic and responsible for dampening the pulsatile output of the heart's left ventricle into a continuous flow of blood into the peripheral blood vessels. In contrast, stiffening of the large arteries has three important hemodynamic consequences (6). Firstly, the pressure in the aorta during the systolic phase of the cardiac cycle increases. This elevated pressure leads to increased ventricular afterload, meaning the heart needs to work harder to overcome the elevated pressure in the aorta. Secondly, increased arterial stiffness reduces the diastolic blood pressure, thereby diminishing the blood supply to the heart muscle. Lastly, the pulse pressure increases, which can damage several end-organs, including the brain and kidneys (6, 11). Therefore, arterial stiffness can be used as a biomarker for CVDs, including heart failure and myocardial infarction, as well as dementia and renal diseases (12).

Different surrogate parameters are commonly used to assess arterial stiffness. First of all, the pulse wave velocity (PWV) is considered the gold standard according to the American Heart Association (13). This value represents the speed at which a pressure pulse propagates from the heart to the peripheral arteries following contraction of the heart's left ventricle. If arteries are stiffer, there will be less elastic recoil of the blood vessel wall, and therefore the PWV will be higher (6, 14) (Figure 1). Secondly, arterial stiffness can be inferred from the shape of the pulse waveform in the aorta, which is called pulse wave analysis (PWA). The augmentation index (AI) is a widely used measure to assess arterial stiffness derived from PWA. If the pressure pulse wave propagates from the heart to the periphery (anterograde or incident wave), diameter reduction and branching of arteries provide resistance to this pulse wave. Subsequently, a second pressure pulse wave is generated, flowing from the periphery to the heart (retrograde or reflected wave). This second pulse gives rise to an augmentation of the initial pulse waveform. The AI is calculated from the magnitude difference between the peak of the anterograde and retrograde pulse, normalized to the pulse pressure. If arteries are stiffer, the anterograde and retrograde pressure pulses will travel faster (as there is an increased pulse wave velocity), and therefore, the AI will be higher (14) (Figure 1). Lastly, if arteries are stiffer, the aortic or central pulse pressure (CPP) increases because higher pressure differences need to be produced by the heart in order to eject the same volume of blood (6, 15) (Figure 1). In contrast to the brachial pulse pressure (PP), which reflects the pulse pressure in the brachial artery and is routinely used for clinical purposes, the CPP represents the pulse pressure in the aorta and is more strongly related to future cardiovascular events (16).

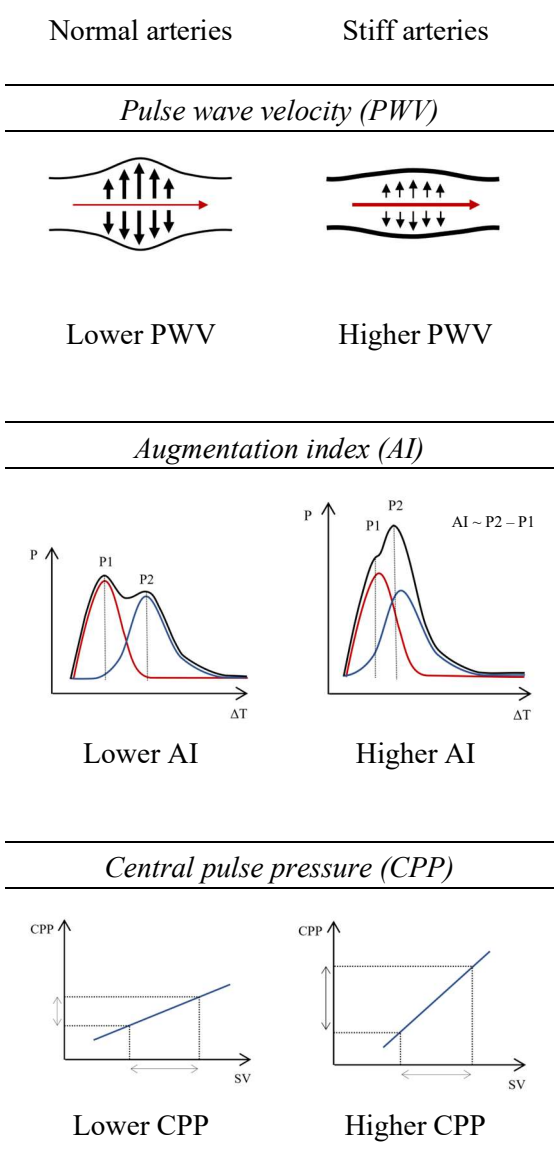

Figure 1: Conceptual drawings of PWV, AI, and CPP – *PWV*: If arteries are stiffer, there is less elastic recoil (black) of the blood vessel wall, and therefore the PWV (red) is higher. *AI*: If arteries are stiffer, the anterograde (red) and retrograde (blue) pressure waves travel faster (see PWV). Therefore, the peaks of the anterograde (P1) and retrograde (P2) pressure waves are closer in time to each other, and the AI is higher. *CPP:* If arteries are stiffer (higher slope of the curve), a higher CPP needs to be generated by the heart to eject the same SV. Adapted from Panchangam et al. (14) and Feher et al. (15). PWV: pulse wave velocity, AI: augmentation index, P: pressure, ΔT: elapsed time, P1: anterograde pressure peak, P2: retrograde pressure peak, CPP: central pulse pressure, SV: stroke volume.

In adults, the PWV as gold standard measure for arterial stiffness has been well characterized, and reference values are available (17). Additionally, a cut-off value of 10 m/s has been proposed, above which an increased risk for cardiovascular events is observed (18). Moreover, an increase in PWV by 1 m/s corresponds to an increased relative risk of 14% in total cardiovascular events (19). However, up until now, no reference values or thresholds for children have been determined. Whereas many studies provide PWV measurements, the wide variety in measurement techniques and devices hampers defining age-, height-, and sex-specific reference values in children (20). Therefore, we provide reference PWV values for Belgian 9- to 11-year-old children using the oscillometric Vicorder® measurement device.

Physical activity (PA) is widely acknowledged for its beneficial effects on the cardiovascular system; engaging in regular PA is associated with decreased cardiovascular mortality and risk of developing CVD (21). PA is defined as any bodily movement produced by the skeletal muscles that requires energy expenditure (22). In contrast, physical inactivity refers to an insufficient activity level to meet the physical activity recommendations documented in the 2020 '*World Health Organization (WHO) guidelines on physical activity and sedentary behavior*'. According to these guidelines, adults should perform at least 150 to 300 minutes per week of moderate-intensity PA, 75 to 150 minutes per week of vigorous-intensity PA, or an equivalent combination of both for substantial health benefits. On the other hand, for children and adolescents aged 6 to 18, the WHO recommends that they perform at least an average of 60 minutes of moderate- to vigorous-intensity PA (MVPA) per day. Moreover, vigorous-intensity activities, as well as those to strengthen muscles and bones, should be incorporated at least three days per week (22). Evidence demonstrates that adhering to these physical activity guidelines, or even exceeding the recommended 60 minutes of daily MVPA, offers numerous beneficial health outcomes for children. These outcomes include lower adiposity (23), better cardiorespiratory fitness (24), bone health (25), cardiometabolic health (26), cognitive function (27), and mental well-being (28). However, it is estimated that 28% of adults (29) and 81% of adolescents (30) worldwide undertake insufficient PA. Moreover, inactivity accounts annually for more than five million deaths worldwide and

is responsible for 6-10% of the major noncommunicable diseases, such as coronary heart disease, diabetes, and breast cancer (31). The WHO guidelines additionally specify that children and adolescents should limit the time spent in sedentary behavior (SB). SB is defined as any waking behavior while in a sitting, reclining, or lying position. Particularly the amount of recreational screen time should be diminished (22). Higher duration of SB and screen time have been associated with negative health effects, including reduced cardiometabolic health (32), unfavorable measures of adiposity (33), academic achievement issues, and social behavioral problems (34).

The WHO physical activity and sedentary behavior guidelines for children focus specifically on two types of movement behavior (i.e., MVPA and SB). However, when dissecting a 24-hour day, we can identify additional movement types. On average, children spend 40% of the 24-hour day sleeping, another 40% in SB, and 15% and 5% in light- and moderate- to vigorous-intensity PA, respectively (35). Consequently, a more extended version of the WHO physical activity recommendations has been included in the '*Canadian 24-hour movement guidelines for children and youth: an integration of physical activity, sedentary behavior, and sleep*'. Apart from guidelines on physical activity (identical to those of the WHO, i.e., at least 60 min/day of MVPA and 3 days/week with vigorous-intensity PA), children should perform several hours of light physical activities per day, obtain between nine and eleven hours of uninterrupted sleep per night, and limit recreational screen time to no more than two hours per day (36). These guidelines might be more useful when considering movement-related health effects since it has been acknowledged that PA and SB do not independently impact but rather interact with each other to influence children's health outcomes (36). In this regard, Carson et al. observed that a higher time spent performing MVPA, relative to other movement-related behaviors, improves cardiovascular risk markers in children (37). Furthermore, a systematic review by Saunders et al. summarized available evidence on the health effects of different PA, SB, and sleep combinations. This review suggested that children with high PA, low SB, and high sleep duration generally have better cardiometabolic health (38).

Different epidemiological studies have shown the beneficial effect of PA on arterial stiffness in adults (39, 40), but evidence for this effect in children remains inconclusive (41). Moreover, critical comparison between the studies investigating this topic is hampered by the variety in measurement parameters, techniques, and devices used to assess arterial stiffness. Additionally, different approaches are widely used to evaluate movement behavior in children, including self-reports, parent-proxy questionnaires, accelerometers, pedometers, and cardiorespiratory fitness tests (42). Furthermore, a limited number of studies have investigated the effect of all different movement-related behaviors on arterial stiffness in children (43, 44) and the molecular mechanisms underlying this association have not yet been thoroughly investigated.

Advanced glycation end-products (AGEs) can be proposed as a potential mediator in the causal pathway between movement behavior and arterial stiffness. AGEs are a heterogenous group of molecules formed through non-enzymatic reactions of glucose or other saccharide derivates with proteins or lipids (45). When the saccharide groups bind to collagen or elastin proteins, they can induce irreversible cross-linking of collagen and elastin fibers within the arterial wall. As a result, the cross-linked fibers will be stiffer and less susceptible to hydrolytic turnover. Secondly, if AGEs bind to their cellular receptor (RAGE), an inflammatory signaling pathway is activated, further increasing arterial stiffening (12). Various environmental and lifestyle factors, such as air pollution, tobacco smoke, a high-sugar diet, and sedentary behavior, induce AGE production (45). In contrast, PA has been shown to reduce the accumulation of AGEs both in adults (46, 47) and children (48). Hence, AGEs are a plausible molecular mechanism underlying the effect of movement behavior on arterial stiffness.

In this study, we aimed to investigate the effect of different types of movement behavior (i.e., sleep, SB, and PA) on arterial stiffness in children aged 9 to 11. Secondly, we aimed to examine the mediating role of AGEs in the association between movement-related behaviors and arterial stiffness. We hypothesized that arteries are less stiff in children who spend more time performing PA, less time in SB, or more time sleeping.

EXPERIMENTAL PROCEDURES

Study characteristics – The study was performed in the context of the ENVIR*ON*AGE (ENVIRonmental influence *ON* early AGEing) birth cohort (49). In this ongoing prospective birth cohort, mother-newborn pairs were recruited at birth in the Hospital East Limburg (Genk, Belgium). Subsequently, the mothers and children were followed up through different stages of their lives. A first follow-up visit occurred when the children were between 4 and 6 years old. Between the ages of 9 and 11, participants were reinvited for a second follow-up visit, during which arterial stiffness measurements of the children were performed. Additionally, the mothers of the children were asked to complete a questionnaire regarding the movement behavior of the children. Between July 2021 and April 2023, 212 children between 9 and 11 years old were followed up a second time. The study was performed in compliance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of Hasselt University (Hasselt, Belgium; ethics approval: B1152021000007). Written informed consent of the guardians and children's assent were obtained.

Arterial stiffness measurements – Before measurements, participants were asked to remove thick clothing. First, the brachial blood pressure was measured five times using an automatic blood pressure monitor (HBP-1320, Omron, Japan) with participants in a supine position with 30° elevated head and shoulders. The first two measurements were discarded, and the last three were averaged to obtain the systolic (SBP) and diastolic (DBP) blood pressure. The pulse pressure (PP) was defined as the difference between the SBP and DBP. Secondly, PWA was performed using the oscillometric Vicorder® measurement device (80Beats Medical, Germany) with participants in a supine position with 30° elevated head and shoulders. In short, a cuff was placed around the participant's arm, capturing the pressure wave profile of the brachial artery. Analysis of this brachial waveform by the Vicorder® software using the generalized transfer function (50) generated a central, aortic pressure waveform. Subsequently, various cardiovascular parameters were derived from this central waveform. At first, the central systolic blood pressure (CSBP) reflected the systolic blood pressure in the aorta. Secondly, the CPP was calculated as the difference between the CSBP and the DBP (which is considered stable across the arterial tree (16)). The central AI was measured as the ratio of the augmentation pressure (defined as the magnitude difference in the two peaks in the central pressure waveform) over the CPP. To account for the influence of the heart rate on AI, this value was normalized to a heart rate of 75 beats per minute, resulting in the AI@75 (51). Finally, the mean arterial pressure (MAP) was defined as the arithmetic mean over the pressure waveform. Lastly, the Vicorder® was used to determine the carotid-femoral PWV. In brief, with participants in supine position, a small cuff was placed around the neck, and a larger one was placed around the thigh, to capture the pulse waves of the carotid and femoral artery, respectively. The transit time was calculated by cross-correlating the rapidly rising lower portions of the systolic section of the pulse waves at the carotid and femoral site. Additionally, the path length was determined by measuring the horizontal distance between the suprasternal notch (SSN) and the center of the femoral cuff using measurement tape to an accuracy of 0.5 cm. Finally, the PWV was calculated as the path length divided by the transit time. Both PWV and PWA measurements by the Vicorder® were performed in triplicate. After removal of low-quality values, the arithmetic mean of three PWV, AI@75, or CPP measurements was used for further analysis.

Movement behavior questionnaire – The movement behavior of the children was categorized into three distinct components: sleep, SB, and PA. The latter was subdivided into walking, cycling and exercise. An online parent-proxy questionnaire (Qualtrics, USA) was used to assess the amount of time the children spent on average performing these various activities outside of the school environment. SB included the time spent using a device with a screen and comprised of watching television, playing videogames, and using a smartphone or computer. Exercise covered the amount of active time in the context of a sports club. All movement behavior variables were expressed in performed hours per week.

AGEs assessment – The level of AGEs was measured by skin autofluorescence using the AGEReader (Diagnoptics, the Netherlands). This validated method allowed for a fast and non-invasive assessment of AGEs (52). In short, the skin at the right ventral side of the forearm was irradiated with low-intensity UV-light at 370 nm, a wavelength that interacts with fluorescent AGEs present in the skin. Next, the ratio between the

emitted (420 to 600 nm) and reflected light (300 to 420 nm) was used to calculate skin autofluorescence as proxy for AGEs, expressed in arbitrary units (a.u.) (53).

Assessment of covariates – The children's height was measured with the Seca 123 fixed stadiometer (Seca, Germany) to an accuracy of 0.1 cm and the weight was assessed using the Karada Scan BF511 digital scale (Omron, Japan). Both measurements were performed when participants wore only light clothes and no shoes. Additionally, ethnicity was determined based on the country of origin of the four grandparents. Children were classified as European if two or more grandparents were European and were classified as non-European if three or more grandparents were born outside Europe. Exposure to smoke was categorized as 'yes' if the child was directly exposed to smoke (tobacco as well as electronic cigarettes) in a family setting at the time of follow-up, and 'no' if this was not the case. To adjust for socioeconomic status, maternal education level was coded 'low' if the mother had no or a primary school degree, 'middle' if the highest obtained degree was a high school degree, or 'high' if the mother received a college or university degree. Next, pubertal maturity was defined as prepubertal, pubertal, or post-pubertal based on the Pubertal Category Scores (PCS). To determine this score, parents were asked to estimate their child's body hair growth, facial hair growth, and voice-deepening for boys, or body hair growth, breast development, and menarche for girls (54, 55). To account for any influence of ambient temperature or light exposure, the season at follow-up visit was determined using meteorological seasons (starting on the first day of the month). A graphical representation of the relationship between arterial stiffness, movement behavior, AGEs, and different covariates (Supplementary Figure 1) was created using DAGitty version 3.0 (University of Nijmegen, the Netherlands) (56).

Statistical analysis – Data were analyzed using RStudio version 4.1.3 (Posit, USA), and graphs were created using GraphPad Prism version 8.0.2 (GraphPad, USA). Age-specific reference curves for PWV were calculated using the LMS method (57). This method is widely used to generate pediatric anthropometric reference curves accounting for nonlinearity and skewed distribution of the dataset. In short, PWV values were transformed into z-scores (Z) using the following formula:

$$Z = \frac{\left(\frac{Y}{M(t)}\right)^{L(t)} - 1}{L(t)S(t)}$$

In this formula, Y represents the average measured PWV per child, whereas L(t), M(t), and S(t) represent the power of the Box-Cox transformation, the median, and the coefficient of variation for every age (t), respectively. Percentile curves were created using RefCurv version 0.4.2 (University of Bonn, Germany) (58). Multivariate linear regression was used to observe the association between movement behavior and arterial stiffness variables. Traditional mediation analysis investigated the effect of AGEs in the association between movement behavior and arterial stiffness. The statistical models were adjusted for a priori chosen covariates, based on literature, including age at follow-up examination, sex, height, weight, MAP, maternal education, exposure to smoke, ethnicity, season, and PCS. A last model adjusted for all types of movement behavior (screen time, sleep, walking, cycling, and exercise), to investigate their effect on arterial stiffness independent of each other. Normality of the residuals was confirmed using a Q-Q plot, whereas homoscedasticity and linearity were evaluated using a residuals-by-predicted plot. A two-sided p-value smaller than 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics – After excluding 4 participants with incomplete arterial stiffness assessment (Figure 2), a total of 208 children (115 girls and 93 boys) between 9 and 11 years old were included in the present study (Table 1, Table 2). The children had a mean (SD) weight of 37.4 (9.0) kg and were 143.5 (7.5) cm tall with a BMI of 18.0 (3.2) kg/m². The mean heart rate, SBP, DBP, and PP were 75.6 (10.4) beats per minute, 108.5 (10.0), 65.4 (6.8), and 42.9 (7.9) mmHg, respectively. The majority of children in the study were classified as European (96%) and were not directly exposed to smoke in a family setting (92%). Girls were more likely than boys to have reached puberty (58% vs. 23%, $p < 0.01$). Additionally, most mothers were highly educated (79%), with only 1% having no or a primary school degree. Apart from PCS and AGEs, no significant differences between boys and girls were observed. Therefore, data from both sexes were

pooled for further analysis. Due to missing data, regression analysis included only 190 out of the 208 participants, and mediation analysis included 188 participants (Figure 2). No significant differences were observed between excluded and included participants (results not shown).

Reference values – Across all ages, the mean PWV equaled 5.1 m/s, with a standard deviation of 0.7 m/s (Table 1). PWV increased significantly with age (0.25 m/s increase per year, 95% CI: 0.13 to 0.37, $p < 0.01$, N = 208). No difference was observed between boys and girls ($p = 0.78$). The LMS method yielded age-specific reference values for PWV (Figure 3, Supplementary Table 1). A mean (SD) AI@75 of 10.8 (6.8) % and CPP of 37.4 (7.1) mmHg were observed (Table 1).

Correlation arterial stiffness parameters – CPP was significantly correlated with PWV (R = 0.158, 95% CI: 0.022 to 0.288, $p = 0.02$) and AI@75 (R = 0.246, 95% CI: 0.114 to 0.370, $p < 0.01$). Albeit not significant, a positive trend was observed between PWV and AI@75 (R = 0.063, 95% CI: -0.074 to 0.197, $p = 0.37$) (Table 3).

Effect of movement behavior on PWV – No statistically significant associations or trends were observed between different types of movement behavior (screen time, sleep, walking, cycling, or exercise) and PWV (Table 4).

Effect of movement behavior on AI@75 – A trend of screen time (1.131% increase in AI@75 per interquartile range (IQR) increase in screen time, 95% CI: -0.107 to 2.370, $p = 0.08$, N = 190) and exercise (β per IQR = -1.183, 95% CI: -2.649 to 0.283, $p = 0.12$) was observed on AI@75. These findings were not significant and disappeared after adjustment for age, sex, height, weight, MAP, maternal education, smoke exposure, ethnicity, PCS, and season (screen time: β per IQR = 0.229, 95% CI: -1.016 to 1.475, $p = 0.72$, exercise: β per IQR = -0.243, 95% CI: -1.665 to 1.179, $p = 0.74$) (Table 4).

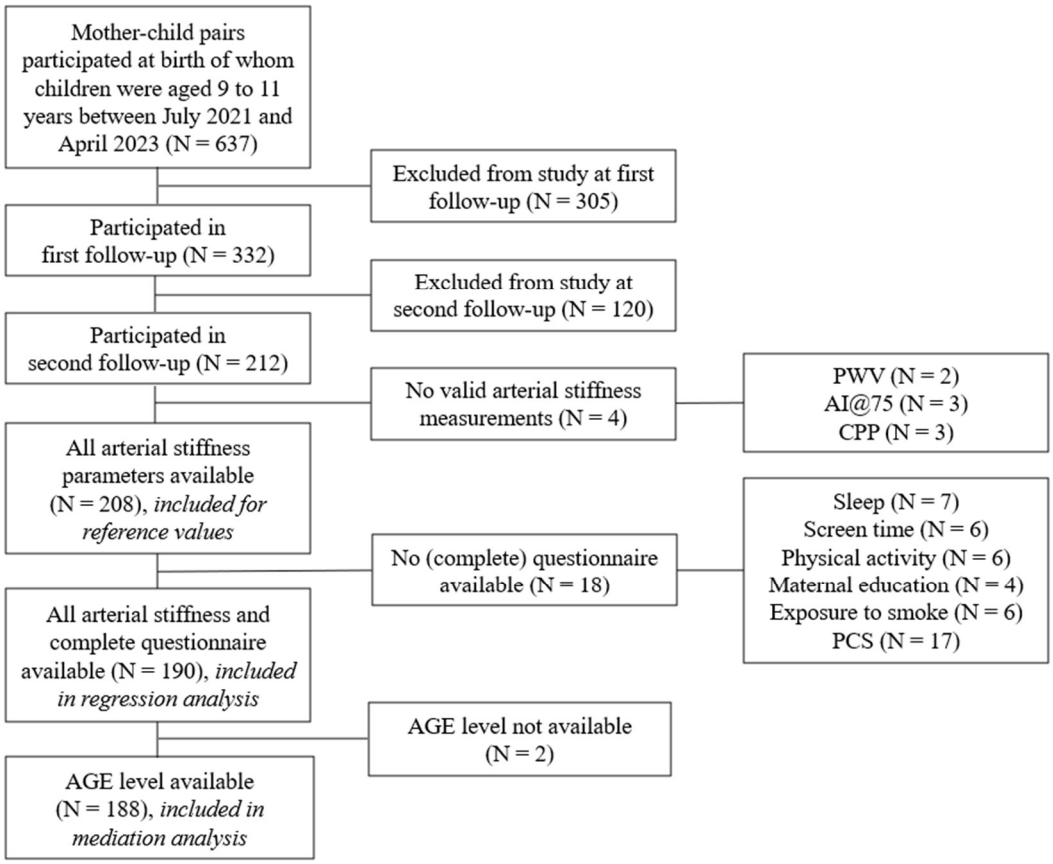

Figure 2: Flowchart of included ENVIR*ON*AGE participants – PWV: pulse wave velocity, AI@75: augmentation index normalized to a heart rate of 75 beats per minute, CPP: central pulse pressure, PCS: Pubertal Category Scores, AGE: advanced glycation end-product.

Table 1: Baseline characteristics of the study population

Variable	N	Total	Girls (55%)	Boys (45%)	P-value difference
Anthropometric characteristics					
Age (year)	208	10.4 ± 0.7	10.4 ± 0.7	10.3 ± 0.7	0.51
Height (cm)	208	143.5 ± 7.5	144.1 ± 7.7	142.6 ± 7.2	0.15
Weight (kg)	208	37.4 ± 9.0	37.4 ± 8.7	37.5 ± 9.4	0.90
Body mass index (kg/m²)	208	18.0 ± 3.2	17.8 ± 3.1	18.2 ± 3.2	0.37
Cardiovascular characteristics					
Heart rate (beats/min)	208	75.6 ± 10.4	76.3 ± 10.6	74.8 ± 10.2	0.36
Systolic blood pressure (mmHg)	208	108.5 ± 10.0	108.1 ± 9.9	108.7 ± 10.0	0.61
Diastolic blood pressure (mmHg)	208	65.4 ± 6.8	65.3 ± 6.7	65.6 ± 7.0	0.87
Pulse pressure (mmHg)	208	42.9 ± 7.9	42.8 ± 8.0	43.1 ± 8.0	0.80
Central systolic blood pressure (mmHg)	208	103.7 ± 9.7	103.6 ± 9.8	103.8 ± 9.7	0.88
Central pulse pressure (mmHg)	208	37.4 ± 7.1	37.3 ± 6.9	37.5 ± 7.5	0.98
Augmentation index @ 75 (%)	208	10.8 ± 6.8	11.5 ± 6.5	10.0 ± 7.0	0.09
Mean arterial pressure (mmHg)	208	83.9 ± 7.6	84.0 ± 7.7	83.9 ± 7.5	0.94
Pulse wave velocity (m/s)	208	5.1 ± 0.7	5.1 ± 0.7	5.1 ± 0.7	0.78
Other characteristics					
Advanced glycation end-products (a.u.)	206	1.00 ± 0.15	0.96 ± 0.14	1.03 ± 0.16	**< 0.01***
Maternal education	204				0.39
Low (no or primary school)		2 (1%)	0 (0%)	2 (2%)	
Middle (secondary school)		40 (20%)	23 (20%)	17 (19%)	
High (college or university)		162 (79%)	91 (80%)	71 (79%)	
Exposure to smoke	202				1.00
No		186 (92%)	103 (92%)	83 (92%)	
Yes		16 (8%)	9 (8%)	7 (8%)	
Ethnicity	208				1.00
European		200 (96%)	111 (97%)	89 (96%)	
Non-European		8 (4%)	4 (3%)	4 (4%)	
Pubertal Category Scores	191				**<0.01***
Pre-pubertal		109 (57%)	46 (42%)	63 (77%)	
Pubertal		82 (43%)	63 (58%)	19 (23%)	
Post-pubertal		0 (0%)	0 (0%)	0 (0%)	
Season at follow-up	208				0.21
Spring		47 (23%)	27 (23%)	20 (22%)	
Summer		65 (31%)	29 (25%)	36 (39%)	
Autumn		43 (21%)	26 (23%)	17 (18%)	
Winter		53 (25%)	33 (29%)	20 (22%)	

Values are represented as mean ± standard deviation or number (proportion), as appropriate. The difference between girls and boys was analyzed using a two-sided t-test or Fisher's exact test. *p < 0.05.

Table 2: Movement behavior characteristics

	N	Min	Q1	Median	Q3	Max	IQR
Screen time (h/week)	202	2.5	16.6	23.0	29.5	45.0	12.9
Sleep (h/week)	201	48.5	65.0	70.0	72.5	85.6	7.5
Walking (h/week)	202	0.0	0.0	3.5	4.5	14.0	4.5
Cycling (h/week)	202	0.0	0.0	2.25	4.5	14.0	4.5
Exercise (h/week)	202	0.0	1.5	4.0	6.0	12.7	4.5

Q1: first quartile, Q3: third quartile, IQR: interquartile range.

Effect of movement behavior on CPP – A 1-IQR higher screen time was significantly associated with 1.767 mmHg (95% CI: 0.448 to 3.086, p = 0.01) higher CPP. However, this association did not remain significant after adjustment for confounders (β per IQR = 0.888, 95% CI: -0.395 to 2.170, p = 0.18). Additionally, a trend was observed for sleep (β per IQR = -1.154, 95% CI: -2.393 to 0.085, p = 0.07 before, and β per IQR = -0.585, 95% CI: -1.724 to 0.553, p = 0.31 after adjustment for confounders). On the other hand, more time spent cycling was associated with higher CPP, although not significant (β per IQR = 0.641, 95% CI: -0.703 to 1.985, p = 0.035 before, and β per IQR = 1.197, 95% CI: -0.015 to 2.409, p = 0.05 after adjustment for potential confounders). This association became significant after additional adjustment for other types of movement behavior (β per IQR = 1.703, 95% CI: 0.248 to 3.158, p = 0.02) (Table 4).

Mediation by AGEs – No mediation by AGEs was observed in the association between different types of movement behavior and arterial stiffness. The indirect effect was not significant, and the magnitude of the estimate was small compared to the direct or total effect (Figure 4).

DISCUSSION

The present study reported a reference PWV value of 5.1 m/s with a standard deviation of 0.7 m/s in 9- to 11-year-old children included in the second follow-up phase of the ENVIR*ON*AGE birth cohort. Until now, two studies available from the literature have provided normative values for PWV using the Vicorder® device in a population of healthy children. For children aged 9 to 11, Thurn et al. (Germany and Türkiye) observed a mean (SD) PWV value of 4.6 (0.5) m/s for boys and 4.5 (0.5) m/s for girls (59), whereas Fischer et al. (Germany) found a median value of 4.4 with an IQR from 3.6 to 5.1 for boys and 4.2 with an IQR from 3.4 to 5.4 for girls (60). The results from the current study were higher than those obtained from literature. However, this discrepancy might be attributable to differences in the measurement of the path length needed to calculate the PWV. In the study by Thurn et al., the path length was calculated by the following formula: SSN to umbilicus + umbilicus to femoral cuff – SSN to carotid cuff (59). On the other hand, Fischer et al. determined the path length as the direct distance between the midpoints of the cuffs around the neck and thigh, using a correction factor of 0.8 (60). In the current study, the path length was measured between the SSN and the femoral cuff. This length is longer than the measurements used in the other two studies, which could explain the higher PWV values observed in the present study.

As expected, we observed an increase in PWV with age, equal to 0.25 m/s per year. However, caution should be taken when interpreting this value, as it could be distorted by the small age range of children included in the present study. To provide a broader perspective, we can look at a meta-analysis performed by Stoner et al. (61). They summarized nine studies providing childhood reference values for PWV, measured with different techniques and devices (including the Vicorder®). The meta-analysis found an overall increase in PWV of 0.12 m/s per year, which is considerably lower than our result. Furthermore, the current study did not observe a significant difference between boys and girls, whereas Thurn et al. and Fischer et al. were able to detect sex-specific differences in PWV (59, 60). It is generally acknowledged that PWV is higher in female than male prepubertal children (62). In contrast, the increase in PWV with age during puberty and adolescence is lower in girls than boys (61).

Therefore, sex differences might have been masked in the current study. However, to preserve power, data from both sexes were pooled for further analyses.

Analysis of the correlation between the different arterial stiffness parameters revealed a positive, but low, correlation between PWV, AI@75, and CPP. These findings support the fact that the three investigated parameters offer a different perspective on arterial stiffness. There are several reasons for these differences. First of all, the stiffness of the blood vessel wall is not homogeneously distributed over the arterial tree (63). Therefore, the distinct measurement location of different parameters might influence their results in terms of arterial stiffness. Secondly, the PWV and AI represent mechanical alterations of the blood vessel wall, whereas the CPP indicates hemodynamic alterations in the aorta. Although arterial stiffness contributes to increased central pulse pressure, the CPP is no direct marker of arterial stiffness (6, 15, 64). Nevertheless, it is still a valuable factor to be assessed when investigating arterial stiffness and cardiovascular health (16). Lastly, the AI is not only influenced by arterial stiffness but also by the magnitude and timing of pressure wave reflections, which are determined by the diameter and elasticity of the smaller arteries (63). Moreover, the AI is known to depend on several other factors, including body height, MAP, and heart rate (13, 65). Therefore, it is important to recognize that PWV, AI@75, and CPP are distinct arterial stiffness parameters, providing different information. They cannot be used interchangeably, and investigating all three parameters is essential for a better understanding of the effect of movement behavior on arterial stiffness in children.

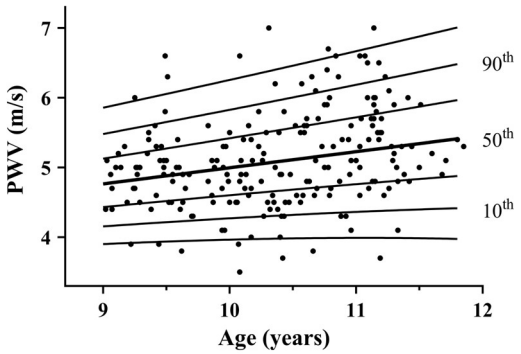

Figure 3: PWV percentile plot by age. Dots represent individual results. The 3rd, 10th, 25th, 50th, 75th, 90th, and 97th age-specific percentiles are shown. N = 208. PWV: pulse wave velocity.

Table 3: Correlation of different arterial stiffness parameters

	PWV	AI@75	CPP
PWV	1.000	0.063	0.158*
AI@75	0.063	1.000	0.246**
CPP	0.158*	0.246**	1.000

Pearson correlation coefficients between PWV, AI@75, and CPP. *p < 0.05, **p < 0.01, N = 208. PWV: pulse wave velocity, AI@75: augmentation index normalized to a heart rate of 75 beats per minute, CPP: central pulse pressure.

In the current study, higher screen time (used as a proxy for SB) was significantly associated with increased CPP. However, this result lost significance after adjustment for relevant confounding variables. Furthermore, a trend was observed between screen time and AI@75, but this effect disappeared after adjustment. On the other hand, no association was found with PWV. Similar results were observed in a Swiss cohort of primary school children by Koechli and colleagues. They found a significant effect of screen time on AI@75, and reported a positive trend with CPP (66). In the same cohort, they did not observe an effect of screen time on PWV (67). Another study by Lona et al. (68) did not find an association between screen time and PWV in 10- to 12-year-old children either. Several other studies investigated the effect of overall time spent in SB on arterial stiffness. In a study by Gomez-Garcia et al. (43), SB (total of time spent performing sedentary play and screen time) was investigated in 4- to 6-year-old children. Without adjustment for confounders, they observed a positive trend with PWV and AI@75. Additionally, Heil and colleagues (41) reported higher PWV in children with more sedentary time in 8- to 11-year-old children. Conversely, Marshall et al. (69) observed a negative trend (higher sedentary time associated with better arterial stiffness) on PWV and CPP, whereas no effect was observed on AI using compositional analysis. In this type of analysis, all kinds of movement behavior (sleep, light-, moderate-, and vigorous-intensity PA) were introduced into a

Table 4: Regression analysis between movement behavior and arterial stiffness parameters

	β per IQR (95% CI)	p
Pulse wave velocity (PWV)		
Screen time		
Model 1†	0.034 (-0.092 to 0.159)	0.60
Model 2	0.003 (-0.125 to 0.131)	0.96
Model 3	-0.002 (-0.130 to 0.127)	0.98
Sleep		
Model 1	-0.004 (-0.121 to 0.113)	0.94
Model 2	0.032 (-0.079 to 0.142)	0.57
Model 3	0.032 (-0.080 to 0.144)	0.58
Walking		
Model 1	0.040 (-0.084 to 0.164)	0.53
Model 2	0.068 (-0.048 to 0.184)	0.25
Model 3	0.049 (-0.091 to 0.189)	0.49
Cycling		
Model 1	0.047 (-0.078 to 0.173)	0.46
Model 2	0.058 (-0.060 to 0.176)	0.34
Model 3	0.027 (-0.117 to 0.170)	0.72
Exercise		
Model 1	-0.053 (-0.202 to 0.095)	0.48
Model 2	-0.074 (-0.216 to 0.069)	0.31
Model 3	-0.071 (-0.215 to 0.074)	0.34
Augmentation index (AI@75)		
Screen time		
Model 1	1.131 (-0.107 to 2.370)	0.08
Model 2	0.229 (-1.016 to 1.475)	0.72
Model 3	0.224 (-1.037 to 1.484)	0.73
Sleep		
Model 1	0.111 (-1.052 to 1.273)	0.85
Model 2	0.472 (-0.629 to 1.573)	0.40
Model 3	0.484 (-0.641 to 1.610)	0.40
Walking		
Model 1	0.275 (-0.962 to 1.512)	0.66
Model 2	0.275 (-0.885 to 1.434)	0.64
Model 3	0.236 (-1.166 to 1.638)	0.74
Cycling		
Model 1	-0.205 (-1.457 to 1.048)	0.75
Model 2	0.236 (-0.947 to 1.419)	0.70
Model 3	0.042 (-1.393 to 1.477)	0.95
Exercise		
Model 1	-1.183 (-2.649 to 0.283)	0.12
Model 2	-0.243 (-1.665 to 1.179)	0.74
Model 3	-0.233 (-1.680 to 1.215)	0.75

single model, which may distort the findings when interpreted only for SB. In conclusion, there is some evidence suggesting that higher time spent in SB, including screen time, might be associated with higher arterial stiffness. These findings align with the WHO and Canadian guidelines on movement behavior (22, 36), which recommend reducing SB, especially the amount of recreational screen time to promote health outcomes in children.

Secondly, albeit non-significantly, our results showed a beneficial trend between higher sleep duration and lower CPP. However, no effects were observed with PWV or AI@75. In the literature, limited studies have investigated the effect of sleep duration on CPP, PWV, or AI in children. Stoner et al. (70) observed a significantly higher PWV in 9- to 11-year-old children with insufficient sleep (< 9h) compared to children who met the sleeping recommendations (9 to 11h). In addition, Gomez-Garcia et al. (43) assessed the amount of time spent sleeping in preschool children and observed a beneficial, although not significant, effect on PWV and AI@75. On the other hand, compositional analysis by Marshall et al. (69) of all different kinds of movement behavior in 8- to 16-year-olds did not reveal a significant association between sleep and PWV, AI, or CPP. Overall, more research is necessary to further explore the association between sleep duration and arterial stiffness in children. The limited available evidence suggests that shorter sleep duration might be associated with stiffer arteries, but additional studies are required.

Next, the effect of physical activity on arterial stiffness was investigated. In our study, we observed lower AI@75 in children with higher exercise time, although this finding was not significant and disappeared after adjustment for confounders. No effect of exercise duration was observed on PWV or CPP. On the other hand, in a study by Baumgartner et al. (71), a significant association was observed between higher exercise duration and lower PWV in adolescents. However, higher training volume (calculated as the product of intensity and duration of exercise) was not significantly associated with lower PWV. Secondly, we observed a higher CPP in children who spent more time cycling, but no effects were observed on PWV or AI@75. In addition, no trends were observed for walk time and the different arterial stiffness parameters. In a study by Gomez-Garcia et al. (43), higher biking time was

Table 4: Regression analysis between movement behavior and arterial stiffness parameters (Cont'd)

	β per IQR (95% CI)	p
Central pulse pressure (CPP)		
Screen time		
Model 1	**1.767 (0.448 to 3.086)**	**0.01***
Model 2	0.888 (-0.395 to 2.170)	0.18
Model 3	0.867 (-0.410 to 2.144)	0.19
Sleep		
Model 1	-1.154 (-2.393 to 0.085)	0.07
Model 2	-0.585 (-1.724 to 0.553)	0.31
Model 3	-0.768 (-1.909 to 0.373)	0.19
Walking		
Model 1	-0.066 (-1.397 to 1.264)	0.92
Model 2	0.130 (-1.070 to 1.330)	0.83
Model 3	-0.770 (-2.192 to 0.651)	0.29
Cycling		
Model 1	0.641 (-0.703 to 1.985)	0.35
Model 2	1.197 (-0.015 to 2.409)	0.05
Model 3	**1.703 (0.248 to 3.158)**	**0.02***
Exercise		
Model 1	-0.461 (-2.046 to 1.124)	0.57
Model 2	0.257 (-1.214 to 1.728)	0.73
Model 3	0.294 (-1.173 to 1.762)	0.69

Regression coefficients per IQR higher movement behavior (13.375 h/week for screen time, 7.5 h/week for sleep, and 4.5 h/week for walking, cycling, or exercise) with 95% confidence intervals, and p-values. *p < 0.05, N = 190.

† Model 1: crude model. Model 2: model 1, additionally adjusted for age, sex, height, weight, MAP, maternal education, exposure to smoke, ethnicity, season, and PCS. Model 3: model 2, additionally adjusted for all other types of movement behavior (screen time, sleep, walking, cycling, and exercise).

IQR: interquartile range, CI: confidence interval, PWV: pulse wave velocity, AI@75: augmentation index normalized to a heart rate of 75 beats per minute, CPP: central pulse pressure, MAP: mean arterial pressure, PCS: Pubertal Category Scores.

associated with lower AI@75 and higher PWV in 4- to 6-year-old children. No significant associations were observed between walk time and PWV or AI@75. They also investigated the effect of total physical activity (composed of walking, cycling, PA at kindergarten, PA outside of kindergarten, and active play), which was associated with lower AI@75 and higher PWV. Various other studies available from the literature investigated the effect of different intensities (light, moderate, and vigorous) of PA on arterial stiffness in children rather than classifying by type of activity. Heffernan et al. (72) found that a higher amount of time spent performing MVPA was significantly correlated with lower PWV in 15 children with autism spectrum disorder. Additionally, a significantly lower PWV was observed with higher vigorous-intensity PA, but this was not found for higher total physical activity in a study by Lona et al. (68). On the other hand, Heil et al. (41) did not observe a significant effect of light-, moderate-, or vigorous-intensity PA on PWV in 8- to 11-year olds. Additionally, Marshall et al. (69) did not observe a significant effect of different intensities of PA on PWV or AI in 8- to 16-year-old children using compositional analysis. Similar results were observed by Koechli and colleagues; vigorous-intensity PA was not significantly associated with PWV (67), AI@75, or CPP (66). Lastly, Giontella et al. (73) assessed physical activity in prepubertal children using the validated Physical Activity Questionnaire for Older Children (PAQ-C) (74, 75), which generates a PA level between 1 (low PA) and 5 (high PA). However, they did not observe a significant correlation between PWV and physical activity level. In conclusion, the effect of PA on arterial stiffness in children has been investigated by various studies. However, the results are inconclusive. Discrepancies between different studies might be attributed to the various measurement techniques and parameters used to assess arterial stiffness and PA, which also complicates the critical comparison of the different studies (42). Additionally, the results of our study regarding the effect of physical activity on arterial stiffness shows controversies. More research using gold standard techniques such as PWV and accelerometry is necessary in order to further unravel the effect of physical activity on arterial stiffness in children. A detailed overview of the studies investigating the association between movement behavior and PWV, AI, AI@75, and CPP included in this discussion can be found in Supplementary Table 2.

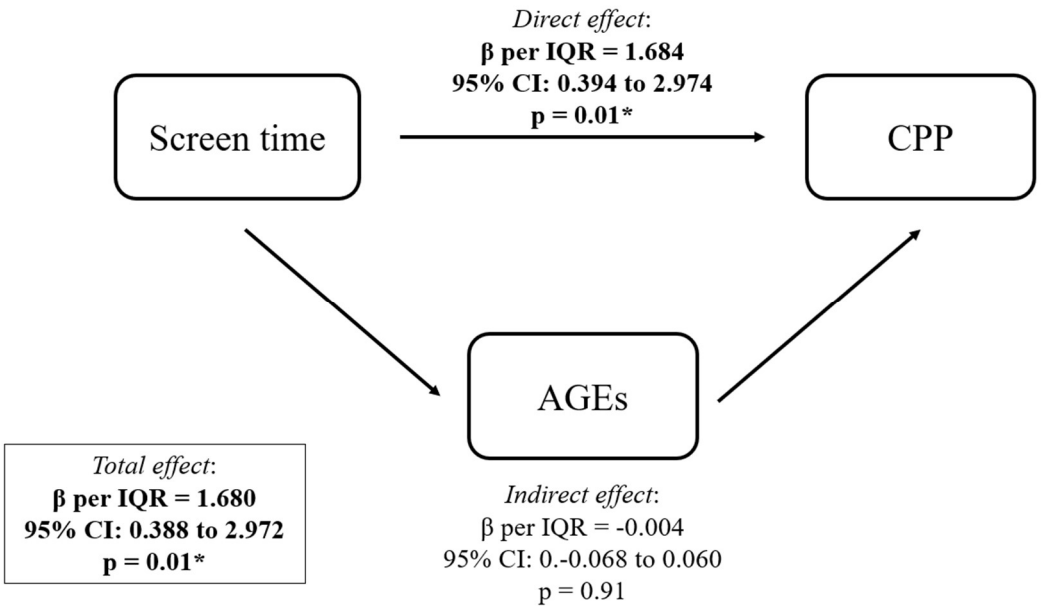

Figure 4: Total, direct, and indirect effect of AGEs in the association between screen time and CPP – Estimates IQR (13.125 h/week) higher screen time, their 95% confidence intervals, and p-values. Model not adjusted for confounders. *p < 0.05, N = 188. AGEs: advanced glycation end-products, CPP: central pulse pressure, IQR: interquartile range, CI: confidence interval.

Another body of evidence on the effect of physical activity on arterial stiffness in children has emerged from interventional studies in which a case group received additional exercise training. A total of six studies were available from the literature, of which five studies investigated the effect on PWV. Two studies observed a significant decrease in PWV in the intervention group compared to the control group (76, 77), and one study observed an insignificant increase (78). On the other hand, PWV increased more in the intervention group compared tot the control group in two other studies (79, 80), although these findings were insignificant. Regarding the AI, one study observed a significant positive effect of the intervention (81), whereas the two other studies also found improved AI, although these findings did not reach statistical significance (76, 78). Lastly, one study observed a beneficial effect of exercise intervention on CPP (80). In general, these studies suggest that exercise interventions can have a positive effect on arterial stiffness, as indicated by improvements in PWV, AI, and CPP (Supplementary Table 3).

In the present study, no mediation by AGEs was observed in the association between movement behavior and arterial stiffness. The indirect effect did not reach significance and had negligible influence on the total effect. This lack of effect might be partially attributable to the use of skin autofluorescence to measure the level of AGEs (see *'strengths and limitations'*). Despite the lack of mediation by AGEs in this study, the biological mechanism remains plausible; AGEs can cross-link collagen and elastin fibers in the blood vessel wall, leading to arterial stiffening (7, 12). In this regard, an animal study investigated the effect of an AGE cross-link breaker (Alagebrium) alone or in combination with exercise on arterial stiffness in rats. The study suggested that both prevention of the formation of new AGEs (with an exercise intervention) and breakdown of old AGEs (using the cross-link breaker) reduce vascular stiffness. Moreover, a combination of exercise and cross-link breaker yielded the best results in terms of arterial stiffness (82). However, these results could not be reproduced in a clinical trial in an older study population; neither exercise nor the AGE cross-link breaker was able to reduce arterial stiffness (83). Several other molecular mechanisms have been proposed to underly the effect of physical activity on arterial stiffness, including the production of the vasodilator nitric oxide (NO) (84) or suppression of the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) (85). Additionally, adiposity has been investigated as a potential

mediator (86, 87, 88). However, more research is needed to further elucidate the biological pathways underlying the effect of movement behavior on arterial stiffness.

Strengths and limitations – A major strength of the current study is the use of the Vicorder® measurement device to evaluate arterial stiffness. Compared to other techniques and devices, the oscillometric Vicorder® has several advantages, including easier, faster, and operator-independent assessments (89). This allows for a more precise estimation of children's arterial stiffness. In addition, measurements were performed by trained operators using a standardized protocol to limit observer bias. Next, we were able to adjust for important potential confounders as seen in literature, such as age at follow-up examination, sex, height, PCS, ethnicity, and MAP. Adjusting for MAP is particularly important because arterial stiffness can vary with blood pressure (15). If the blood pressure during arterial stiffness measurement is higher, arteries will appear stiffer than at lower blood pressure (7). To investigate the effect of movement behavior on arterial stiffness independent of blood pressure at the time of arterial stiffness assessment, MAP was included in statistical analysis.

Nevertheless, the present study has some limitations that should be acknowledged. The first disadvantage is the use of a parent-proxy questionnaire to evaluate movement behavior. Overall, questionnaires have low validity and reliability in assessing the PA behavior (90). Especially in children, who show mostly brief (less than 6 seconds) bouts of high-intensity PA (91), questionnaires might be insufficient. Additionally, the questionnaire used in this study queried the total duration of movement-related behaviors but was not able to assess its intensity or frequency. These last two factors might also be important to consider when investigating the effect of physical activity (92). The use of accelerometer devices might have been more appropriate since they can accurately distinguish between light-, moderate-, and vigorous-intensity PA, SB, and sleep (93, 94). A second disadvantage of the study is the assessment of the level of AGEs through skin autofluorescence (with the AGEReader measurement device). This fast and non-invasive method has been validated as a biomarker of cumulative skin AGEs (52), but the technique has some limitations. First of all, skin pigmentation influences the absorption of excitation and emission light resulting in higher measurement values in subjects with darker skin colors (53). By the same principle, the use of sunscreen also generates false positive results (95). Lastly, endogenous fluorescent molecules present in the skin may interfere with the measurement (53). Furthermore, we only measured the AGE level one single time, whereas obtaining three consecutive recordings at three different sites on the forearm of the children might have been more appropriate (53). Another limitation is the cross-sectional design of the current study, as longitudinal studies or intervention trials would provide stronger evidence for causal associations.

CONCLUSION

The present study suggests that different types of movement behavior might be associated with arterial stiffness in 9- to 11-year-old children. Therefore, more active movement behavior (lower sedentary behavior, higher sleep duration, and higher physical activity) in childhood potentially reduces the risk of developing cardiovascular diseases later in life. However, further research using gold standard techniques is needed to investigate the specific effect of different aspects of movement behavior (including duration, intensity, and frequency of physical activity, sedentary behavior, and sleep) on various arterial stiffness parameters. Additionally, more research is required to elucidate the underlying molecular mechanisms. While AGEs were considered as potential mediator in the present study, no significant mediation was observed. Future studies could explore inflammatory or adiposity markers to better understand the biological pathway through which movement behavior influences arterial stiffness in children.

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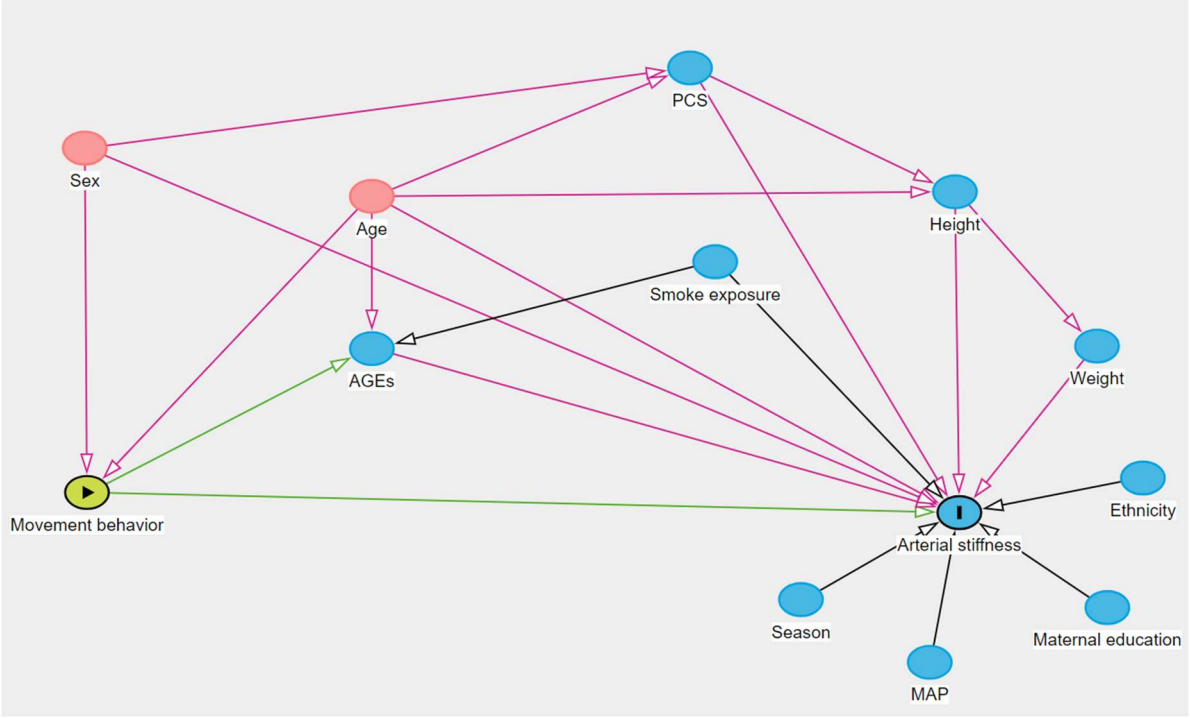
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Directed acyclic graph. Graphical representation of the causal relationship between movement behavior (exposure, ►), arterial stiffness (outcome, ▌), AGEs (mediator), and various covariates. Connections were based on literature search. Green arrows indicate the causal path between the exposure and outcome, whereas pink arrows indicate the biasing path. Blue spheres represent covariates influencing the outcome variable. Pink spheres are confounding variables that influence both the exposure and outcome variable. PCS: Pubertal Category Scores, AGEs: advanced glycation end-products, MAP: mean arterial pressure.

Supplementary Table 1: Age-specific reference values for PWV

Age	N	L	M	S	3rd	10th	25th	50th	75th	90th	97th
9.0	36	-0.161	4.765	0.106	3.91	4.16	4.44	4.76	5.12	5.47	5.85
9.5	33	-0.021	4.880	0.114	3.94	4.22	4.52	4.88	5.27	5.65	6.05
10.0	45	0.119	4.996	0.122	3.96	4.27	4.60	5.00	5.42	5.83	6.26
10.5	41	0.260	5.111	0.129	3.98	4.32	4.68	5.11	5.57	6.01	6.47
11.0	46	0.400	5.226	0.136	3.99	4.36	4.76	5.23	5.72	6.19	6.67
11.5	7	0.540	5.342	0.144	3.99	4.40	4.84	5.34	5.87	6.37	6.87

Median and percentiles of PWV are expressed in m/s. The 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles per age (years) are shown. PWV: pulse wave velocity, N: number of children in each age category, L: power of the Box-Cox transformation, M: median, S: coefficient of variation.

Supplementary Table 2: Overview of literature presented in discussion

Pulse wave velocity (aPWV or cfPWV)

Reference	Location	N (F%)	Age	Effect
Baumgartner et al. (2021) (71)	Germany	395 (25%)	14.0 ± 1.9	Higher amount of time spent performing structured exercise in the context of a sports club (min/week), as assessed by a questionnaire, was significantly associated with lower aPWV (β = -0.085, p = 0.033). However, higher training volume (MET-hours/week) was not significantly associated with aPWV (β = -0.0004, p = 0.493).
Giontella et al. (2019) (73)	Italy	300 (50%)	8.6 ± 0.7	Higher physical activity levels, as assessed by a questionnaire, were not significantly correlated with cfPWV (r = -0.045, p > 0.05). Higher physical activity volume, expressed as MET-minutes per week, was not significantly correlated with cfPWV (r = -0.056, p > 0.05).
Gomez-Garcia et al. (2021) (43)	Uruguay	816	5.8 ± 0.4	Higher time spent in sedentary behavior, as assessed by a questionnaire, was not significantly correlated with aPWV (measured by Mobil-O-Graph, r = 0.076, p = 0.104). Additionally, higher sleep duration was not significantly correlated with aPWV (r = 0.081, p = 0.090). Lastly, aPWV was not significantly associated with higher bike time (r = 0.060, p = 0.160), higher walk time (r = 0.041, p = 0.248), or higher total physical activity duration (r = 0.094, p = 0.060).
Heffernan et al. (2018) (72)	USA	15	7 ± 2	Higher amount of time spent performing MVPA, as assessed by accelerometry, was significantly correlated with lower aPWV (r = -0.462, p < 0.05).
Heil et al. (2020) (41)	Germany	80 (51%)	9.4 ± 1.1	aPWV was not significantly associated with amount of time spent performing vigorous-intensity PA (β = -0.209, p = 0.103), moderate-intensity PA (β = -0.147, p = 0.243), light-intensity PA (β = -0.031, p = 0.803), or sedentary behavior (β = 0.326, p = 0.282), as assessed by accelerometry.
Koechli et al. (2019) (67)	Switzerland	1171 (51%)	7.2 ± 0.4	aPWV was not significantly associated with vigorous-intensity PA (β = 0.0002, p = 0.367), or screen time (β = 0.0003, p = 0.998) assessed by a questionnaire.
Lona et al. (2020) (68)	Switzerland	262 (54%)	11.4 ± 0.3	aPWV was significantly inversely associated with vigorous-intensity PA (per 10 min/day) assessed by questionnaires (β = -0.009, p = 0.047). However, aPWV was not significantly associated with total physical activity (β = 0.002, p = 0.378) or sedentary behavior (β < 0.001, p = 0.995).
Marshall et al. (2022) (44)	UK	101 (45%)	12.4 ± 1.6	Compositional analysis of all different movement behaviors, assessed by accelerometry and diaries, revealed no significant associations of time spent in sleep (β = 0.05, p = 0.67), sedentary behavior (β = -0.08, p = 0.51), light- (β = 0.04, p = 0.60), and moderate- to vigorous-intensity physical activity (β = -0.02, p = 0.76) on cfPWV.
Stoner et al. (2020) (70)	New Zealand	421 (50%)	9.7 ± 0.7	Short sleepers (less than 9h, measured using accelerometry) had a significant higher cfPWV (β = 0.174, p = 0.017) compared to children who met sleep recommendations (9 to 11h).

Supplementary Table 2: Overview of literature presented in discussion (Cont'd)

Augmentation index (AI or AI@75)

Reference	Location	N (F%)	Age	Effect
Gomez-Garcia et al. (2021) (43)	Uruguay	816	5.8 ± 0.4	AI@75 measured by the Mobil-O-Graph device was significantly correlated with physical activity (r = -0.134, p = 0.013) and bike time (r = -0.163, p = 0.004), but not with walk time (r = -0.036, p = 0.277), sedentary behavior (r = 0.076, p = 0.104) or sleep (r = -0.018, p = 0.382) assessed by a questionnaire.
Koechli et al. (2021) (66)	Switzerland	1324 (51%)	7.2 ± 0.4	AI@75 was not significantly associated with the time spent performing vigorous-intensity physical activity, as assessed by questionnaires, (β = -0.007, p = 0.233). However, screen time was significantly associated with AI@75 (β = 0.016, p = 0.001).
Marshall et al. (2022) (44)	UK	101 (45%)	12.4 ± 1.6	Compositional analysis of all different movement behaviors, assessed by accelerometry and diaries, revealed no significant associations of time spent in sleep (β = 0.90, p = 0.45), sedentary behavior (β = 0.00, p = 0.99), light- (β = -0.83, p = 0.22), and moderate- to vigorous-intensity physical activity (β = -0.07, p = 0.87) with AI.

Central pulse pressure (CPP)

Reference	Location	N (F%)	Age	Effect
Koechli et al. (2021) (66)	Switzerland	1324 (51%)	7.2 ± 0.4	CPP was not significantly associated with the time (min/day) spent performing vigorous-intensity PA (β = -0.0004, p = 0.934), screen time (β = 0.006, p = 0.100), as assessed by a questionnaire.
Marshall et al. (2022) (44)	UK	101 (45%)	12.4 ± 1.6	Compositional analysis of all different movement behaviors, assessed by accelerometry and diaries, revealed no significant associations of time spent in sleep (β = -1.52, p = 0.41), sedentary behavior (β = -1.52, p = 0.41), light- (β = 1.78, p = 0.12), and moderate- to vigorous-intensity physical activity (β = 0.04, p = 0.96) with CPP.

aPWV: aortic pulse wave velocity, cfPWV: carotid-femoral pulse wave velocity, N: number of study participants, F%: percentage girls, MET: metabolic equivalent, MVPA: moderate- to vigorous-intensity physical activity, PA: physical activity, AI: augmentation index, AI@75: augmentation index normalized to a heart rate of 75 beats per minute, CPP: central pulse pressure.

Supplementary Table 3: Overview of literature presented in discussion

Pulse wave velocity (aPWV)

Reference	Location	N (F%)	Age	Effect
Hacke et al. (2019) (79)	Germany	135 (53%)	4.8 ± 0.8	A control group was compared to an intervention group that received a supervised exercise session 45 min twice a week for six months. The intervention group showed no significant difference in aPWV (difference in mean change = 0.5 m/s, p = 0.425) compared to the control group.
Ketelhut et al. (2020) (76)	Germany	105 (51%)	8.2 ± 0.6	A control group was compared to an intervention group, receiving an exercise session of 45 min twice a week for 37 weeks. The intervention group showed a significant decrease in aPWV (difference in mean change = -0.2 m/s, p = 0.037) compared to the control group.
Ketelhut et al. (2020) (78)	Germany	17 (0%)	15.3 ± 1.3	In a study population of rowing athletes, a control group was compared to an intervention group, receiving an additional HIIT (50 min) or MICT (80 min) twice a week for 8 weeks. Although not significant, aPWV improved in the intervention group (difference = -0.2 m/s, p = 0.05), but not in the control group (difference = 0.0 m/s, p = 0.86).
Ketelhut et al. (2020) (77)	Germany	46 (44%)	10.7 ± 0.6	A control group was compared to an intervention group which received an additional 20 min of HIIT during the physical education courses at school. The intervention group showed a significant decrease in aPWV (difference in mean change = -0.2 m/s, p = 0.032) compared to the control group.
Minghetti et al. (2022) (80)	Switzerland	68 (51%)	5.2 ± 0.6	A control group was compared to an intervention group who received a weekly exercise session of 45 minutes for 25 weeks. No significant difference was observed in aPWV for the intervention group compared to the control group (difference in mean change = 0.07 m/s, 95% CI: -0.32 to 0.46).

Augmentation index (AI or AI@75)

Reference	Location	N (F%)	Age	Effect
Meucci et al. (2013) (81)	USA	22 (45%)	10.1 ± 1.3	A control group was followed-up along with two intervention groups, receiving a 4 and 8 week supervised, play-based physical activity program for 6 h/day, 5 days/week. AIx@75 decreased significantly after 4 weeks (mean difference = -1.7%, $p < 0.05$) and 8 weeks (mean difference = -2.6%, $p < 0.05$) of intervention and did not change significantly in the control group (mean difference = -1.0%, $p > 0.05$).
Ketelhut et al. (2020) (76)	Germany	105 (51%)	8.2 ± 0.6	A control group was compared to an intervention group, receiving an exercise session of 45 min twice a week for 37 weeks. No significant difference in AI (difference in mean change = -3.2%, p = 0.088) was observed for the intervention group compared to the control group.

Supplementary Table 3: Overview of literature presented in discussion (Cont'd)

Augmentation index (AI or AI@75, cont'd)

Reference	Location	N (F%)	Age	Effect
Ketelhut et al. (2020) (78)	Germany	17 (0%)	15.3 ± 1.3	In a study population of rowing athletes, a control group was compared to an intervention group, receiving an additional HIIT (50 min) or MICT (80 min) twice a week for 8 weeks. No significant differences were observed in AI for the intervention (difference = -2.2%, p = 0.23) or control group (difference = 7.7%, p = 0.20).

Central pulse pressure (CPP)

Reference	Location	N (F%)	Age	Effect
Minghetti et al. (2022) (80)	Switzerland	68 (51%)	5.2 ± 0.6	A control group was compared to an intervention group who received a weekly exercise session of 45 minutes for 25 weeks. The intervention group showed a significant decrease in CPP (difference in mean change = -0.08 mmHg, 95% CI: -0.14 to -0.02) compared to the control group.

aPWV: aortic pulse wave velocity, N: number of study participants, F%: percentage girls, HIIT: high-intensity interval training, MICT: moderate-intensity continuous training, CI: confidence interval, AI: augmentation index, AI@75: augmentation index normalized for a heart rate of 75 beats per minute, CPP: central pulse pressure.