PHYSICAL ACTIVITY AND CARDIOVASCULAR HEALTH IN PRESCHOOLERS

PHYSICAL ACTIVITY AND CARDIOVASCULAR HEALTH INDICATORS DURING EARLY CHILDHOOD

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LAY ABSTRACT

Heart disease develops slowly over time, starting in childhood. This thesis explored if physical activity can begin to prevent heart disease even in preschoolers, and how much physical activity preschoolers need in order to keep their hearts and blood vessels healthy. We found that preschool-aged children who were more active had better heart health, including better blood vessel health and higher fitness. While all activity was good, more intense, moderate-to-vigorous physical activity (sometimes called energetic play) was more beneficial and slowed down the stiffening of their blood vessels as the children grew. We determined that preschool-aged children should engage in at least 4 hours of physical activity at any intensity or 80 minutes of moderate-to-vigorous physical activity every day to avoid poor heart health. The findings in this thesis highlight the importance of regular physical activity participation to promote heart health even in preschool-aged children.

ABSTRACT

Cardiovascular disease begins to develop in childhood. Physical activity positively impacts cardiovascular health and lowers cardiovascular disease risk in school-aged children and adults; however, there is insufficient evidence to determine the effects of physical activity on cardiovascular health during early childhood. The specific dose of physical activity required for favourable cardiovascular health in this age group is also unknown. The purpose of this thesis was to explore the relationships between physical activity and cardiovascular health indicators during early childhood.

In the first study, we found that physical activity engagement has beneficial effects on cardiovascular fitness, blood pressure, autonomic function, and arterial stiffness during early childhood. We then determined in the second study that to avoid unfavourable cardiovascular health, preschool-aged children should engage in at least 240 minutes of activity at any intensity, 80 minutes of moderate-to-vigorous physical activity, or 8700 steps per day. Finally, in the third study we evaluated a novel technique for examining arterial wall properties, carotid artery longitudinal wall motion, and determined that it was weakly associated with an established indicator of arterial stiffness (pulse wave velocity) in early childhood.

These findings highlight that the benefits of physical activity on cardiovascular health begin in early childhood and the minimal amount of activity to avoid unfavourable cardiovascular health is higher than current recommendations. For the most part, physical activity benefited boys and girls similarly, although it is unclear if the physical activity targets apply equally to boys. We also determined that carotid artery longitudinal wall motion may not be an indicator of arterial stiffness in young children. Future studies

should determine if the favourable effects of physical activity on cardiovascular health during early childhood carry over to later years and if achieving the physical activity targets determined in this thesis are associated with additional health benefits.

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Finally, I dedicate this thesis to my nieces, Hazel, 6, and Claire, 3. May you continue to HOPP and play through life.

TABLE OF CONTENTS

Lay Abstract ... iii
Abstract ... iv
Acknowledgements ... vi
Table of Contents ... viii
List of Figures ... x
List of Tables ... xi
List of Abbreviations ... xiii
List of Equations ... xiv
Declaration of Academic Achievement ... xv

CHAPTER 1: INTRODUCTION ... 1

1.1 Preamble ... 1

1.2 Defining childhood age groups ... 3

1.3 Non-invasive cardiovascular health indicators ... 3

1.3.1 Arterial stiffness ... 4

1.3.1.1 Pulse wave velocity ... 5

1.3.1.2 Carotid artery stiffness ... 7

1.3.1.3 Carotid artery longitudinal wall motion ... 8

1.3.2 Arterial blood pressure ... 11

1.3.3 Heart rate ... 12

1.3.3.1 Resting heart rate ... 13

1.3.3.2 Heart rate recovery ... 14

1.3.4 Cardiovascular fitness ... 15

1.4 Physical activity ... 17

1.4.1 Physical activity measurement ... 17

1.4.2 Physical activity volume and intensity in early childhood ... 19

1.5 Physical activity and cardiovascular health in children ... 20

1.5.1 Arterial stiffness and physical activity ... 20

1.5.2 Blood pressure and physical activity ... 23

1.5.3 Heart rate and physical activity ... 25

1.5.4 Cardiovascular fitness and physical activity ... 26

1.5.5 Physical activity guidelines ... 28

1.5.5.1 Current recommendations ... 28

1.5.5.2 Compliance with physical activity guidelines ... 29

1.5.5.3 Cardiovascular health in preschoolers meeting the guidelines ... 30

1.6 Study Objectives and Hypotheses 31

1.7 Methodological note 33

CHAPTER 2: Physical activity and trajectories of cardiovascular health indicators during early childhood 34

CHAPTER 3: Physical activity thresholds that discriminate between favourable and unfavourable cardiovascular health indicators in preschool-aged children 47

CHAPTER 4: Associations between carotid artery longitudinal wall motion and arterial stiffness indicators in young children 74

CHAPTER 5: DISCUSSION 81

5.1 Discussion overview 81

5.2 Physical activity and cardiovascular health in early childhood 81

5.3 Similarities and differences between boys and girls 85

5.4 Arterial stiffness during early childhood 89

5.5 Conclusions 95

CHAPTER 6: REFERENCES 97

Appendix A: Copyright permissions 116

A1. American Academy of Pediatrics author rights (Chapter 2) 116

A.2 Elsevier author rights (Chapter 4) 117

LIST OF FIGURES

CHAPTER 1: Introduction

Figure 1-1. Theoretical progression of arterial wall damage across the lifespan. 3

Figure 1-2. Typical CALM pattern shown as a percentage of a single cardiac cycle. 9

CHAPTER 2: Physical activity and trajectories of cardiovascular health indicators during early childhood

Figure 1. Flow diagram showing participants progressing through the study from recruitment to the final visit. 37

Figure 2. A, Model-predicted PWV over the 3 years of the study for different amounts of TPA. B, Model-predicted PWV over the 3 years of the study for different amounts of MVPA. 42

CHAPTER 4: Associations between carotid artery longitudinal wall motion and arterial stiffness indicators in young children

Figure 1. Average CALM pattern and single-wall radial motion of the sample expressed as the displacement across a single cardiac cycle. 76

CHAPTER 5: Discussion

Figure 5-1. Hypothesized progression in arterial stiffness across the lifespan for individuals engaging in high, average, and low amounts of physical activity. 91

LIST OF TABLES

CHAPTER 2: Physical activity and trajectories of cardiovascular health indicators during early childhood

Table 1.	Baseline characteristics.	38
Table 2.	Physical activity variables across the 3 years for participants meeting wear time criteria.	39
Table 3.	Cardiovascular health indicators across 3 annual assessments.	40
Table 4.	Linear mixed-effects model results for the effect of physical activity on cardiovascular health indicators.	41
Supplemental Table 5.	Comparison of variables at baseline between children who completed the study and those lost to follow-up.	46

CHAPTER 3: Physical activity thresholds that discriminate between favourable and unfavourable cardiovascular health indicators in preschool-aged children

Table 1.	Descriptive characteristics for participants with valid physical activity data.	68
Table 2.	Cut-off criteria and number of participants classified as unfavourable for each indicator.	69
Table 3.	Physical activity thresholds that discriminate unfavourable cardiovascular health in the combined sample.	70
Table 4.	Physical activity thresholds that discriminate between favourable and unfavourable cardiovascular health in girls.	71
Appendix Table 1.	Participant characteristics, cardiovascular health indicators, and physical activity for all participants (N=269), regardless of valid accelerometry data.	72
Appendix Table 2.	ROC curve results for physical activity discriminating between favourable and unfavourable cardiovascular health indicators in boys.	73

CHAPTER 4: Associations between carotid artery longitudinal wall motion and arterial stiffness indicators in young children

Table 1.	Participant characteristics.	77
Table 2.	CALM outcome variables and arterial stiffness indictors.	77
Table 3.	Pearson correlations between CALM outcomes and arterial stiffness.	78
Table 4.	Multiple linear regression results for arterial stiffness as predictors of CALM outcomes after adjusting for age, sex, and height.	78

LIST OF ABBREVIATIONS

AU	Arbitrary units
AUC	Area under the curve
bpm	Beats per minute
BMI	Body mass index
CALM	Carotid artery longitudinal wall motion
CDC	US Centers for Disease Control and Prevention
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiogram
HOPP	Health Outcomes and Physical activity in Preschoolers
HR	Heart rate
HRR	Heart rate recovery
ICC	Intraclass correlation coefficient
LPA	Light physical activity
MIDA	Maximum instantaneous diastolic acceleration
MIDV	Maximum instantaneous diastolic velocity
MPA	Moderate physical activity
MVPA	Moderate-to-vigorous physical activity
PWV	Pulse wave velocity
ROC	Receiver operating characteristic
SBP	Systolic blood pressure
TPA	Total physical activity
VO_2max	Maximal oxygen consumption
VPA	Vigorous physical activity
WHO	World Health Organization
Wt.	Weight

LIST OF EQUATIONS

Equation 1 $$Pulse\ wave\ velocity = \frac{Distance\ between\ sites}{Pulse\ transit\ time}$$

Equation 2 $$Arterial\ compliance\ = \frac{\Delta Diameter}{\Delta Pressure} = \frac{(AD_{max} - AD_{min})}{(P_s - P_d)}$$

Equation 3 $$Arterial\ distensibility\ = \frac{\Delta Diameter}{ADmin\ x\ \Delta Pressure} = \frac{(AD_{max} - AD_{min})}{AD_{min}\ \times (P_s - P_d)}$$

Equation 4 $$\beta - Stiffness\ Index\ = \frac{\ln(P_s/P_d)}{\Delta Diameter/ADmin} = \frac{\ln(P_s/P_d)}{(AD_{max} - AD_{min})/AD_{min}}$$

AD_{max} and AD_{min} are maximum and minimum arterial diameter, respectively; P_s and P_d are systolic and diastolic arterial blood pressure, respectively.

DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis was prepared in the "sandwich thesis" format outlined in the McMaster University School of Graduate Studies Guide for the Preparation of Master's and Doctoral Theses, published in December 2016. The first chapter is an introduction, which sets the context for the complete body of research. Chapters 2, 3, and 4 consist of three original research papers for which the candidate is first author. At the time of the thesis preparation, Chapters 2 and 4 were published in peer-reviewed journals and appear in the thesis as published, while Chapter 3 was submitted for peer-review and appears in the thesis as submitted. Chapter 5 is a concluding chapter and summarizes and discusses the main findings of this thesis and includes future research directions. The contributions of the candidate and coauthors are outlined below for each paper:

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CHAPTER 1: INTRODUCTION

1.1 Preamble

Cardiovascular disease remains the number one cause of morbidity and mortality worldwide (Benjamin et al., 2018; IHME, 2018). While overt cardiovascular disease events (myocardial infarction, stroke, etc.) typically do not occur until adulthood, cardiovascular disease develops slowly over time starting in childhood (Lloyd-Jones et al., 2010). Initial evidence that damage to arteries begins very early in life came from autopsy studies which found evidence of alterations in the arterial walls of children starting from birth. For example, almost all 1- to 5-year-olds had fragmented internal elastic membrane and proliferation of smooth muscle cells in the coronary arteries (Angelini, Thiene, Frescura, & Baroldi, 1990) and fatty streaks were present in the aortas of 3-year-olds (Newman, Wattigney, & Berenson, 1991).

Prospective cohort studies demonstrate that cardiovascular health in childhood and adolescence predicts cardiovascular health in adulthood (Ferreira, van de Laar, Prins, Twisk, & Stehouwer, 2012; Magnussen, Smith, & Juonala, 2013). For example, the Cardiovascular Risk in Young Finns Study found that brachial artery pulse pressure in childhood (3-18 years old) predicted carotid artery stiffness in adulthood (24-39 years old) (Juonala et al., 2005; Raitakari et al., 2003). Indicators like blood pressure also track from childhood to adulthood (Chen, Xiaoli & Wang, Youfa, 2008) and children and adolescents who are hypertensive are at an increased risk of premature death in adulthood (Franks et al., 2010).

Recognizing that cardiovascular disease is progressive and begins to develop in childhood, the American Heart Association released the "Defining and Setting National Goals for Cardiovascular Health Promotion and Disease Reduction: The American Heart Association's Strategic Impact Goal Through 2020 and Beyond" statement (Lloyd-Jones et al., 2010) and a companion statement focused specifically on cardiovascular health promotion in children (Steinberger et al., 2016). These statements shifted focus from primary and secondary prevention of cardiovascular disease to primordial prevention of cardiovascular disease, which includes reducing exposure to cardiovascular disease risk factors and engaging in positive health behaviours, such as physical activity. Similar to the American Heart Association statement, Nilsson and Laurent have proposed the concept of early and supernormal vascular aging, representing extremes of the progression on vascular aging, with a focus on arterial stiffening (Laurent, Boutouyrie, Cunha, Lacolley, & Nilsson, 2019; Nilsson, Boutouyrie, & Laurent, 2009). Again, this concept recognizes that arterial stiffness progressively increases across the lifespan, but the rate of artery stiffening can be both accelerated and slowed (Figure 1-1). Physical activity has been shown to positively impact cardiovascular health and lower cardiovascular disease risk in both school-aged children (Poitras et al., 2016; Tarp et al., 2018) and adults (Nocon et al., 2008); however, there is less evidence of this in early childhood. This literature review will provide an overview of non-invasive cardiovascular disease indicators that can be used to assess cardiovascular health in young children and examine previous literature related to physical activity and its association with cardiovascular health indicators during early childhood.

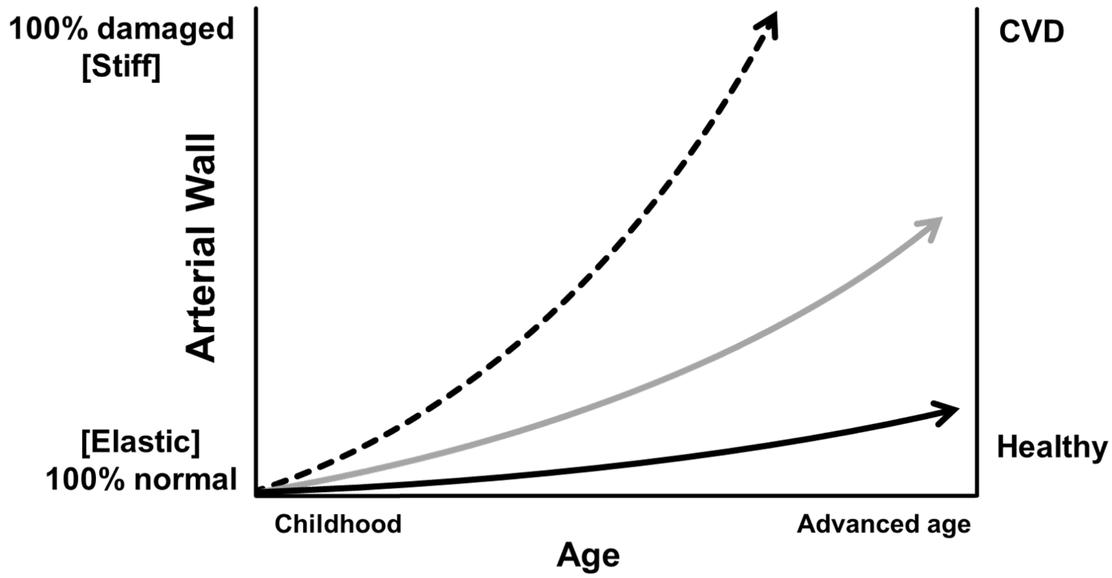

Figure 1-1. Theoretical progression of arterial wall damage across the lifespan. The grey arrow represents average vascular aging, while the dashed arrow represents accelerated vascular aging leading to CVD events. The solid black arrow shows a slowed progression in vascular aging. CVD, cardiovascular disease. [Adapted from Nilsson et al. (2009), *Hypertension* and Laurent et al. (2019), *Hypertension*]

1.2 Defining childhood age groups

There are no universally accepted definitions for describing age groups of children. In this thesis, early childhood refers to the period up to 8 years old; these children are referred to as young children. The term 'preschool-aged children' will generally refer to 3-5-year-olds, and 'school-aged children' will refer to 5-17-year-olds.

1.3 Non-invasive cardiovascular health indicators

Cardiovascular disease is an umbrella term for diseases affecting the heart and/or blood vessels (World Health Organization, 2017). Examples of cardiovascular diseases include coronary artery disease, peripheral artery disease, and stroke (US Department of Health and Human Services, 2018). While these overt cardiovascular diseases typically do not occur until adulthood, cardiovascular health can be assessed on a continuum using non-invasive indicators. This thesis will examine arterial stiffness, blood pressure, autonomic function, and cardiovascular fitness as cardiovascular health indicators, with a focus on arterial stiffness. The following section will define these indicators and briefly describe their associations with cardiovascular disease in adults and traditional cardiovascular disease risk factors in school-aged children and provide descriptive information (e.g. effect of age and sex differences) for these indicators in young children.

1.3.1 Arterial stiffness

Arterial stiffness refers to the rigidity of the artery wall (Mackenzie, Wilkinson, & Cockcroft, 2002). It is primarily determined by the composition and alignment of elastin and collagen and resting vasomotor tone (Zieman, Melenovsky, & Kass, 2005). Fragmentation of elastin fibers and replacement of these fibers with less elastic collagen fibers increases the stiffness of the artery wall (Chirinos, Segers, Hughes, & Townsend, 2019; Tanaka, 2019; Zieman et al., 2005). Arterial stiffness progressively increases with age (Avolio et al., 1983; Diaz, Zócalo, Bia, Wray, & Fischer, 2018; Kucharska-Newton, Stoner, & Meyer, 2019) and is predictive of cardiovascular disease events and all-cause mortality, even after adjusting for traditional cardiovascular disease risk factors (e.g. blood pressure) (Vlachopoulos, Aznaouridis, & Stefanadis, 2010). Arterial stiffness can be assessed using non-invasive techniques, including pulse wave velocity (PWV), which estimates stiffness in a segment of the arterial tree, and local assessments in a small portion of an artery, typically the carotid artery (O'Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002).

1.3.1.1 Pulse wave velocity

When the heart contracts, a pulse wave is generated that propagates through the arteries in the body. The stiffer the artery, the faster the pulse wave travels (O'Rourke et al., 2002). Thus, we can assess the stiffness of a segment of the arterial tree by determining the speed the pulse travels between two sites, which is called pulse wave velocity (PWV) (Laurent et al., 2006). PWV can be assessed non-invasively by detecting the pulse over the skin at specific points in the arterial tree (Laurent et al., 2006). The time delay between the arrival of the pulse wave at the two sites (pulse transit time) is determined and divided into the measured distance between the two sites to calculate PWV (Equation 1) (O'Rourke et al., 2002).

Equation 1 $$Pulse\ wave\ velocity = \frac{Distance\ between\ sites}{Pulse\ transit\ time}$$

PWV can be assessed centrally (carotid-to-femoral), peripherally (carotid-to-radial or femoral-to-dorsalis pedis), or using a whole-body approach (carotid-to-foot, -ankle, or -toe) (Laurent et al., 2006). Carotid-to-femoral PWV, also often referred to as central PWV or aortic PWV, is the gold standard assessment of arterial stiffness and has the largest body of evidence linking stiffness to cardiovascular disease (Laurent et al., 2006). In a metanalysis of over 17,000 adults, carotid-to-femoral PWV was found to be a significant predictor of cardiovascular events and mortality, over and above traditional risk factors (Ben-Shlomo et al., 2014), and every 1 m/s increase in carotid-to-femoral PWV was found to correspond to a 14-15% increase in risk for cardiovascular events and mortality (Vlachopoulos et al., 2010).

Although fewer studies have examined whole-body PWV (e.g. carotid-to-foot), this is a more simple and feasible method (Phillips et al., 2015) that still includes the central arteries while eliminating the need to detect a femoral pulse, which may be considered intrusive or uncomfortable, particularly for young children and/or their parents (Savant, Furth, & Meyers, 2014). Recently, Klassen *et al.* (2018) found that carotid-to-toe PWV had a moderate-to-strong correlation with carotid-to-femoral PWV in 20-28-year-old males and females; although carotid-to-toe PWV overestimated carotid-to-femoral PWV at lower values and underestimated carotid-to-femoral PWV at higher values. Whole-body PWV was reported to have substantial reliability (ICC=0.76) in 2-6-year-old boys and girls (Currie, Proudfoot, Timmons, & MacDonald, 2010).

School-aged children with hypertension have higher whole-body PWV (Phillips et al., 2015) and carotid-to-femoral PWV was found to be associated with obesity, blood lipids, and inflammation in 8- and 9-year-olds (Correia-Costa et al., 2016). Most studies show that carotid-to-femoral PWV increases with age in school-aged children (Curcio et al., 2016; Mora-Urda, Molina, Mill, & Montero-López, 2017; Reusz et al., 2010); however, there is little research examining the change in PWV over time during early childhood. In a cross-sectional analysis, Hidvégi *et al.* (2012) reported that PWV, estimated by a brachial pulse wave analysis technique, was not different in 3- to 8-year-olds; however, this pulse wave analysis technique has been found to reflect upper limb (carotid-to-radial) PWV in adults, not central stiffness (Trachet et al., 2010). In school-aged children, carotid-to-radial PWV has been shown to not be associated with age (Curcio et al., 2016). Studies have reported there are no differences in carotid-to-femoral (Curcio et al., 2016; Mora-Urda et al., 2017; Reusz et al., 2010) or carotid-to-radial

(Ayer, Harmer, Marks, Avolio, & Celermajer, 2010; Curcio et al., 2016) PWV between prepubertal boys and girls.

1.3.1.2 Carotid artery stiffness

Arterial stiffness can also be assessed locally in a small section of a single artery through simultaneous acquisition of both pressures and diameters at the same site (O'Rourke et al., 2002). The common carotid artery is most often assessed as it is an accessible artery and carotid artery stiffness is associated with cardiovascular events and mortality in adults (van Sloten et al., 2015). Typically, carotid artery stiffness is determined non-invasively using an ultrasound to measure diameters and a tonometer to measure pressures in the artery of interest (O'Rourke et al., 2002). There are a variety of ways to calculate local artery stiffness; three common calculations are arterial compliance (Equation 2), arterial distensibility (Equation 3), and β-stiffness index (Equation 4), as described in the equations that follow, where AD_{max} and AD_{min} are maximum and minimum arterial diameter, respectively and P_s and P_d are systolic and diastolic arterial blood pressure, respectively (O'Rourke et al., 2002). Arterial compliance and distensibility quantify the absolute and relative change in diameter (ΔDiameter),

Equation 2 $$\text{Arterial compliance} = \frac{\Delta Diameter}{\Delta Pressure} = \frac{(AD_{max} - AD_{min})}{(P_s - P_d)}$$

Equation 3 $$\text{Arterial distensibility} = \frac{\Delta Diameter}{ADmin\ x\ \Delta Pressure} = \frac{(AD_{max} - AD_{min})}{AD_{min} \times (P_s - P_d)}$$

Equation 4 $$\beta - Stiffness\ Index = \frac{\ln(P_s/P_d)}{\Delta Diameter/ADmin} = \frac{\ln(P_s/P_d)}{(AD_{max} - AD_{min})/AD_{min}}$$

respectively, for a given change in pressure (ΔPressure) across the heart cycle, while β-stiffness index takes into account the absolute systolic and diastolic blood pressures in the artery. Stiffer arteries have lower compliance and distensibility and higher β-stiffness index. These equations make a number of assumptions, including that the artery is a uniform, circular, hollow structure (Laurent et al., 2006). Carotid artery β-stiffness index was reported to have higher day-to-day reliability (ICC=0.80) than carotid artery compliance (ICC=0.61) and distensibility (ICC=0.63) in 2-6-year-olds (Currie et al., 2010). In school-aged children, carotid artery β-stiffness index is associated with obesity, blood lipids, and inflammation (Cote et al., 2015; Mikola et al., 2017). Like central PWV, carotid artery stiffness has been found to increase with age in children (Calabrò et al., 2017; Curcio et al., 2016; Doyon et al., 2013); however, it is unknown if carotid artery stiffness increases during the early childhood period. At age 8, Ayer *et al.* (2010) found that boys (n=207) have higher carotid artery stiffness, assessed by distensibility, than girls (n=198), while Curcio *et al.* (2016) reported no sex differences in carotid artery β-stiffness index a small sample of 4-8 year-old boys (n=13) and girls (n=9).

1.3.1.3 Carotid artery longitudinal wall motion (CALM)

The specialized equipment required and challenges in signal acquisition have limited the use of PWV and local carotid artery stiffness in clinical and research settings (Williams et al., 2018). While a number of alternative techniques have been developed (e.g. augmentation index and pulse wave analysis estimate arterial stiffness from a single pulse wave signal acquired at the carotid or wrist (Mackenzie et al., 2002)), these still require specialized equipment and skilled personnel that are not typically available in clinical settings. Recently, carotid artery longitudinal wall motion (CALM) has been proposed as a measure of vascular health that may be a novel indicator of arterial stiffness. CALM may address some of the feasibility concerns associated with other indices of arterial stiffness because it only requires high resolution longitudinal images of the carotid artery wall, which could be acquired in a typical ultrasound assessment (Wang, Vahala, & Hao, 2018). CALM refers to the movement of the artery wall in the axial plane, toward and away from the heart (Cinthio et al., 2018). This longitudinal movement is similar in magnitude to radial distension (Cinthio et al., 2006). CALM typically presents with a triphasic pattern (Figure 1-2) of bidirectional movement, with the wall moving anterograde, in the same direction of blood flow, and retrograde, against the direction of blood flow (Cinthio et al., 2018). Au *et al.* (2016) hypothesized that throughout the cardiac cycle, the systolic anterograde movement was a result of the local

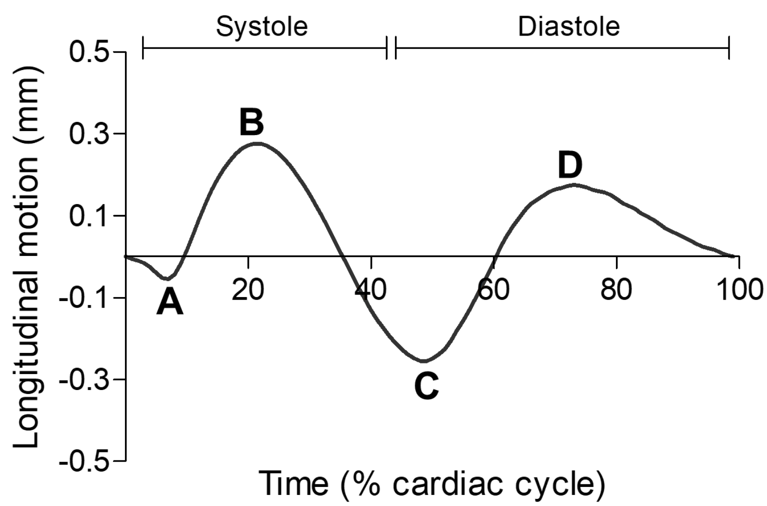

Figure 1-2. Typical CALM pattern shown as a percentage of a single cardiac cycle. Positive deflections indicate displacement in the anterograde direction (i.e. toward the head, in the direction of blood flow) and negative deflections indicate displacement in the retrograde direction (i.e. toward the heart). Systolic anterograde CALM displacement is the difference in magnitude from A to B. Systolic retrograde CALM displacement is the difference in magnitude between B and C. Diastolic CALM displacement is the difference in magnitude from C to D. Maximum displacement is the total amplitude of the trace. CALM, carotid artery longitudinal wall motion.

blood flow in the carotid artery during early systole, followed by a retrograde displacement of the arterial wall as the artery is pulled back down towards the heart by the twisting motion of the heart during late systole. The third phase of the movement occurs when the artery wall recoils back towards its starting position during diastole (Au et al., 2016).

CALM measurements have good to excellent beat-to-beat and intra- and interobserver reliability in adults (ICC>0.86) (Au, Yli-Ollila, & MacDonald, 2018; Zahnd et al., 2011). The day-to-day reproducibility of retrograde and maximal displacements is acceptable (Cronbach's alpha 0.78 and 0.73, respectively); however, anterograde displacements have lower day-to-day reproducibility (Cronbach's alpha 0.57) (Yli-Ollila, Laitinen, Weckström, & Laitinen, 2013).

There is evidence that the magnitude of the segmental displacements depends on the elastic properties of the artery (Cinthio et al., 2018) such that associations have been found between arterial stiffness, measured by carotid-to-femoral PWV and carotid distensibility, and CALM displacements in adults (Au, Valentino, McPhee, & MacDonald, 2017; Taivainen et al., 2017; Zahnd et al., 2012). CALM displacements are reduced in adults with cardiovascular disease (Au et al., 2017; Svedlund & Gan, 2011; Zahnd, Saito, Nagatsuka, Otake, & Sato, 2018) and traditional cardiovascular disease risk factors, such as high blood pressure and abnormal blood lipids (Taivainen et al., 2018; Zahnd et al., 2011, 2012). The magnitude of CALM displacements are also predictive of cardiovascular events over the course of 12 months in adults with suspected coronary artery disease (Svedlund, Eklund, Robertsson, Lomsky, & Gan, 2011). CALM displacements are reduced with age in adults (Au et al., 2017; Zahnd et al., 2012); however, CALM has not been assessed in children. Only one study reported comparisons between males and females and found CALM displacements did not differ in a small sample of healthy adults (n=16) (Svedlund & Gan, 2011).

1.3.2 Arterial blood pressure

Arterial systolic blood pressure (SBP) is the highest pressure in an artery and occurs during left ventricular contraction, while arterial diastolic blood pressure (DBP) is the lowest pressure in an artery and occurs during relaxation of the left ventricle (Tortora & Grabowski, 2003). Blood pressure is affected by cardiac output, systemic vascular resistance, and blood volume. These factors are regulated by the parasympathetic and sympathetic nervous systems, baroreceptors and chemoreceptors, blood vessel vasodilators and vasoconstrictors, and a number of hormones (Tortora & Grabowski, 2003). Clinically, blood pressure is typically assessed in the brachial artery in a seated position (Pickering et al., 2005).

High blood pressure is a leading risk factor for both cardiovascular disease and all-cause morbidity, even after adjusting for other risk factors (Lim et al., 2012). Both SBP and DBP are predictive of cardiovascular events in adults, with SBP also predictive of all-cause mortality (Psaty et al., 2001). Blood pressure in children and adolescents is associated with target organ damage, including left ventricular hypertrophy (Flynn, 2018). Elevated blood pressure in childhood is also predictive of hypertension, metabolic syndrome, and signs of atherosclerosis such as increased arterial wall thickness, in adulthood (Flynn, 2018).

SBP and DBP increase during childhood (Flynn et al., 2017). Blood pressure is also related to height, with higher blood pressures measured in taller individuals. As such, percentiles are used to classify blood pressure in children according to their age, sex, and height (Rosner, Cook, Portman, Daniels, & Falkner, 2008). For children under the age of 13, normal blood pressure is defined as both SBP and DBP <90th percentile; elevated blood pressure is defined as SBP and/or DBP ≥90th percentile to <95th percentile or ≥120/80 mmHg to <95th percentile; and hypertension is SBP and/or DBP ≥95th percentile or ≥130/80 mmHg (Flynn et al., 2017). Absolute SBP and DBP tend not to differ between boys and girls under the age of 10 years (Malina, Bouchard, & Bar-Or, 2004), although SBP has been reported to increase at a faster rate in boys compared to girls from age 4 years onwards (Shen et al., 2017).

1.3.3 Heart rate

Impaired cardiac autonomic function is a key feature of many cardiovascular diseases, including hypertension and heart failure (Peçanha, Silva-Júnior, & Forjaz, 2014; Tadic, Cuspidi, & Grassi, 2018). Heart rate at rest and during recovery from exercise (referred to as heart rate recovery [HRR]) can be used to assess autonomic function (Lahiri, Kannankeril, & Goldberger, 2008). Heart rate is regulated through the autonomic nervous system, with greater activation of the sympathetic and parasympathetic nervous systems increasing and decreasing heart rate, respectively (Tortora & Grabowski, 2003). Resting heart rate largely reflects sympathovagal balance, whereas HRR largely reflects the responsiveness of the parasympathetic nervous system following exercise (Lahiri et al., 2008). Generally, high sympathetic activity and low parasympathetic activity are associated with negative cardiovascular outcomes (Lahiri et al., 2008; Tadic et al., 2018).

1.3.3.1 Resting heart rate

In a healthy individual at rest, the parasympathetic nervous system is dominant and largely determines heart rate (Tadic et al., 2018). A higher resting heart rate, therefore, suggests an imbalance of parasympathetic (vagal) and sympathetic outflow, with greater sympathetic compared to parasympathetic activation (Tadic et al., 2018). Resting heart rate is an independent predictor of cardiovascular morbidity and mortality in adults (Cooney et al., 2010), with higher resting heart rates indicating poorer cardiovascular health (Tadic et al., 2018). In a large metanalysis of over 1 million adults in prospective cohort studies, Zhang *et al.* (2016) found that cardiovascular and all-cause mortality increased 8-9% for every 10 beats/min increase in resting heart rate. In children and adolescents, high resting heart rate is associated with cardiovascular disease risk factors like higher blood pressures and adiposity (Farah et al., 2015; Kwok et al., 2013). Resting heart rate decreases throughout childhood; from age 3 to 10 resting heart rate decreases at a rate of approximately 3 beats per minute per year (Sarganas, Schaffrath Rosario, & Neuhauser, 2017). On average, seated resting heart rate is higher in girls than boys during early childhood (Sarganas et al., 2017), although supine, basal heart rate has been shown to not be different between boys and girls under the age of 10 (Malina et al., 2004). While there is no consensus on body position for resting heart rate measurement (Palatini et al., 2006), supine, but not seated, heart rate was found to predict cardiovascular mortality in a large sample of older adults (Li et al., 2019). Furthermore, in 8- and 9-year-olds (n=40), reliability was higher for supine (boys: ICC 0.95, CV 2.8%; girls: ICC 0.92, CV 3.3%) compared to seated (boys: ICC 0.66, CV 4.7%; girls: ICC 0.32, CV 7.2%) heart rate (Silva, Bertollo, Reichert, Boullosa, & Nakamura, 2017).

1.3.3.2 Heart rate recovery

The decline of heart rate following exercise, or HRR, is another measure of autonomic function (Lahiri et al., 2008). HRR is assessed as the difference between peak heart rate achieved during exercise and heart rate at 0.5, 1, 2, or 5 minutes after cessation of exercise (Peçanha et al., 2014). Upon initiation of exercise, the initial increase in heart rate is primarily due to parasympathetic withdrawal, followed by increased sympathetic activation (Peçanha et al., 2014). Upon cession of exercise, the decrease in heart rate during the first minute of recovery is predominately due to parasympathetic reactivation (Imai et al., 1994; Peçanha et al., 2014), followed by a combination of sympathetic withdrawal and parasympathetic reactivation in the second minute of recovery (Peçanha et al., 2014). Higher (faster) HRR is indicative of better autonomic function (Lahiri et al., 2008). Slow HRR is predictive of mortality in adults (Cole, Blackstone, Pashkow, Snader, & Lauer, 1999), and young adults with cardiovascular disease risk factors, including obesity and high fasting glucose, are more likely to develop impaired HRR (Carnethon et al., 2012). In 12-19-year-olds (n=933), HRR following a submaximal treadmill test was reported to be inversely associated with SBP, blood lipids, and inflammation (Lin et al., 2008).

HRR is faster in children than adults and decreases across childhood (Mimura & Maeda, 1989; Singh, Rhodes, & Gauvreau, 2008). In a sample of 485 5- to 18-year-old children, 1-minute HRR decreased by 2 bpm for each year in age and HRR was approximately 5 bpm faster in boys than girls, even with adjustment for BMI, baseline heart rate, age, and fitness (Singh et al., 2008). HRR increases along with fitness and training status in adults (Daanen, Lamberts, Kallen, Jin, & Van Meeteren, 2012) and is also faster in more fit children (Singh et al., 2008), which has led to its use as a proxy of cardiovascular fitness.

1.3.4 Cardiovascular fitness

Cardiovascular fitness refers to the ability of the heart, lungs, and circulatory system to supply oxygen to working muscles efficiently (Heyward, 2006, p. 380). In adults, cardiovascular fitness is one of the strongest predictors of cardiovascular disease events and mortality and improves risk classification when added to traditional risk factors (Ross et al., 2016). The gold standard for assessment of cardiovascular fitness is maximum oxygen uptake (VO_2max), which can be determined using a metabolic cart for gas collection during an incremental exercise test on a treadmill or cycle ergometer (Armstrong & McManus, 2017). When it is neither possible nor feasible to conduct a VO_2max test, predictive tests can be employed which typically depend upon the linear relationships between oxygen uptake, exercise time during a progressive test, and increases in heart rate (Heyward, 2006). As such, time to exhaustion on a standardized exercise test can also be used to assess cardiovascular fitness and is more feasible in young children as it does not require a facemask or mouthpiece to collect breath samples, which may not be tolerated well in young children (van der Cammen-van Zijp et al., 2010). Time to exhaustion on the Bruce Protocol (Bruce, Kusumi, & Hosmer, 1973), a progressive treadmill test, strongly correlates with VO_2max in 10-18-year-olds ($r=0.88$), providing evidence that this is a valid fitness test for children (Cumming, Everatt, & Hastman, 1978). Furthermore, in 7-13-year-olds, time to exhaustion on tests performed on different days strongly correlated ($r=0.94$), suggesting this test is also reliable in children (Cumming et al., 1978). Some adaptations to the Bruce Protocol are generally made when using this test with young children, these include: permitting children to hold the handrails, positioning an investigator behind the child during the test, and allowing the child to run once the speed is too fast for walking (van der Cammen-van Zijp et al., 2010).

While the Bruce Protocol is the most common lab-based exercise test used in preschool-aged children, the 20-metre shuttle run is a popular field-based assessment of cardiovascular fitness (Ortega et al., 2015). In this test, the participant runs back and forth for 20 meters under a standardized amount of time, which progressively gets shorter as you move through the test (Cadenas-Sanchez et al., 2016). In general, more fit children should be able to complete more laps in the allotted time, with the number of laps completed often converted to an estimated VO_2max using published equations that also include age, sex, and body mass or BMI (Batista et al., 2013). In school-aged children, half of the studies comparing laboratory-measured VO_2max with estimated VO_2 max from the 20-m shuttle reported correlations <0.70, with weaker correlations reported (0.11 lower) when only the performance (i.e. laps completed) is correlated with measured VO_2max (Mayorga-Vega, Aguilar-Soto, & Viciana, 2015). In preschool-aged children, results are typically reported in laps completed as the equations were not created or validated using this age group, which suggests this test may have lower validity for cardiovascular fitness in preschool-aged children in comparison to the Bruce Protocol.

Time to exhaustion on the Bruce Protocol increases from age 4 to around age 10 in girls, and from 4 to age 13-15 in boys (Cumming et al., 1978). Comparisons between 4-6-year-old boys and girls for both time to exhaustion on the Bruce protocol (Cumming et al., 1978; van der Cammen-van Zijp et al., 2010; Wessel, Strasburger, & Mitchell, 2001) and stages completed on the 20-m shuttle run (Bürgi et al., 2011; Leppänen et al., 2016;

Niederer et al., 2012) are largely equivocal. A systematic review by Ruiz *et al.* (2009) identified 17 high quality studies which examined the prospective relationship between cardiovascular fitness (using both laboratory and field-based assessments) in children and adolescents and subsequent cardiovascular disease risk factors 2-24 years later. They concluded that there was strong evidence that cardiovascular fitness in childhood and adolescence predicted future blood pressure, blood lipids, adiposity, and arterial stiffness (Ruiz et al., 2009).

1.4 Physical activity

Arterial stiffness, blood pressure, autonomic function, and cardiovascular fitness are predictive of cardiovascular disease in adults, associated with traditional cardiovascular disease risk factors in school-aged children, and are feasible to use in young children. There is a large body of literature demonstrating that the volume and intensity of physical activity can impact each of these cardiovascular indicators; however, little of this work has been conducted in early childhood. Prior to reviewing the associations between physical activity and cardiovascular health indicators, the measurement of physical activity and typical volumes and intensities reported in early childhood will be introduced in the following section.

1.4.1 Physical activity measurement

The accepted definition of physical activity is “any bodily movement produced by the skeletal muscles that results in energy expenditure” (Caspersen, Powell, & Christenson, 1985, p. 126). Physical activity can be measured using questionnaires (with parent-proxy reports used to assess young children's activity), direct observation, doubly labelled water (i.e., energy expenditure), or devices such as pedometers or accelerometers (Sirard & Pate, 2001). Accelerometers have become the de facto standard for physical activity measurement in children, allowing for a device-measured assessment of physical activity over several days in a free-living setting (Troiano, McClain, Brychta, & Chen, 2014).

ActiGraph devices are the most widely used research-grade accelerometers (Bassett, Troiano, Mcclain, & Wolff, 2015). In children, the devices are typically worn on a band around the waist (Migueles et al., 2017). Movement is measured as accelerations in G-forces (*g*), which are then converted to proprietary activity counts. Activity counts were traditionally summed across a 1-minute period, referred to as an epoch, due to storage limitations in older model accelerometers (Rowlands, 2007). Today, most accelerometers can store raw accelerations and the epoch can be selected during post-processing (Troiano et al., 2014). Most studies in children now use shorter epochs (≤15-seconds) to capture the sporadic nature of children's physical activity and play (Migueles et al., 2017; Rowlands, 2007). To quantify physical activity, activity counts are summed across the user-specified epoch and the count is classified as light (LPA), moderate (MPA), or vigorous (VPA) intensity by applying age-appropriate thresholds, or cut-points, that are associated with activity intensity (Rowlands, 2007). MPA and VPA are often combined and referred to as moderate-to-vigorous physical activity (MVPA). Total physical activity (TPA) is the sum of LPA, MPA, and VPA.

While accelerometry has become the standard for measurement of physical activity in children, few parameters of the collection and processing of accelerometry data have been standardized (Migueles et al., 2017). In particular, variations in epoch and cut-point selection can dramatically impact the ultimate values produced in analysis, which creates challenges when comparing absolute physical activity quantities across studies (Migueles et al., 2017). Within the preschool age group alone, at least 9 cut-points for ActiGraph devices have been created to classify activity intensity, which has resulted in large variability in estimated levels of physical activity volume and intensity during early childhood (Migueles et al., 2017).

1.4.2 Physical activity volume and intensity in early childhood

Due to the lack of consistency in data collection and processing, reported levels of physical activity are highly variable in early childhood. A review of objectively measured physical activity in 2-5-year-olds identified 37 studies that used direct observation, heart rate monitors, and most often, accelerometry to quantify physical activity (Hnatiuk, Salmon, Hinkley, Okely, & Trost, 2014). Across the studies, the average proportion of waking time spent in LPA and MVPA ranged from 2-41% (approximately 13 minutes to 5.4 hours) and 4-33% (approximately 30 minutes to 4.2 hours), respectively. The median time spent in LPA and MVPA was estimated to be 2.2 hours and 47 minutes, respectively, for an estimated TPA of approximately 3 hours per day (Hnatiuk et al., 2014). However, when examining only the studies that used the Pate cut-points (Pate, Almeida, McIver, Pfeiffer, & Dowda, 2006), which are the most accurate for classifying MVPA in 4-6 year-olds (Janssen et al., 2013), average MVPA engagement appears to be just under 100 minutes per day (Hnatiuk et al., 2014).

MVPA and TPA increase from age 3 in boys and girls (Hnatiuk, Lamb, Ridgers, Salmon, & Hesketh, 2019; Schmutz et al., 2018), with significant declines in MVPA beginning around age 6 years in girls and age 9 years in boys (Farooq et al., 2019). Most studies show that boys are more active than girls during early childhood (Bingham et al., 2016; Hinkley, Crawford, Salmon, Okely, & Hesketh, 2008). When the mean MVPA across 33 studies (total n=12 178; 3-6-year-olds) derived from 7 different cut-points were transformed to the Pate cut-points, the transformed estimated MVPA was 94 and 84 minutes per day for boys and girls, respectively (Ravagnani et al., 2017). This suggests a difference in MVPA of approximately 10 minutes per day between preschool-aged boys and girls (Ravagnani et al., 2017).

1.5 Physical activity and cardiovascular health in children

The number of studies examining the impact of physical activity on health during early childhood has increased in recent years (Carson et al., 2017); however, there remains limited evidence on cardiovascular health indicators (Pate et al., 2019). In the following section, previous literature related to physical activity and its associations with the cardiovascular health indicators arterial stiffness, blood pressure, heart rate, and cardiovascular fitness during early childhood, will be summarized and supplemented by findings from existing research in school-aged children.

1.5.1 Arterial stiffness and physical activity

There is little previous research examining the effect of physical activity on arterial stiffness in early childhood. To date, the study that examined the youngest participants was a 6-month physical activity intervention in 3-6-year-olds (n=92 in intervention, n=43 in control) (Hacke et al., 2019). Overall, this preschool-based intervention (1 hour, 2 times per week) did not have an effect on PWV, measured by pulse wave analysis; however, due to the lack of device-measured physical activity it is not known if the intervention actually increased physical activity levels. A subgroup analysis revealed that there was a reduction in PWV (-0.1 m/s) following the intervention in children of lower socioeconomic status. It is unclear if the children of lower socioeconomic status had poorer initial arterial health (i.e. higher baseline PWV) as this data was not presented (Hacke et al., 2019).

In an observational study, Idris *et al.* (2015) found no relationship between parent-reported physical activity and sport participation and carotid artery distensibility in children when they were 5 years old (n=595) nor in a follow-up cross-sectional analysis 3 years later at age 8 (n=237). In contrast, using accelerometry-measured physical activity, Haapala *et al.* (2017) found that both MPA and VPA were inversely (favourably) associated with arterial stiffness, assessed by pulse contour analysis, in 6-8-year-olds. Likewise, inverse associations were reported between step count and carotid-to-femoral PWV in 9-10-year-olds (Sakuragi et al., 2009). Results from studies using self-reported physical activity in 10-14-year-olds have been mixed (Schack-Nielsen, Mølgaard, Larsen, Martyn, & Michaelsen, 2005), which highlights the importance of using device-measured physical activity and considering the method of physical activity measurement when evaluating and comparing the results of studies.

Device-measured physical activity also facilitates a better understanding of the role of the intensity of physical activity on arterial stiffness. In the study by Haapala *et al.* (2017), the authors reported that only MPA and VPA, not LPA, were significantly associated with arterial stiffness. While this finding provides evidence that higher intensities of physical activity have a stronger relationship with arterial stiffness, it does not clarify the effect of activity at any intensity (i.e. TPA). In 8-11-year-olds, both TPA and MVPA, measured via accelerometry, accounted for a similar amount of variance in small artery compliance; however, neither were predictors of large artery compliance (Nettlefold, McKay, Naylor, Bredin, & Warburton, 2012). Work in other vascular health parameters, such as flow-mediated dilation, suggests that higher intensity physical activity may be more beneficial for arterial heath in school-aged children (Hopkins et al., 2009). It is also not clear if physical activity has a similar effect on arterial stiffness for both boys and girls. While many of the previously mentioned studies adjusted for sex in the analyses, only two examined boys and girls separately or included sex as a moderator (Haapala et al., 2017; Idris et al., 2015). In separate analyses for girls and boys, Haapala *et al.* (2017) found that the effects of physical activity on arterial stiffness remained significant for girls (n=79), while the effect was attenuated, and no longer significant (p=0.06), for boys (n=57), although this may have been due to the small sample size. This finding contrasts with previous findings in older children, where 15-year-old boys in the lowest quartile of device-measured MVPA had significantly stiffer carotid arteries compared to the most active boys, with no relationship observed in girls (Ried-Larsen, Grøntved, Froberg, Ekelund, & Andersen, 2013).

While the overall evidence suggests that active school-aged children have lower arterial stiffness, there are limited prospective studies with repeated measures of arterial stiffness to determine if active children have a slower age-related increase in arterial stiffness than their less active peers. A 3-year follow up of adolescents initially aged 12-16 years old (n=257) showed an association between decreased physical activity and an increase in carotid-to-radial PWV in boys, but not girls (Chen et al., 2012). This study, however, utilized a crude measurement of physical activity where participants self-rated their physical activity engagement on a scale of 1 (not at all) to 5 (very often). In a longitudinal analysis that combined data from intervention and control participants enrolled in a diet and lifestyle counseling randomized control trial (n=503), Mikola *et al.* (2017) did not find a significant effect of self-reported leisure time physical activity on carotid artery distensibility from age 11 to 19 years old. There is currently a lack of prospective, observational research examining the relationships between device-measured physical activity and a) arterial stiffness during early childhood and b) the rate of change in arterial stiffness during childhood.

1.5.2 Blood pressure and physical activity

More work has been done looking at the associations of physical activity and blood pressure during early childhood than arterial stiffness; however, the findings are inconsistent, and most have not been conducted utilizing device-measured physical activity (Bell, Fletcher, Timperio, Vuillermin, & Hesketh, 2018). Two recently published systematic reviews (2017 and 2018) addressed relationships between physical activity and blood pressure during early childhood (Bell et al., 2018; Carson et al., 2017). These reviews searched randomized and non-randomized control trials, as well as prospective and cross-sectional studies, where the mean age of the sample was either 1 month to 4.9 years or had an upper range of 6 years (Carson et al., 2017) or the majority of sample was 3.0-5.5 years (Bell et al., 2018). Combined, these reviews identified nine unique studies that examined relationships between physical activity and blood pressure. The majority of these studies showed no associations between physical activity and blood pressure in preschool-aged children. Specifically, for SBP, most studies found no association (Bel-Serrat et al., 2013; Klesges, Haddock, & Eck, 1990; Sääkslahti et al., 1999, 2004; Vale, Trost, Rêgo, Abreu, & Mota, 2015; Wilson et al., 1992), with mixed (Idris et al., 2015) and unfavourable (Kolpakov et al., 2011) associations also reported. For DBP, null (Idris et al., 2015; Klesges et al., 1990; Sääkslahti et al., 1999, 2004), mixed (Wilson et al., 1992), favourable (Scheffler, Ketelhut, & Mohasseb, 2007), and unfavorable (Kolpakov et al., 2011) associations were reported.

Only three of the studies included in the reviews utilized device-measure physical activity, while the remaining studies used parent-reported physical activity. Vale *et al.* (2015) found that inactive 3- to 6-year-olds, defined as engaging in less than 60 minutes of MVPA per day, were not more likely to have elevated SBP compared to their active peers. In another study of 3- to 6-year-olds (n=137), Klesges *et al.* (1990) did not find significant correlations between TPA, measured on 1 day using accelerometry, and SBP or DBP. Conversely, a cross-sectional study (n=287) from Kolpakov *et al.* (2011) found both preschool-aged boys and girls with low physical activity levels, as determined by pedometer step count, had lower SBP and DBP than the children who were more active. These cross-sectional studies did not perform sex-based analyses (Klesges et al., 1990; Vale et al., 2015) or adjust for potential confounders in their analyses (Klesges et al., 1990; Kolpakov et al., 2011; Vale et al., 2015), including age or height, which have both been previously identified as important determinants of blood pressure (Rosner et al., 2008).

In school-aged children (5-17 years old), a systematic review of the relationships between physical activity and health indicators using only device-measured physical activity found that the associations between physical activity and blood pressure were more consistent in cross-sectional compared to longitudinal studies (Poitras et al., 2016). Most of the cross-sectional studies found favourable associations between TPA and blood pressure (SBP and DBP) as well as MVPA and SBP; however, associations between MVPA and DBP were mixed (Poitras et al., 2016). Two longitudinal studies found favourable associations between TPA and DBP, but not SBP, and no associations were found between MVPA or VPA and SBP, DBP, or mean arterial pressure in three longitudinal studies (Poitras et al., 2016). Another review including prospective studies found that only half of the included studies reported favourable effects between device-measured physical activity and blood pressure in school-aged children, with most of these favourable effects found in only boys (Skrede, Steene-Johannessen, Anderssen, Resaland, & Ekelund, 2019). During early childhood, there are currently a lack of both sex-based analyses and longitudinal studies examining device-measured physical activity and blood pressure.

1.5.3 Heart rate and physical activity

While many studies that assess physical activity and cardiovascular health include resting heart rate as a descriptive variable, the associations between physical activity and resting heart rate are not often examined. One study of 3-6-year-olds did not find an association between physical activity, measured with an accelerometer over 1 day, and resting heart rate (Klesges et al., 1990). In contrast, engaging in at least 1 sport activity per week was associated with a slower resting heart rate by 2 beats per minute in 3-10-year-old boys, but not girls (Sarganas et al., 2017). Investigations between self- or parent-report physical activity and resting heart rate in 8-18 (Kwok et al., 2013) and 14-17-year-olds (Christofaro, Andrade, Vanderlei, Fernandes, & Mota, 2018) yielded favourable associations when boys and girls were analyzed separately (Kwok et al., 2013) or after adjusting for sex/gender (Christofaro et al., 2018).

Much like resting heart rate, there is a paucity of literature on physical activity and HRR in children. Kühne *et al.* (2016) examined the effect of MVPA on a submaximal field-based test of HRR in 732 3-6-year-olds. Children ran around in circles for 2 minutes and HRR was calculated as the difference between the highest heart rate in the final minute of running and heart rate 1 minute following cessation of running while lying on the floor (Kühne et al., 2016). Every 10 additional minutes of daily MVPA, assessed using combined accelerometry and heart rate, was associated with a 1 bpm faster HRR, indicating that more active preschoolers have faster HRR compared to their less active peers. In this study, the effect of MVPA on HRR was not different between boys and girls. While this study had repeated measures (baseline, 6 months, 12 months), it was a randomized controlled trial with all children enrolled in preschools that were participating in either a basic or comprehensive physical activity/nutrition intervention (Kühne et al., 2016). Furthermore, the exercise intensity was self-selected, while it is recommended that HRR be assessed following a standardized, maximal exercise test (Peçanha et al., 2014). In a cross-sectional study of adolescents (n=54), positive associations were found between the time decay in heart rate in the first 30 seconds after a cycling test to exhaustion and accelerometry-measured MPA and VPA, but not LPA (Oliveira, Barker, & Williams, 2018). It was not reported if the effects were the same for boys and girls (Oliveira et al., 2018). There are currently a lack of prospective observational studies examining the effect of physical activity on HRR following maximal exercise in children, including during early childhood.

1.5.4 Cardiovascular fitness and physical activity

Results from cross-sectional (Fang et al., 2017; Leppänen et al., 2016) and longitudinal (Bürgi et al., 2011; Leppänen et al., 2017) studies consistently show that more active preschool-aged children are more fit (Carson et al., 2017). This is supported by a systematic review and metanalysis that examined the favourable effects of physical activity interventions, most of which were aimed at improving motor skills, on cardiovascular fitness in children under the age of 6 years (García-Hermoso, Alonso-Martinez, Ramírez-Vélez, & Izquierdo, 2019). The favourable associations between physical activity and fitness reported to date during early childhood echo the results of a systematic review of device-measured physical activity and fitness in school-aged children (Poitras et al., 2016). This review demonstrated associations between physical activity and cardiovascular fitness, with more consistent relationships with MVPA than TPA and lower intensities of activity (Poitras et al., 2016).

There is some evidence that the intensity of physical activity has an impact on cardiovascular fitness during early childhood. In a cross-sectional analysis of 4-6-year-olds enrolled in the control arm of the Ballabeina Study (n=217), TPA, MPA, and VPA were associated with a field-based measure of cardiovascular fitness, the 20-meter shuttle run (Bürgi et al., 2011). However, only VPA was predictive of improvements in cardiovascular fitness 9 months later (Bürgi et al., 2011). Likewise, an analysis of the control group of children enrolled in the MINISTOP trial found that MVPA and VPA in 4-year-olds were associated with better performance on the 20-m shuttle run 1 year later at age 5 (n=307) (Leppänen et al., 2017), whereas lower intensities of physical activity (LPA and MPA) were not associated with cardiovascular fitness at baseline (Leppänen et al., 2016) or 1 year later (Leppänen et al., 2017). Neither study (Bürgi et al., 2011; Leppänen et al., 2017) found the effect of physical activity on fitness was different between boys and girls. While both the Ballabeina and MINISTOP studies measured physical activity via accelerometry, they used a field-based assessment of cardiovascular fitness and involved data from the control arm of an intervention trial (Bürgi et al., 2011;

Leppänen et al., 2017). No prospective cohort studies have examined physical activity and fitness during early childhood. Indeed, even across the school-aged years, only one longitudinal study (Carson et al., 2017) that examined the associations between physical activity and fitness was identified in a systematic review (Poitras et al., 2016), identifying a gap in the currently available knowledge related to the effects of device-measured physical activity on cardiovascular fitness over time in children of all ages.

1.5.5 Physical activity guidelines

1.5.5.1 Current recommendations

Given the health benefits of engaging in physical activity in childhood, many countries and organizations have developed and promoted physical activity recommendations, which vary by age group and organization. Guidelines in the last decade have, for the most part, recommended that preschool-aged children (typically encompassing either 3- and 4-year-olds or 3-5-year-olds) engage in at least 180 minutes (3 hours) of physical activity at any intensity daily (TPA) (Ministry of Health, 2017; US Department of Health and Human Services, 2018) with some recommending that this 180 minutes include at least 60 minutes of MVPA (Okely et al., 2017; Tremblay et al., 2017; UK Chief Medical Officers, 2019; World Health Organization, 2019). While these guidelines suggest volume and intensity of physical activity in minutes per day, physical activity assessment via step count is a popular and feasible alternative method of physical activity assessment and recommendation (Tudor-Locke, Hatano, Pangrazi, & Kang, 2008). Despite this, the current physical activity guidelines make no recommendations for number of steps that preschool-aged children should engage in per day to achieve health benefits.

The Canadian, Australian, and World Health Organization physical activity guidelines for the early years (0-5 years old) are now part of 24-hour movement guidelines, which provide recommendations on physical activity, sedentary and screen time, as well as sleep (Okely et al., 2017; Tremblay et al., 2017; World Health Organization, 2019). While a further understanding of the interconnectedness of these behaviours and health outcomes in early childhood is warranted, there remain significant gaps in our understanding of the volume of physical activity required to confer health benefits in early childhood; thus, the current thesis focuses specifically on physical activity recommendations.

1.5.5.2 Compliance with physical activity guidelines

The reported percentage of preschoolers meeting the physical activity guidelines for the early years varies considerably due to differences in accelerometry processing, although most studies report that the majority of preschoolers meet the guidelines (Chaput et al., 2017; Cliff et al., 2017; Colley et al., 2013; Dias et al., 2019; Garriguet et al., 2016; Quan et al., 2019; Vale et al., 2013). In a pooled analysis of data of 1052, 3- and 4-year-olds from the United Kingdom, Switzerland, Belgium, and the USA, in the International Children's Accelerometry Database, Dias *et al.* (2019) found that 70% were engaging in at least 180 minutes of activity each day, and 79% were engaging in at least 60 minutes of MVPA. Furthermore, consistent with the previously reported sex-differences in physical activity, it was identified that more boys than girls engaged in at least 180 minutes of TPA (77% versus 63%) and 60 minutes of MVPA (85% versus 72%).

1.5.5.3 Cardiovascular health in preschoolers meeting the guidelines

Few published studies have compared cardiovascular health indicators between preschoolers meeting and not meeting physical activity guidelines. No differences in BMI were found between 3-4-year-olds who did and did not meet the guideline of 180 minutes of activity at any intensity, including at least 60 minutes of MVPA (Chaput et al., 2017), although overweight or obese 4-6-year-old girls were less likely to engage in at least 60 minutes of MVPA (Vale et al., 2013). Similarly, 3-6-year-olds not attaining 60 minutes of daily MVPA were not more likely to have elevated blood pressure compared to those who did; however, overweight children who did not get 60 minutes of daily MVPA were 3 times more likely to have elevated SBP than non-overweight, active children (Vale et al., 2015). de Moraes *et al.* (2015) found no increased risk of hypertension in children who engaged in less than 60 minutes of MVPA per day at baseline (average age 6 years; range 2-9 years) but when physical activity was reassessed 2 years later, children who engaged in less than 60 minutes of MVPA per day had an increased risk of hypertension.

Overall while there were some differences in overweight preschoolers, there is little evidence that preschoolers who meet the 180 minutes of activity at any intensity and/or 60 minutes of MVPA physical activity guidelines for the early years have better cardiovascular health than those who do not meet the guidelines. This finding may reflect the target activity recommendation in the guidelines themselves. More specifically, the recommendation of 180 minutes at any intensity is set at the average physical activity level of a preschool-aged child rather than an empirically-derived dose of physical activity associated with health benefits (Carson et al., 2017; Institute of Medicine, 2011; Pate et al., 2019). Indeed, one of the conclusions the systematic review undertaken as part of the development of the Canadian 24-Hour Movement Guidelines for the Early Years

(0–4 years) was that while there is an exposure/outcome gradient for physical activity and health (i.e. favourable associations between physical activity and health showing more is better), it was not possible to determine the specific amount of activity required for health benefits in preschoolers due to a lack of available data (Carson et al., 2017). A similar conclusion was reached in the systematic review conducted as part of the Physical Activity Guidelines for Americans, with the additional caveat provided that there is insufficient evidence to determine the effects of physical activity on cardiometabolic health (e.g. blood lipids, respiratory function, and blood pressure) in children under the age of 6 (Pate et al., 2019). This review also highlighted that there is a pressing need to identify cardiometabolic health markers that are sensitive enough to detect change in young children in order to evaluate the relationship between physical activity and cardiometabolic health in this age group (Pate et al., 2019).

1.6 Study Objectives and Hypotheses

Physical activity positively impacts cardiovascular health and lowers cardiovascular disease risk in school-aged children; however, there is little evidence that physical activity impacts the progression of cardiovascular disease starting in early childhood nor is there evidence to support how much physical activity young children should engage in to promote cardiovascular health. Therefore, the overarching purpose of this thesis is to explore the relationships between physical activity and indicators of cardiovascular health during early childhood.

In Chapter 2 (Study 1), we examined the impact of physical activity on trajectories of cardiovascular health indicators during early childhood. Specifically, using a longitudinal observational cohort study design, we aimed to determine the effects of device-measured TPA and MVPA on arterial stiffness, blood pressure, HRR, and cardiovascular fitness, over a 3-year period during early childhood. We hypothesized that young children who engage in greater amounts of TPA and MVPA would have more favorable trajectories of cardiovascular health indicators (arterial stiffness, blood pressure, HRR, and cardiovascular fitness).

In Chapter 3 (Study 2), we conducted a study to determine the amount of physical activity preschoolers should engage in to avoid unfavourable cardiovascular health. Specifically, we aimed to determine if there are thresholds of physical activity, measured as TPA, MVPA, and steps per day, which discriminate between favourable and unfavourable cardiovascular health indicators in preschool-aged children. We hypothesized the physical activity variables (TPA, MVPA, and steps/day) would discriminate between favourable and unfavourable cardiovascular health (arterial stiffness, blood pressure, resting heart rate, HRR, and BMI).

In Chapter 4 (Study 3), we assessed the associations between a novel technique for examining arterial wall properties, CALM, and established indicators of arterial stiffness in early childhood, in order to determine if CALM measurement could be a feasible alternative to the current arterial stiffness measurement techniques in young children. We aimed to determine the associations between established arterial stiffness indicators (PWV and carotid artery β-stiffness index) and CALM outcomes, specifically CALM displacements during systole and diastole and velocity and acceleration during diastole, in young children. We hypothesized that CALM outcomes would be inversely associated with arterial stiffness (PWV and β-stiffness index) in young children.

1.7 Methodological note

Data from Chapters 2, 3, and 4 were collected as part of the Health Outcomes and Physical activity in Preschoolers (HOPP) study. The HOPP study is a prospective cohort study that enrolled 418 3-, 4-, and 5-year-old children from south-central Ontario between 2010-2012. Each year, participants completed a series of assessments over 2 visits, between which the child wore an accelerometer for 7 days. These visits were repeated annually for a total of 3 years (year 1, year 2, year 3). A flow chart showing participant progression through the study can be found in Chapter 2, Figure 1. Chapter 2 contains data from all participants across all years of the study. Chapter 3 contains data from 236 unique participants who were 4 years old (4.0-4.9) at either year 1 or year 2 of the study. Chapter 4 includes data from a subset of participants at year 3 (n=191, 5.0-8.0 years old) as the higher frame rate ultrasound images required for the analysis of CALM was an addition to the original protocol.

CHAPTER 2: PHYSICAL ACTIVITY AND TRAJECTORIES OF CARDIOVASCULAR HEALTH INDICATORS DURING EARLY CHILDHOOD

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Physical Activity and Trajectories of Cardiovascular Health Indicators During Early Childhood

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abstract

OBJECTIVES: Cardiovascular disease prevention should begin in childhood. However, the influence of physical activity on cardiovascular health in early childhood is unknown. Our purpose in this study was to determine the effect of physical activity on trajectories of cardiovascular health indicators during early childhood.

METHODS: This prospective, observational cohort study (Health Outcomes and Physical Activity in Preschoolers) enrolled 418 3- to 5-year-olds with annual assessments for 3 years. Total physical activity (TPA) and moderate-to-vigorous physical activity (MVPA) were measured over 7 days via accelerometry. Cardiovascular health indicators included cardiovascular fitness (exercise time on a maximal treadmill test [treadmill time] and 1-minute heart rate recovery), resting arterial stiffness (whole-body pulse wave velocity and carotid β stiffness index), and seated systolic blood pressure. Data were analyzed by using linear mixed-effects modeling; effects are reported as unstandardized estimates (Est).

RESULTS: There were main effects of TPA and MVPA on treadmill time (Est = 0.004 [$P = .005$] and 0.008 [$P = .001$], respectively) and heart rate recovery (Est = 0.05 [$P < .001$] and 0.08 [$P < .001$], respectively). There was a main effect of TPA on pulse wave velocity (Est = −0.001; $P = .02$) and an MVPA × time interaction (Est = −0.002; $P = .01$). For carotid β stiffness index, the effect of a TPA × time interaction was not significant (Est = −0.002; $P = .051$); however, there was a significant MVPA × time interaction (Est = −0.003; $P = .03$). MVPA was associated with a slower rate of change in systolic blood pressure for girls (Est = 0.06; $P = .009$).

CONCLUSIONS: Children who engage in higher levels of physical activity during early childhood have better cardiovascular health indicators, with more intense physical activity (ie, MVPA) attenuating the stiffening of arteries.

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Ms Proudfoot coordinated the study, collected, analyzed, and interpreted the data, drafted the initial manuscript, and reviewed and revised the manuscript; Dr King-Dowling collected, analyzed, and interpreted the data and critically reviewed and revised the manuscript for important intellectual content; Drs Bray and Cairney conceptualized and designed the study and critically reviewed and revised the manuscript for important intellectual content; Drs MacDonald and Timmons conceptualized and designed the study, supervised the study, interpreted the data, and critically reviewed and revised the manuscript for important intellectual content; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT: Cardiovascular disease begins to develop in early childhood. Although physical activity has been shown to have beneficial effects on cardiovascular health for older children and adults, the effect of physical activity on cardiovascular health in young children is unknown.

WHAT THIS STUDY ADDS: We show that engagement in physical activity during early childhood has favorable effects on trajectories of cardiovascular fitness, arterial stiffness, and blood pressure over a 3-year span.

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It is now well accepted that the initiating events of the cardiovascular disease process begin in early childhood.[1] Autopsy studies initially identified fatty streaks in the arteries of children as young as 1 year.[2] More recently, clusters of cardiovascular risk factors have been identified in 1- to 5-year-olds.[3] Together, these findings highlight the importance of strategies aimed at early prevention of cardiovascular diseases; however, little longitudinal data exist to guide the development and assessment of these strategies.

There is an abundance of evidence among adults that engagement in physical activity is an effective way to prevent and slow the progression of cardiovascular diseases.[4,5] Moreover, school-aged children who are more active have better indicators of cardiovascular health, such as lower resting blood pressures,[6,7] higher cardiovascular fitness,[8,9] and more favorable indices of arterial stiffness.[10,11] These positive associations observed in both adults and children led the American Heart Association to include physical activity as part of its strategy of primordial prevention of cardiovascular disease.[1] This recommendation acknowledges that engagement in physical activity can slow the decline in cardiovascular health that begins at birth. However, there is little evidence to support this recommendation during early childhood (herein defined as 8 years and younger[12]).

Few studies have examined the relationships between physical activity and cardiovascular health indicators during early childhood. Furthermore, a recent systematic review rated the quality of evidence examining the impact of physical activity on cardiovascular health indicators in early childhood as very low[13] because of a lack of control for confounding variables, such as age and height, and the use of primarily cross-sectional designs. No study, to date, has longitudinally evaluated the impact of physical activity on the trajectories of cardiovascular health indicators during early childhood.

The Health Outcomes and Physical Activity in Preschoolers (HOPP) study[14] was a prospective, observational cohort study that measured physical activity and a variety of health indicators, including cardiovascular health, in annual visits across early childhood, thereby addressing a number of the aforementioned shortcomings. Using data collected as part of the HOPP study, the purpose of this study was to determine the influence of physical activity on trajectories of cardiovascular health indicators during early childhood. Specifically, we aimed to determine the effect of objectively measured total physical activity (TPA) and moderate-to-vigorous physical activity (MVPA) on cardiovascular fitness, arterial stiffness, and blood pressure over a 3-year period during early childhood. We chose to evaluate TPA and MVPA because these are the focus of physical activity guidelines for the early years[15–17] and are relevant for public health promotion. We hypothesized that young children who engage in greater amounts of TPA and MVPA would have more favorable trajectories of cardiovascular health indicators.

METHODS

Study Design

Details of the HOPP study design and methodology have been published.[14] Briefly, each year for 3 years, children completed a series of assessments distributed over 2 visits separated by 19 ± 14 days. At the first visit, anthropometry and fitness assessments were conducted. At the end of the visit, the child was given a physical activity monitor to wear for 1 week. After the activity monitoring period, the child attended a second visit, at which resting arterial stiffness and blood pressure were assessed. Data were collected from August 2010 to September 2014. Written informed consent from the child's legally authorized representative (eg, parent) was obtained at the initial visit. The Hamilton Integrated Research Ethics Board approved the study.

Participants

The HOPP study was conducted on a community-based sample through enrollment of children when they were 3, 4, or 5 years old. Recruitment took place from August 2010 to August 2012 in south-central Ontario, Canada, through early-years centers, preschools, day cares, local schoolboards, community events, and word of mouth. Children with diagnosed medical conditions or known developmental or cognitive delays were excluded. There were 418 children who completed the initial visit; 42 participants withdrew or were lost to follow-up by the final visit (5% attrition per year; Fig 1).

Physical Activity

Free-living physical activity was assessed by using accelerometry. Children were instructed to wear an accelerometer (ActiGraph GT3X line) over their right hip for 7 days during all waking hours except prolonged water activities. Data were downloaded in 3-second epochs and analyzed with ActiLife software (version 6.6.3; ActiGraph). Nonwear time, identified as periods of at least 60 minutes of continuous 0 counts or as indicated by the parent in a logbook, was removed. Data were analyzed in the vertical plane for average daily TPA and MVPA by using the Pate cut-points.[18,19] The 15-second epoch cut-points were divided by 5 to set thresholds at ≥8 counts per 3 seconds for TPA and

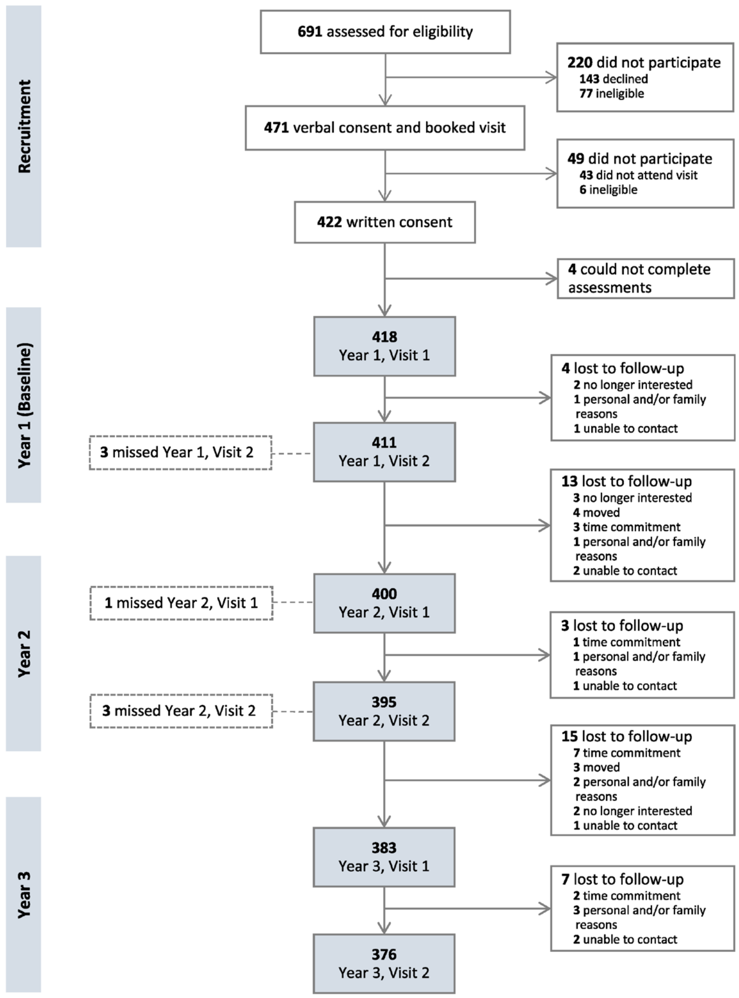

FIGURE 1
Flow diagram showing participants progressing through the study from recruitment to the final visit.

≥84 counts per 3 seconds for MVPA. At least 3 days of valid wear (defined as at least 10 hours of wear) were required for inclusion in subsequent statistical analyses.[20]

Cardiovascular Health Indicators

To provide a comprehensive perspective of cardiovascular health, we assessed 3 related indicators: cardiovascular fitness (exercise time on a treadmill test and heart rate recovery [HRR]), arterial stiffness (pulse wave velocity [PWV] and carotid artery β stiffness index), and seated brachial artery systolic blood pressure (SBP).

Cardiovascular Fitness

Exercise time on a graded maximal treadmill test (hereafter, treadmill time), the Bruce Protocol,[21] and HRR 1 minute after test termination were used as indicators of cardiovascular fitness.[22] To accommodate the children's abilities and small size, participants held handrails, and a researcher was positioned behind the child for safety.[23] A heart rate (HR) monitor (Polar Electro) was worn on the chest throughout. The treadmill (General Electric Marquette Series 2000) increased in speed and incline every 3 minutes until the child could no longer maintain running cadence to position themselves at the front of the treadmill or refused to continue. The time of cessation was recorded as the treadmill time. The child was seated immediately after, and HRR was calculated as the difference in peak HR achieved during the test and HR at 1 minute of recovery. Fitness data were excluded from analyses if the participant did not reach an HR of at least 180 beats per minute.[24] HRR data were also excluded if the child did not sit calmly during recovery (eg, coughing, being upset, or moving). A longer treadmill time and faster HRR indicate more favorable cardiovascular fitness.

Arterial Stiffness

Children lay supine, and a movie was displayed overhead to encourage them to remain still and quiet during the assessment. After at least 10 minutes of rest, arterial stiffness was measured regionally in a large segment of the arterial tree (whole-body PWV) and locally in the common carotid artery (β stiffness index). PWV was determined as the speed of the pulse wave between the carotid and dorsalis pedis arteries. A handheld pressure transducer (tonometer model SPT-301; Millar Instruments) was placed on the neck over the right carotid artery, and a photoplethysmograph probe (MLT1020PPG; ADInstruments) was placed on the right foot over the dorsalis pedis artery. The time delay between the arrival of the pulse wave at these 2 sites was determined by applying a 5- to 30-Hz bandpass filter,[25] as previously described[26] (LabChart 7 Pro version 7.3.4; ADInstruments). The time delay for 20 consecutive beats was averaged and divided into the measured distance between the 2 sites ([suprasternal notch to sensor on foot] – [suprasternal notch to location of tonometer on carotid]).[27,28] A faster PWV is indicative of greater arterial stiffness.

Local stiffness in the common carotid artery was calculated by using β stiffness index[27]:

$$\beta - Stiffness\ \ Index = \frac{\ln(P_s/P_d)}{(AD_{max} - AD_{min})/AD_{min}}$$

where P_s and P_d are carotid artery systolic and diastolic blood pressure, respectively, and AD_{max} and AD_{min} are the maximum and minimum artery diameters over a heart cycle. We chose to use the β stiffness index because this takes into account the child's artery size and blood pressure, which increase with age,[29,30] and it is more reliable than compliance and distensibility in young children.[26] Carotid artery systolic and diastolic blood pressure were determined by calibrating 10 heart cycles of carotid artery pressure waveforms, obtained from the tonometer, to discrete measures of supine brachial artery blood pressure, as previously described.[26] Simultaneous to tonometry, a 12-MHz linear ultrasound probe (Vivid q; General Electric Medical Systems) was placed over the left common carotid artery with an orientation to view the long axis. Video clips of brightness mode ultrasound were captured for 10 consecutive heart cycles (23 frames per second). Frames corresponding to the maximum and minimum artery diameters over a heart cycle were extracted (Sante DICOM Editor, Santesoft version 3.3.1), and arterial wall boundaries were analyzed by using semiautomated edge tracking software (Artery Measurement System version 2.0).

Seated Brachial Blood Pressure

Automated measures of seated blood pressure (Dinamap Pro 100; Critikon Inc) were obtained in the right arm in triplicate with a 1-minute delay between each measure. The second and third measures were averaged[31]; additional measures were taken if SBP differed by more than 5 mm Hg. Seated SBP was used as a cardiovascular health indicator.[32]

TABLE 1 Baseline Characteristics

	All	Girls	Boys
N	418	208	210
Age, y, mean (SD)	4.5 (0.9)	4.5 (0.9)	4.5 (0.9)
White race and/or ethnicity, %	87	87	87
Greater than or equal to median income, %	78	79	77
Height,[a] cm, mean (SD)	106.5 (7.7)	105.9 (7.9)	107.2 (7.5)
z score[b]	0.42 (0.95)	0.38 (0.89)	0.47 (1.0)
Wt,[a] kg, mean (SD)	17.9 (3.2)	17.6 (3.1)	18.2 (3.2)
z score[b]	0.26 (0.93)	0.21 (0.84)	0.31 (1.0)
BMI, mean (SD)	15.7 (1.3)	15.6 (1.3)	15.7 (1.3)
z score,[b] mean (SD)	0.07 (0.98)	0.11 (0.94)	0.02 (1.0)
Normal wt, No. (%)[b]	334 (80)	167 (80)	167 (80)
Overweight, No. (%)[b]	45 (11)	30 (14)	15 (7)
Obese, No. (%)[b]	20 (5)	4 (2)	16 (8)
Underweight, No. (%)[b]	19 (6)	7 (4)	12 (6)

[a] Height and wt were collected by using standard procedures.[14]
[b] *z* scores and wt classification were determined from US Centers for Disease Control and Prevention data.[33]

Statistical Analyses

Linear mixed-effects modeling with the restricted maximum likelihood method was used to determine the effect of physical activity on trajectories of cardiovascular health indicators (SAS University Edition release 3.6; SAS Institute, Inc, Cary, NC). This method accommodates missing data by creating estimates using all data available for each participant. The same approach was taken for modeling each cardiovascular health indicator (treadmill time, HRR, PWV, β stiffness index, and SBP). TPA and MVPA were always in separate models. We determined the effect of time (ie, study years 1, 2, and 3) and physical activity (TPA or MVPA) on each cardiovascular health indicator (model 1) and subsequently assessed for a TPA × time or MVPA × time interaction (model 2). All models were adjusted for the child's sex and/or gender (parent reported), age at enrollment, and height *z* score[33] as a surrogate for growth at each visit. To determine if the impact of physical activity on cardiovascular health differed by sex and/or gender, TPA × sex and/or gender and MVPA × sex and/or gender interactions and 3-way interactions with time (TPA × time × sex and/or gender and MVPA × time × sex and/or gender) were added. To test if the effects of physical activity were confounded by body size, we further adjusted models by BMI. A random intercept at the participant level was included in all models.

RESULTS

Participant characteristics at baseline are shown in Table 1. Physical activity (exposure) and indicators of cardiovascular health (outcome) variables are presented in Tables 2 and 3, respectively. Children lost to follow-up (25–42 depending on outcome measure) had shorter treadmill time and lower β stiffness index at baseline but were otherwise not different compared with those retained in the cohort (Supplemental Table 5). Results are summarized below with linear mixed-effects models 1 and 2, presented in Table 4. Estimates are unstandardized, and all effects were independent of the child's age at enrollment and height *z* score. Estimates (Est) were similar in all models after adjustment for BMI (results not shown).

TABLE 2 Physical Activity Variables Across the 3 Years for Participants Meeting Wear Time Criteria

Variable	Year 1	Year 2	Year 3
All			
n valid[a]	365	368	358
Valid d, mean (SD)	5.6 (1.3)	5.9 (1.3)	6.0 (1.1)
Wear time, min per d, mean (SD)	722.8 (40.9)	721.2 (42.3)	739.2 (48.2)
TPA, min per d, mean (SD)	256.5 (37.8)	255.9 (37.2)	256.5 (40.7)
MVPA, min per d, mean (SD)	96.4 (21.7)	99.5 (21.7)	102.3 (24.4)
Girls			
n valid[a]	175	182	177
Valid d, mean (SD)	5.6 (1.3)	5.7 (1.3)	5.9 (1.2)
Wear time, min per d, mean (SD)	719.7 (40.1)	717.0 (44.2)	736.2 (46.0)
TPA, min per d, mean (SD)	242.3 (32.5)	243.8 (36.6)	245.0 (35.6)
MVPA, min per d, mean (SD)	88.2 (18.2)	91.9 (20.7)	94.3 (19.8)
Boys			
n valid[a]	190	186	181
Valid d, mean (SD)	5.6 (1.3)	6.0 (1.3)	6.2 (1.0)
Wear time, min per d, mean (SD)	725.6 (41.5)	725.3 (39.9)	742.0 (50.3)
TPA, min per d, mean (SD)	269.5 (37.8)	267.7 (34.1)	267.8 (42.3)
MVPA, min per d, mean (SD)	104.0 (21.9)	107.0 (20.2)	110.2 (25.9)

[a] Number of participants who met accelerometer wear time criteria of ≥10 h on ≥3 d.

Treadmill time increased over the study. TPA and MVPA had positive main effects on treadmill time (Table 4), showing higher levels of physical activity are associated with greater endurance. A significant TPA × time × sex and/or gender interaction (Est = −0.005; P = .049) revealed that TPA had a positive effect on the rate of change of treadmill time for girls but not boys. HRR did not change over the study period; there were positive main effects of TPA and MVPA on HRR, and this association was not moderated by sex and/or gender.

PWV and β stiffness index increased over the 3 years, indicating arteries became stiffer over time. There was an inverse (favorable) main effect of TPA on PWV, and this relationship was consistent over time (Fig 2A). The significant MVPA × time interaction indicates that the rate of change of PWV differs on the basis of levels of MVPA, with higher amounts of MVPA being associated with a slower increase in PWV (Fig 2B). Similarly, there was a significant MVPA × time interaction on the β stiffness index. The TPA × time interaction on the β stiffness index was not significant (P = .051). There were no moderating effects of sex and/or gender on either stiffness outcome.

SBP rose over the study period. Although neither TPA nor MVPA were associated with SBP on average or over time (Table 4), a significant MVPA × time × sex and/or gender interaction (Est = 0.06; P = .009) revealed that over time, MVPA had a favorable effect on the rate of change of SBP for girls. The TPA × time × sex and/or gender interaction was not significant (Est = 0.02; P = .06).

DISCUSSION

Our findings demonstrate that in early childhood, trajectories of cardiovascular fitness and arterial stiffness are favorably impacted by higher levels of physical activity independent of age, height, and BMI. The positive effects of physical activity on cardiovascular health were found for boys and girls, suggesting that both benefit from engagement in physical activity and particularly MVPA; however, MVPA appears to be associated with SBP in girls only.

Cardiovascular Fitness

Cardiovascular fitness is an important indicator of cardiovascular risk, and it has been suggested that it be included as a vital sign when assessing patient health and risk in adults.[34] We found that higher objectively measured physical activity levels during early childhood have a positive effect on cardiovascular fitness, as measured by a laboratory-based assessment of exercise time on a graded maximal treadmill test and HRR independent of age and growth. Our findings are consistent with the Ballabeina[35] and Mobile-Based Intervention Intended to Stop Obesity in Preschoolers[36] studies that reported positive associations between baseline MVPA and field-based measures of fitness 9 to 12 months later in 4- to 6-year-olds. However, these studies involved data from the control arm of an intervention trial and did not control for physical activity at follow-up. By including repeated assessments of physical activity and fitness, we determined that the positive effect of MVPA on fitness is consistent over a 3-year span in early childhood for boys and girls, but TPA appears to improve endurance in girls only. This may reflect boys' average higher physical activity and fitness levels (~25 minutes more TPA and lasting 30 seconds longer on the treadmill than girls), thereby requiring more intense physical activity to improve fitness.

Our study advances our understanding of the role of physical activity on cardiovascular health with novel determinations of the relationship between physical activity and HRR during early childhood. Although we included HRR as a proxy of fitness, it is more commonly used as an indicator of autonomic function because the drop in HR after exercise is primarily due to parasympathetic reactivation.[37] Therefore, our results suggest that young children who engaged in greater amounts of physical activity also have better autonomic function, as previously identified in studies of older children[38] and adults.[39,40]

TABLE 3 Cardiovascular Health Indicators Across 3 Annual Assessments

Variable	Year 1		Year 2		Year 3	
	Mean (SD)	*n* Valid[a]	Mean (SD)	*n* Valid[a]	Mean (SD)	*n* Valid[a]
All						
Cardiovascular fitness						
Peak HR, beats per min	196 (7)	386	200 (7)	391	200 (7)	378
Treadmill time, min[b,c]	9.3 (2.2)	386	11.7 (2.3)	392	13.2 (2.1)	380
HRR, beats per min[b]	65 (14)	382	65 (13)	384	65 (13)	373
Arterial stiffness						
PWV, m/s	4.7 (0.5)	385	5.0 (0.5)	389	5.0 (0.4)	375
β stiffness index, AU[b,d,e]	3.15 (0.98)	335	3.44 (0.90)	375	3.65 (0.90)	373
Blood pressure, mm Hg						
SBP[b,e]	94 (7)	340	99 (7)	377	100 (7)	374
DBP[e]	58 (6)	340	59 (6)	377	60 (6)	374
Girls						
Cardiovascular fitness						
Peak HR, beats per min	197 (7)	194	200 (6)	192	201 (6)	189
Treadmill time, min	9.0 (2.1)	194	11.4 (2.1)	193	12.9 (1.9)	190
HRR, beats per min	63 (15)	192	61 (14)	189	62 (14)	188
Arterial stiffness						
PWV, m/s	4.7 (0.5)	189	5.0 (0.5)	194	5.1 (0.4)	186
β stiffness index, AU	3.06 (0.89)	165	3.25 (0.86)	183	3.52 (0.88)	186
Blood pressure, mm Hg						
SBP	94 (7)	167	98 (7)	185	100 (7)	186
DBP	58 (6)	167	59 (6)	185	60 (6)	186
Boys						
Cardiovascular fitness						
Peak HR, beats per min	196 (7)	192	199 (7)	199	198 (7)	189
Treadmill time, min	9.6 (2.4)	192	12.0 (2.5)	199	13.6 (2.3)	190
HRR, beats per min	67 (14)	190	68 (12)	195	69 (12)	185
Arterial stiffness						
PWV, m/s	4.7 (0.5)	196	5.0 (0.5)	192	5.0 (0.4)	189
β stiffness index	3.23 (1.05)	170	3.63 (0.89)	192	3.78 (0.90)	187
Blood pressure, mm Hg						
SBP	94 (7)	173	100 (7)	192	101(7)	188
DBP	58 (6)	173	60 (6)	192	59 (6)	188

AU, arbitrary unit; DBP, diastolic blood pressure.
[a] Number of valid assessments.
[b] Variable is different between boys and girls, as determined by the significant effect of sex and/or gender in mixed-effects models (Table 4).
[c] In Year, 1 all participants started at the first stage of the Bruce Protocol. In the second and third years, children started at different stages on the basis of age: 4-year-olds started at stage I, 5-year-olds started at stage II, and 6- and 7-year-olds started at stage III. To account for starting at stage II or III, 3 or 6 min, respectively, were added to the treadmill time.
[d] For the first 44 assessments, an older ultrasound device was used (System FiVe, General Electric Medical Systems). There were no differences in β stiffness index between the 2 devices.
[e] Because of a faulty hose connection, blood pressure data were removed for 44 participants in Year 1 and 10 participants in Year 2. These were also removed for the β stiffness index.

Arterial Stiffness

Both PWV and carotid artery stiffness are strong predictors of cardiovascular events, even after accounting for traditional risk factors.[41,42] In a cross-sectional study involving 5- and 8-year-olds, Idris et al[43] found no relationship between parent-reported physical activity and sport participation and carotid artery stiffness. In contrast, significant inverse relationships between objectively measured physical activity and arterial stiffness were reported in cross-sectional examinations of 6- to 8-year-olds[10] and 10-year-olds.[11] Using a longitudinal design and objective physical activity assessment, we found that age-related increases in arterial stiffness were attenuated in children who engaged in greater amounts of MVPA but not TPA (Fig 2). This suggests that more intense physical activity is required to slow the progressive stiffening of arteries and results in better vascular health trajectories. Indeed, a recent systematic review concluded that positive associations between physical activity and most indices of cardiovascular health in school-aged children are more robust at higher intensities of activity.[44] Future work should further delineate the role of intensity and determine if certain bout lengths of MVPA are optimal for promoting cardiovascular health in young children.

Although not a specific objective of our article, we note that PWV and β stiffness index increased with age, in contrast to Hidvégi et al,[45] who did not find differences across 3- to 8-year-olds in PWV measured by a pulse wave analysis technique. Our results align with the literature in school-aged children and adults, which consistently show central arteries stiffen with age.[46–48]

TABLE 4 Linear Mixed-Effects Model Results for the Effect of Physical Activity on Cardiovascular Health Indicators

	TPA		MVPA	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
Treadmill time				
Age at baseline	1.61 (0.08)**	1.61 (0.08)**	1.59 (0.08)**	1.59 (0.08)**
Sex and/or gender	0.39 (0.15)*	0.39 (0.15)*	0.37 (0.15)*	0.37 (0.15)*
Height	0.25 (0.07)**	0.25 (0.07)**	0.24 (0.07)**	0.24 (0.07)**
Time	1.98 (0.04)**	1.95 (0.31)**	1.96 (0.05)**	1.91 (0.21)**
PA	0.004 (0.001)*	0.004 (0.003)	0.008 (0.002)*	0.007 (0.005)
PA × time	—	0.0001 (0.001)	—	0.0004 (0.002)
HRR				
Age at baseline	−0.87 (0.61)	−0.87 (0.61)	−1.13 (0.61)	−1.15 (0.61)
Sex and/or gender	4.53 (1.13)**	4.53 (1.13)**	4.55 (1.14)**	4.52 (1.14)**
Height	−0.97 (0.55)	−0.98 (0.55)	−1.07 (0.56)	−1.05 (0.56)
Time	−0.15 (0.38)	−0.36 (2.65)	−0.38 (0.38)	1.15 (1.76)
PA	0.05 (0.01)**	0.05 (0.02)*	0.08 (0.02)**	0.11 (0.04)*
PA × time	—	0.0009 (0.01)	—	−0.02 (0.02)
PWV				
Age at baseline	0.09 (0.02)**	0.09 (0.02)**	0.10 (0.02)**	0.09 (0.02)**
Sex and/or gender	−0.008 (0.04)	−0.008 (0.04)	−0.02 (0.04)	−0.02 (0.04)
Height	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)
Time	0.16 (0.01)**	0.19 (0.10)	0.17 (0.02)**	0.34 (0.07)**
PA	−0.001 (0.0004)*	−0.0007 (0.0009)	−0.0007 (0.0007)	0.003 (0.0002)
PA × time	—	−0.0001 (0.0004)	—	−0.002 (0.0007)*
β stiffness index				
Age at baseline	0.13 (0.04)*	0.13 (0.04)*	0.13 (0.04)*	0.12 (0.04)*
Sex and/or gender	0.23 (0.07)*	0.23 (0.08)*	0.24 (0.08)*	0.24 (0.08)*
Height	0.10 (0.04)*	0.10 (0.04)*	0.10 (0.04)*	0.10 (0.04)*
Time	0.25 (0.03)**	0.64 (0.20)*	0.25 (0.03)**	0.53 (0.13)**
PA	0.0007 (0.0008)	0.004 (0.002)*	0.0007 (0.001)	0.007 (0.003)*
PA × time	—	−0.002 (0.0008)	—	−0.003 (0.001)*
SBP				
Age at baseline	1.32 (0.31)**	1.32 (0.31)**	1.31 (0.31)**	1.30 (0.31)**
Sex and/or gender	1.03 (0.58)	1.05 (0.58)	1.21 (0.59)*	1.21 (0.59)*
Height	1.27 (0.29)**	1.26 (0.29)**	1.27 (0.29)**	1.27 (0.29)**
Time	3.16 (0.22)**	2.48 (1.53)	3.17 (0.22)**	3.30 (1.01)**
PA	0.005 (0.006)	−0.0005 (0.01)	−0.003 (0.01)	-7.6×10^{-6} (0.02)
PA × time	—	0.003 (0.006)	—	−0.001 (0.01)

Effects are reported as unstandardized estimates (SEs). Age at baseline (fixed variable); child's age (in years) at the first visit; sex and/or gender (fixed variable), girls coded as 0 and boys coded as 1; height, entered as a *z* score at each year; time, study year 1, 2, or 3; and MVPA and TPA, entered in min per day were measured. PA, physical activity; —, not applicable.
[a] Model 1 shows the estimates for main effects of physical activity.
[b] Model 2 includes estimates for interactions between physical activity and time.
* $P < .05$; ** $P < .001$.

Brachial Blood Pressure

Our findings show that SBP increases more slowly for girls engaging in greater amounts of MVPA but not boys. The association between MVPA and SBP seems to emerge over time, which is consistent with cross-sectional studies, which more often find relationships between physical activity and SBP in school-aged[6] but not preschool-aged[13,49–51] children. It is unclear why we found this relationship only in girls; however, given that physical activity was associated with arterial stiffness in both boys and girls, blood pressure, which has low day-to-day reliability in young children,[26] may not have been a sensitive enough indicator to detect changes in vascular health in this cohort of boys.

Strengths and Limitations

This study is strengthened by repeated measures over time, a high retention of participants over the study (90%), objective measurement of physical activity, a laboratory-based maximal fitness test, and sensitive indicators of arterial health. Notwithstanding these strengths, we acknowledge the following limitations: Only 22% of our families were below the median income for the geographic area, and 13% of participants were non-white, potentially limiting the generalizability of our sample. Accelerometry data lack information during water-based activities, such as swimming, and are sensitive to epoch and cut-point selection; however, it remains the best available tool for assessment of free-living physical activity in children. We were unable to determine peak oxygen consumption, the gold

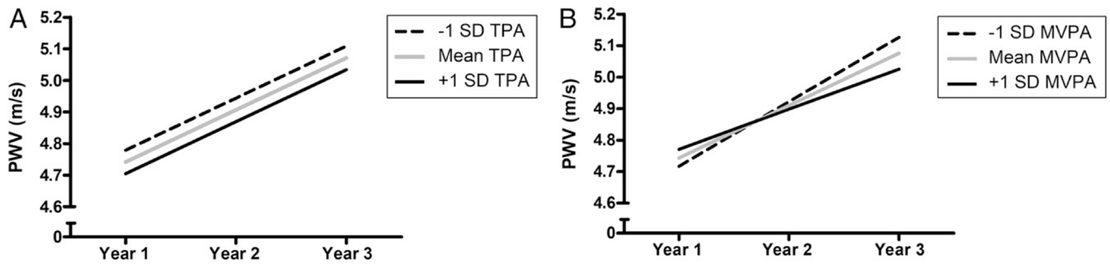

FIGURE 2
A, Model-predicted PWV over the 3 years of the study for different amounts of TPA. B, Model-predicted PWV over the 3 years of the study for different amounts of MVPA. Gray lines represent the model-predicted trajectory of PWV for a child engaging in average (mean) amounts of TPA and MVPA; dashed lines represent low (mean −1 SD) and solid-black lines represent high (mean +1 SD) levels of TPA and MVPA. The rate of change in PWV is consistent across amounts of TPA, whereas the rate of change in PWV is greater for children engaging in low amounts of MVPA. Models were adjusted for the child's sex and/or gender, age at enrollment, and height *z* score.

standard measure of fitness, during the maximal exercise test because collecting breath samples in young children was not feasible.[52] Nevertheless, exercise time on the Bruce Protocol is a valid indicator of fitness in children,[53] and HRR was used to complement our results. Finally, our measure of PWV incorporated central and peripheral arteries, whereas the gold standard measure includes only central arteries (carotid to femoral). As a result, we may have underestimated the impact of physical activity on PWV because studies in adults report a relationship between physical activity and central, but not peripheral, PWV.[54,55]

CONCLUSIONS

Engagement in physical activity results in greater cardiovascular fitness, better autonomic function, and lower arterial stiffness during early childhood. More intense physical activity (ie, MVPA) provides additional benefits because it is associated with slowing the progressive stiffening of arteries, which is a marker of atherosclerosis. MVPA also slows the increase in SBP in girls. This study adds to the evidence that physical activity is beneficial to cardiovascular health[4,5,44] and fills an important gap in the literature demonstrating that the protective effects of physical activity on cardiovascular health begin early in childhood. Future research should evaluate if the effects of physical activity on cardiovascular health indicators during early childhood carry over into later childhood and adulthood.

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ABBREVIATIONS

HOPP: Health Outcomes and Physical Activity in Preschoolers
HR: heart rate
HRR: heart rate recovery
MVPA: moderate-to-vigorous physical activity
PWV: pulse wave velocity
SBP: systolic blood pressure
TPA: total physical activity

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Supplemental Information

SUPPLEMENTAL TABLE 5 Comparison of Variables at Baseline Between Children Who Completed the Study and Those Lost to Follow-up

	Completed Study		Lost to Follow-up		P[a]
	Mean (SD)	n Valid[b]	Mean (SD)	n Valid[b]	
Age, y	4.5 (0.9)	376	4.4 (0.9)	42	.44
Sex, n	187 (female); 189 (male)	376	21 (female); 21 (male)	42	.97
Height, cm	106.7 (7.8)	376	104.7 (7.0)	42	.10
Wt, kg	18.0 (3.2)	376	17.5 (3.0)	42	.33
BMI	15.7 (1.3)	376	15.9 (1.4)	42	.34
TPA, min per d	256.0 (37.8)	335	261.8 (38.6)	30	.42
MVPA, min per d	96.1 (21.4)	335	100.2 (25.4)	30	.31
Treadmill time, min	9.4 (2.3)	351	8.5 (2.2)	35	.03
HRR, beats per min	65 (14)	348	64 (16)	34	.75
PWV, m/s	4.69 (0.50)	354	4.75 (0.47)	31	.55
Stiffness index, AU	3.18 (0.99)	310	2.76 (0.76)	25	.04
SBP, mm Hg	94 (7)	313	94 (8)	27	.73

AU, arbitrary unit; DBP, diastolic blood pressure.

[a] Comparisons were made by using independent t tests except for sex, for which a χ^2 test was used.

[b] Number of valid assessments.

CHAPTER 3: PHYSICAL ACTIVITY THRESHOLDS THAT DISCRIMINATE BETWEEN FAVOURABLE AND UNFAVOURABLE CARDIOVASCULAR HEALTH INDICATORS IN PRESCHOOL-AGED CHILDREN

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Abstract

Introduction: Physical activity is favourably associated with cardiovascular health during the preschool years; however, the amount of activity required to promote cardiovascular health is not clear. The purpose of this study was to determine the threshold of physical activity which discriminates between favourable and unfavourable cardiovascular health indicators in preschool-aged children.

Methods: This observational study includes cross-sectional data from 236 4-year-olds (114 girls) collected as part of the Health Outcomes and Physical activity in Preschoolers study from 2010-2013. Receiver operating characteristic area under the curve (AUC) analysis determined the ability of physical activity (total physical activity [TPA], moderate-to-vigorous physical activity [MVPA], and steps/day; measured via accelerometry) to discriminate between favourable and unfavourable cardiovascular health (BMI, blood pressure, supine resting heart rate, arterial stiffness, and heart rate recovery). Sex-specific and combined analyses were performed in 2019.

Results: For the combined sample, 241 minutes/day of TPA and 82 minutes/day of MVPA classified unfavourable heart rate recovery; 8987 and 8640 steps/day classified resting heart rate and heart rate recovery, respectively (AUC: 0.623-0.672, $p<0.05$). Similar results were found separately in the girls, with the addition that 83 minutes/day MVPA and 8787 steps/day classified elevated blood pressure (AUC: 0.645-0.793, $p<0.05$). For boys, there were no significant AUC ($p>0.05$).

Conclusions: Current physical activity guidelines for preschoolers are insufficient to promote cardiovascular health, as we found 4-year-olds should engage in at least 240 minutes of TPA, 80 minutes of MVPA, or 8700 steps/day. It is unclear if these thresholds apply equally to boys and girls.

Introduction

Physical activity has a role in cardiovascular disease prevention across the lifespan.[1] In early childhood, physical activity is favourably associated with several cardiovascular health indicators including adiposity, cardiovascular fitness, metabolic health, blood pressure, and arterial health.[2-4] In recognition of the importance of physical activity, several countries, and the WHO, have generated physical activity guidelines for the early years. In general, these guidelines recommend preschool-aged children (3 and 4-year-olds) engage in at least 180 minutes/day of activity at any intensity,[5,6] with some guidelines further recommending the 180 minutes of total physical activity (TPA) include at least 60 minutes of moderate-to-vigorous physical activity (MVPA).[7-10]

The activity targets in these early years guidelines are largely based on a combination of normative physical activity levels for this age group and evidence of positive associations between physical activity and health.[2] There is, however, scant empirical evidence to suggest that engaging in at least 180 minutes of TPA and/or 60 minutes of MVPA are optimal for promoting health in this age group.[2,11] Indeed, meeting current physical activity guidelines is not associated with weight status in preschool-aged children[12,13]. In contrast, for school-aged children and adolescents (6-17 years-old), empirical physical activity targets have been derived by determining the amount of physical activity which objectively discriminates between favourable and unfavourable health, defined using established criterion for health (e.g. BMI cut-offs for overweight or obesity) or high levels of an indicator within a sample (e.g. >1 SD from the mean). These studies show 46-68 minutes/day of MVPA discriminates between school-aged children who are normal weight and overweight/obese,[14–18] and between those with healthy and unhealthy cardiovascular fitness[19] and arterial stiffness.[20] Taken together, these studies provide objective evidence in support of the 60 minutes of MVPA recommended in physical activity guidelines for school-aged children and adolescents.[21–23] We lack similar empirically-derived physical activity targets for promoting health in general, and cardiovascular health more specifically, in preschool-aged children.[11] Further, targets for step counts, which are a more accessible activity index,[24] have been derived for preschool children based on the amount of steps associated with meeting current activity guidelines,[25–28] rather than the amount of steps associated with better health indicators.

Given that cardiovascular health in childhood predicts cardiovascular health in adulthood (e.g., blood pressure tracks from childhood to adulthood[29]), cardiovascular disease detection and prevention should begin as early as possible.[1] Therefore, the purpose of this study was to determine the minimal amount of physical activity associated with the promotion of favourable cardiovascular health in preschool-aged children. Using data collected from the Health Outcomes and Physical activity in Preschoolers (HOPP) study,[30] we determined the thresholds of physical activity, measured as TPA, MVPA, and steps/day, which discriminated between favourable and unfavourable cardiovascular health indicators in 4-year-olds. We hypothesized that physical activity variables would discriminate between favourable and unfavourable cardiovascular health for each indicator.

Methods:

Study design

This cross-sectional analysis used data collected as part of the HOPP study, a prospective cohort study with annual visits to the Child Health and Exercise Medicine Program laboratory and Vascular Dynamics Laboratory at McMaster University, Hamilton, Ontario, Canada.[30] Data were collected from August 2010-2013. Children completed a series of assessments of cardiovascular health indicators and wore an accelerometer for 1 week to measure free-living physical activity. To determine the threshold (amount) of physical activity which discriminates between favourable and unfavourable cardiovascular health, we first defined 'unfavourable' health using a combination of established and sample-specific cut-off values for traditional (BMI and blood pressure) and novel (supine resting heart rate, arterial stiffness, and heart rate recovery [HRR]) cardiovascular health indicators, respectively. The Hamilton Integrated Research Ethics Board approved the study. Written, informed consent was obtained from each child's legally authorized representative.

Participants

The HOPP study recruited a community-based sample of children from south-central Ontario, Canada when they were 3-5 years-old.[30] Children with diagnosed medical conditions or known developmental or cognitive delay were not enrolled. The current study includes data from participants at one timepoint when they were 4.0-4.9 years old, identified from either their first (n=139) or second (n=130) annual study visit. We focused specifically on 4-year-olds to maximize our sample size and reduce the confounding effects of growth on our sample-specific cut-off values.

Physical activity

Participants were instructed to wear an accelerometer (ActiGraph GT3X line) over their right hip for 7 days. Data were downloaded in 3-second epochs and non-wear time was removed for analysis. Periods of 60 consecutive minutes of 0 counts, or as parent-reported in a diary, were classified as non-wear time. Days where the device was worn for at least 10 hours were considered valid; at least 3 days of valid wear were required to be included in the statistical analyses. Pate cut-points were applied in the vertical axis to quantify TPA and MVPA[31,32] by dividing the cut-points by 5 to account for our 3-second epoch. Activity counts ≥8 and ≥84 counts/3 seconds were classified as TPA and MVPA, respectively. TPA and MVPA in minutes/day were averaged across valid days. Step counts/day were summed and averaged across all valid days.

Cardiovascular health indicators

Cardiovascular health was measured using established (BMI, seated blood pressure)[1] and non-traditional (resting heart rate,[33] arterial stiffness,[34] HRR from a maximal exercise test[35]) indicators.

BMI. Height and weight were measured in duplicate to the nearest 0.1 cm and kg, respectively, using a stadiometer and digital scale (BWB-800, Tanita Corporation). BMI was calculated as average weight in kg/height in meters squared; z-scores were calculated using the WHO reference standards.[36]

Seated blood pressure. Seated brachial blood pressure in the right arm was measured three times using an automated device (Dinamap Pro 100, Critikon Inc.), with a 1-minute delay between measures. The first measure was discarded and the remaining two were averaged; if the final 2 systolic blood pressures differed by >5 mmHg, an additional measure was taken.[37] Systolic and diastolic blood pressure percentiles were calculated based on normal weight children,[38] as recommended in the 2017 American Academy of Pediatrics Guidelines.[39]

Resting heart rate. A 1-lead ECG setup was placed on the child's chest (Powerlab, ADInstruments). Resting heart rate was determined as a 30-beat average following at least 10 minutes of supine rest (LabChart7 Pro v7.3.4 ADInstruments).

Arterial stiffness. Arterial stiffness was measured using whole-body pulse wave velocity, the procedures of which have been detailed elsewhere.[4,30] A hand-held pressure transducer (tonometer model SPT-301 Millar Instruments) and photoplethysmograph probe (MLT1020PPG, ADInstruments) were placed on the skin over the right common carotid and dorsalis pedis arteries, respectively. The filter method was used to detect the arrival of the pulse at each location[40] and the time delay between arrival was averaged across 20 consecutive heart cycles (LabChart7 Pro v7.3.4 ADInstruments). The time delay was then divided into the measured distance between the sites using the subtraction method [(distance from suprasternal notch to dorsalis pedis artery) - (distance from suprasternal notch to common carotid artery)].[41,42]

Heart rate recovery. HRR was determined 1 minute following a maximal treadmill test,[43] the Bruce Protocol.[44] Participants progressed through the Bruce Protocol stages, which increase in speed and incline every 3 minutes, until they could no longer maintain a cadence to stay at the front of the treadmill or refused to continue. Heart rate was monitored throughout (Polar Electro) and participants were seated immediately following the test. HRR was calculated as the peak heart rate attained during the test minus heart rate 1 minute into recovery. Data were excluded from the analyses if the child did not reach a peak heart rate of at least 180 bpm,[45] or they did not sit still calmly during recovery (e.g. coughing, upset, or moving).

Cut-offs for unfavourable cardiovascular health

Each cardiovascular health indicator was transformed from a scale to the binary variable of ‘favourable’ or ‘unfavourable’ using cut-off values. Established cut-off values were available for the traditional cardiovascular health indictors of BMI and blood pressure. Unfavourable BMI was defined as a BMI z-score >1, which includes children at risk of overweight, overweight, or obese using the WHO standards.[36,46] Children were classified as having unfavourable blood pressure if they had elevated blood pressure or hypertension, defined as systolic and/or diastolic blood pressure ≥90th percentile for age, sex, and height.[39]

As there were no available cut-off values for the non-traditional indicators for preschool-aged children, we created sample-specific cut-offs. We utilized a common approach and calculated cut-offs for unfavourable supine resting heart rate, arterial stiffness, and HRR as 1 SD away from the sex-specific sample mean (Appendix Table 1),[17,47,48] in the direction known to be associated with negative health status in older children or adults. Using this method, unfavourable indicators were defined as a resting heart rate >104 bpm for boys and girls; pulse wave velocity >5.3 m/s for boys and girls; and HRR <54 bpm for boys and <49 bpm for girls.

Statistical analysis

Independent t-tests were used to compare boys and girls across all variables. Receiver operating characteristic (ROC) area under the curve (AUC) analysis was performed to determine the ability of each physical activity variable (TPA, MVPA, and step count) to discriminate between favourable and unfavourable cardiovascular health for each indicator. The ROC curve has a range of sensitivity (probability of detecting true positives) and specificity (probability of correctly detecting true negatives) values across all possible levels of physical activity. An AUC of 1 is a perfect test; AUC <0.5 means that the classifier variable (i.e. physical activity) does not predict the presence or absence of the unfavourable health indicator more than chance.[49] For all significant AUC, the threshold of physical activity with the greatest combined sensitivity and specificity was determined using the Youden Index, which is the maximum value of J (sensitivity + specificity -1).[50] We performed the ROC analyses including all participants, followed by separate analyses for boys and girls. Analyses were conducted in 2019 with all available data using SPSS (PASW Statistics 18). Significance was set at $p < 0.05$.

Results

Two hundred sixty-nine unique participants were identified from the HOPP cohort as being 4.0 to 4.9 years old. Of these, 236 (88%) met the criteria for valid accelerometry; physical characteristics and average physical activity of these 236 participants are presented in Table 1. Participant characteristics of the 269 participants used to create cut-offs for the non-traditional indicators (regardless of available physical activity data) can be found in Appendix Table 1. The number of participants classified as 'unfavourable' for each cardiovascular health indicator is shown in Table 2.

ROC curve results for boys and girls combined are presented in Table 3. For the combined sample, a threshold of 241 mins/day of TPA and 82 minutes/day of MVPA discriminated between favourable and unfavourable HRR; there were no other significant AUC for TPA or MVPA. There was a significant AUC for step counts classifying both unfavourable resting heart rate and HRR, with thresholds of 8987 and 8640 steps/day, respectively. Sensitivity and specificity in the combined sample ranged from 55.2 to 76.3% and 50.0 to 80.2%, respectively. Sensitivity was higher for steps/day as the discriminator variable; specificity was higher for MVPA.

For boys, ROC curve analysis revealed that neither TPA, MVPA, nor step counts classified unfavourable cardiovascular health for any of the indicators (AUC 0.422 to 0.602, $p>0.05$ for all; Appendix Table 2). For girls (Table 4), a threshold of 241 minutes of TPA discriminated between favourable and unfavourable HRR, while 83 and 82 minutes of MVPA discriminated between favourable and unfavourable blood pressure and HRR, respectively. There were significant AUCs for step counts classifying elevated blood pressure, high resting heart rate, and unfavourable HRR in girls; thresholds of 8787, 8627, and 8599 steps/day, respectively, maximized the sensitivity and specificity. For girls, sensitivity and specificity ranged from 77.3 to 92.9% and 51.2 to 71.9%, respectively. Sensitivity was similar across all classifier variables (i.e., TPA, MVPA, and steps/day) and specificity was higher for MVPA as a classifier variable.

Discussion

The purpose of this study was to determine the minimal amount of physical activity associated with the promotion of favourable cardiovascular health in preschool-aged children. We found that 241 minutes (4 hours) of TPA and 82-83 minutes of MVPA, were optimal for favourable HRR in the sample overall, and for girls alone, and for favourable blood pressure in girls. While most current physical activity guidelines suggest that 3- and 4-year-olds should engage in at least 180 minutes of activity at any intensity (TPA),[5,6] including at least 60 minutes of MVPA,[7–10] our findings suggest that these recommendations may not be sufficient to promote cardiovascular health in preschool-aged children in general, and in girls specifically.

We did not find a threshold of physical activity that discriminated between children who were at risk for or overweight, nor those with unfavourable arterial stiffness. This suggests that there may not be a threshold of physical activity associated with these outcomes in preschool-aged children, despite these associations existing in school-aged children.[14–17,20] Likewise, studies comparing preschoolers meeting and not meeting the current guidelines have shown there are no differences in BMI[13] or social-cognitive outcomes,[51] which provides some evidence that the level of physical activity recommended by current guidelines may be too low. Alternatively, there may not yet be a measurable impact of physical activity on weight and arterial stiffness, given the young age of our sample.

There was not a threshold of TPA, MVPA, or step counts that discriminated between any of our cardiovascular health indicators in boys. Overall the boys were more active than the girls (Table 1), and using the thresholds derived in the current study, 73% of boys met the 240 minutes of TPA threshold and 89% met the MVPA threshold, compared to 56 and 69% for the girls, respectively. As most boys in our sample may have surpassed the level of physical activity which confers cardiovascular health benefits, studies including boys on the lower range of physical activity may be required to determine if these physical activity thresholds are also appropriate for preschool-aged boys. Indeed, ROC analysis involving school-aged children has found that thresholds of physical activity required for healthy body weight and fitness are higher in boys than girls[14–16,18,19]; thus, we recommend preschool-aged boys meet at least the activity targets established in the current study.

While physical activity guidelines focus on physical activity in minutes/day, providing activity targets in the form of a daily step count may allow for more feasible and affordable assessment of physical activity.[24] To date, step count recommendations for preschool-aged children have been created by determining the number of steps that correspond with meeting the physical activity guidelines (i.e., 180 minutes of TPA and/or 60 minutes MVPA) rather than the associations between step counts and health. Previous studies found 6000[26] and 9000[28] steps/day are associated with meeting 180 minutes of activity, and 6700,[26] 9934,[27] and 13 874[25] steps/day with 60 minutes of MVPA, with the between study variability likely due to differences in physical activity measurement. Rather than equating steps/day with activity in minutes/day, the current study provides step count recommendations based on associations with cardiovascular health. Our results suggest that achieving at least 8599-8987 steps/day (average approximately 8700 steps) is optimal for avoiding elevated blood pressure and unfavourable resting heart rate and HRR in 4-year-olds.

The sensitivity and specificity of the physical activity thresholds in the current study are similar to those reported in school-aged children.[14–17,19,20] Targets created using ROC curve analysis in school-aged children generally have higher sensitivity than specificity,[14–16,18,20] which we also found in our sample of girls. This suggests that the physical activity thresholds are better at classifying those with unfavourable cardiovascular health (i.e., children with unfavourable cardiovascular health are below the physical activity thresholds) but may misclassify children who are in the healthy range (i.e., there are children below the activity thresholds who do not have unfavourable cardiovascular health). However, given the low risk of engaging in physical activity[52] and the wide range of associated health benefits, it seems acceptable to have an activity target that may, for some children, exceed the minimum level necessary to avoid unfavourable cardiovascular health. A lower physical activity target may not be sufficient for children who may be more susceptible to cardiovascular disease.[53]

Our findings suggest preschool-aged children should achieve targets of approximately 240 minutes of TPA, 80 minutes MVPA, or 8700 steps/day for cardiovascular health. Future research should validate these thresholds in independent samples of 4-year-olds as well as younger children (i.e., 3-year-olds). Furthermore, future research should determine if there is an optimal amount of physical activity associated with other aspects of health and development that are important in early childhood, including motor, cognitive, social and emotional development.

Limitations

We present the first empirical physical activity targets for preschool-aged children using device-measured physical activity and a combination of established and non-traditional cardiovascular health indicators. However, there are limitations worthy of discussion. First, our sample was primarily white, limiting the generalizability of our findings. Second, the cross-sectional analysis does not allow for determination of causality. Third, physical activity measurement via accelerometry is sensitive to cut-point and epoch selection. We utilized a 3-second epoch to capture the short bursts of activity that are characteristic of this age group (95% of activity bouts are ≤15 seconds)[54] rather than a 15-second epoch. Based on previous work from our group that found shorter epoch lengths capture more MVPA, but less TPA/day,[54] we estimate that the threshold for TPA would be approximately 37% higher (329 minutes, or 5.5 hours) and MVPA would be approximately 5% lower (76 minutes) if calculated using a 15-second epoch. Lastly, the values we used to classify children as having unfavourable resting heart rate, pulse wave velocity, and HRR are specific to our sample as existing cut-offs do not exist; however, 1 SD away from the mean is a common approach for creating cut-offs.[17,47,48] Where available we also utilized indicators with established cut-off values (BMI, blood pressure).

Conclusions

In summary, current physical activity guidelines may be insufficient for maintaining cardiovascular health in young children. Our empirically derived targets suggest 4-year-olds should engage in at least 240 minutes of TPA, 80 minutes of MVPA, or 8700 steps/day to promote cardiovascular health.

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Table 1. Descriptive characteristics for participants with valid physical activity data

	n valid[a]	All N=236	Boys n=122	Girls n=114
Age, years	236	4.5 (0.3)	4.5 (0.3)	4.5 (0.3)
White, n (%)	236	213 (90)	109 (89)	104 (91)
Height, cm	236	106.7 (5.1)	**107.9 (5.0)**	**105.6 (5.0)***
Weight, kg	236	17.9 (2.5)	**18.3 (2.6)**	**17.4 (2.2)***
BMI, kg/m^2	236	15.7 (1.3)	15.7 (1.3)	15.6 (1.2)
WHO BMI z-score	236	0.21 (0.87)	0.26 (0.91)	0.16 (0.83)
normal weight, n (%)		193 (82)	101 (83)	92 (81)
at risk overweight, n (%)		36 (15)	15 (12)	21 (18)
overweight, n (%)		7 (3)	6 (5)	1 (1)
CDC BMI z-score[b]	236	0.05 (0.99)	0.001 (1.0)	0.11 (0.93)
normal weight, n (%)		185 (78)	96 (79)	89 (78)
overweight, n (%)		31 (13)	11 (9)	20 (18)
obese, n (%)		10 (4)	8 (7)	2 (2)
underweight, n (%)		10 (4)	7 (6)	3 (3)
Systolic blood pressure, mmHg	209[c]	96 (7)	**98 (8)**	**95 (7)***
Percentile		62 (23)	65 (24)	59 (22)
Diastolic blood pressure, mmHg	209[c]	59 (6)	59 (6)	58 (6)
Percentile		71 (20)	**74 (19)**	**67 (20)***
Resting heart rate, bpm	235[d]	94 (10)	93 (11)	94 (10)
Pulse wave velocity, m/s	229[e]	4.8 (0.5)	4.9 (0.5)	4.8 (0.5)
Heart rate recovery, bpm	221[f]	65 (13)	**67 (12)**	**64 (14)***
Physical activity				
Wear time, min/day	236	714 (36)	716 (39)	712 (33)
TPA, min/day	236	257 (36)	**267 (34)**	**247 (34)****
MVPA, min/day	236	98 (21)	**104 (20)**	**91 (20)****
Step counts, steps/day	235[g]	9032 (1784)	**9377 (1723)**	**8665 (1781)***

Mean (SD) unless noted. Boldface indicates statistically significant difference between boys and girls (*$p<0.05$, **$p<0.001$).

[a] Number of valid assessments for each variable.

[b] CDC z-scores and weight classification were determined from U.S. Centre for Disease Control and Prevention data.[55]

[c] Due to a faulty hose connection, blood pressure data were not available for n=17 participants; n=10 children refused blood pressure measurement.

[d] n=1 refused measurement.

[e] n=4 refused measurement; n=2 removed for poor signal quality; n=1 did not have path length measurement.

[f] n=11 did not reach a peak heart rate of 180 bpm and were excluded; n=2 heart rate monitor malfunctioned; n=2 child was not resting post exercise.

[g] n=1 accelerometer was not initialized to record steps.

BMI, body mass index; CDC, Centers for Disease Control and Prevention; MVPA, moderate-to-vigorous physical activity; TPA, total physical activity; WHO, World Health Organization.

Table 2. Cut-off criteria and number of participants classified as unfavourable for each indicator

Variable	Cut-off criteria	n unfavourable[a] All	Boys	Girls
Established indicators				
BMI z-score	z-score >1, WHO standards	43	21	22
Blood pressure	Systolic and/or diastolic blood pressure ≥90th percentile for age, sex, and height	45	31	14
Non-traditional				
Resting heart rate	>1 SD from sex-specific sample mean	38	16	22
Pulse wave velocity	>1 SD from sex-specific sample mean	42	27	15
Heart rate recovery	<1 SD from sex-specific sample mean	29	14	15

[a] Includes only children with valid accelerometry (n=236).
BMI, body mass index; SD, standard deviation; WHO, World Health Organization

Table 3. Physical activity thresholds that discriminate unfavourable cardiovascular health in the combined sample

	AUC (95% CI)	*p*-value	Threshold	Youden Index	Se (%)	Sp (%)
TPA						
BMI	0.465 (0.375 to 0.555)	.47	–	–	–	–
Blood pressure	0.513 (0.411 to 0.615)	.79	–	–	–	–
Resting heart rate	0.535 (0.436 to 0.634)	.49	–	–	–	–
Pulse wave velocity	0.537 (0.441 to 0.633)	.45	–	–	–	–
Heart rate recovery	**0.634 (0.529 to 0.739)**	**.02**	**241 min/day**	**.253**	**58.6**	**66.7**
MVPA						
BMI	0.465 (0.369 to 0.561)	.47	–	–	–	–
Blood pressure	0.543 (0.444 to 0.643)	.37	–	–	–	–
Resting heart rate	0.589 (0.491 to 0.687)	.08	–	–	–	–
Pulse wave velocity	0.526 (0.433 to 0.620)	.60	–	–	–	–
Heart rate recovery	**0.672 (0.560 to 0.783)**	**.003**	**82 min/day**	**.354**	**55.2**	**80.2**
Step count						
BMI	0.486 (0.394 to 0.578)	.77	–	–	–	–
Blood pressure	0.562 (0.470 to 0.654)	.21	–	–	–	–
Resting heart rate	**0.623 (0.528 to 0.719)**	**.02**	**8987 steps/day**	**.263**	**76.3**	**50.0**
Pulse wave velocity	0.577 (0.480 to 0.674)	.12	–	–	–	–
Heart rate recovery	**0.632 (0.519 to 0.746)**	**.02**	**8640 steps/day**	**.252**	**65.5**	**59.7**

Boldface indicates statistical significance ($p<0.05$)
AUC, area under the curve; BMI, body mass index; MVPA, moderate-to-vigorous physical activity; Se, sensitivity; Sp, specificity; TPA, total physical activity

Table 4. Physical activity thresholds that discriminate between favourable and unfavourable cardiovascular health in girls

	AUC (95% CI)	*p*-value	Threshold	Youden Index	Se (%)	Sp (%)
TPA						
BMI	0.469 (0.337 to 0.601)	.66	–	–	–	–
Blood pressure	0.656 (0.517 to 0.795)	.06	–	–	–	–
Resting heart rate	0.540 (0.404 to 0.677)	.56	–	–	–	–
Pulse wave velocity	0.552 (0.398 to 0.706)	.52	–	–	–	–
Heart rate recovery	**0.715 (0.591 to 0.838)**	**.008**	**241 min/day**	**.373**	**80.0**	**57.3**
MVPA						
BMI	0.496 (0.358 to 0.634)	.95	–	–	–	–
Blood pressure	**0.703 (0.559 to 0.848)**	**.02**	**83 min/day**	**.476**	**78.6**	**69.0**
Resting heart rate	0.593 (0.457 to 0.729)	.18	–	–	–	–
Pulse wave velocity	0.551 (0.410 to 0.693)	.52	–	–	–	–
Heart rate recovery	**0.793 (0.683 to 0.904)**	**<.001**	**82 min/day**	**.586**	**86.7**	**71.9**
Step count						
BMI	0.498 (0.363 to 0.632)	.97	–	–	–	–
Blood pressure	**0.726 (0.606 to 0.846)**	**.007**	**8787 steps/day**	**.440**	**92.9**	**51.2**
Resting heart rate	**0.645 (0.516 to 0.774)**	**.03**	**8627 steps/day**	**.311**	**77.3**	**53.8**
Pulse wave velocity	0.565 (0.428 to 0.702)	.42	–	–	–	–
Heart rate recovery	**0.726 (0.598 to 0.854)**	**.005**	**8599 steps/day**	**.406**	**86.7**	**53.9**

Boldface indicates statistical significance ($p<0.05$)
AUC, area under the curve; BMI, body mass index; MVPA, moderate-to-vigorous physical activity; Se, sensitivity; Sp, specificity; TPA, total physical activity

Appendix Table 1. Participant characteristics, cardiovascular health indicators, and physical activity for all participants (N=269), regardless of valid accelerometry data

	n valid[a]	All N=269	Boys N=135	Girls N=134
Age, years	269	4.5 (0.3)	4.5 (0.3)	4.5 (0.3)
White, n (%)	268	240 (90)	120 (89)	120 (90)
Height, cm	269	106.7 (5.1)	**107.6 (5.0)**	**105.8 (4.9)***
Weight, kg	269	17.9 (2.5)	**18.2 (2.6)**	**17.6 (2.3)***
BMI, kg/m^2	269	15.7 (1.3)	15.7 (1.3)	15.7 (1.3)
WHO BMI z-score	269	0.24 (0.89)	0.27 (0.90)	0.22 (0.83)
Normal weight, n (%)		217 (81)	112 (83)	105 (78)
at risk overweight, n (%)		44 (16)	16 (12)	28 (21)
Overweight, n (%)		8 (3)	7 (5)	1 (1)
CDC BMI z-score	269	0.09 (0.98)	0.01 (1.0)	0.18 (0.93)
Normal weight, n (%)		209 (78)	107 (79)	102 (76)
overweight, n (%)		38 (14)	12 (9)	26 (19)
Obese, n (%)		12 (4)	9 (7)	3 (2)
Underweight, n (%)		10 (4)	7 (5)	3 (2)185
Systolic blood pressure, mmHg	234	97 (7)	**98 (8)**	**95 (7)***
Percentile		63 (23)	65 (23)	60 (22)
Diastolic blood pressure, mmHg	234	59 (6)	59 (6)	58 (6)
Percentile		70 (21)	**73 (20)**	**67 (21)***
Resting heart rate, bpm	264	94 (10)	93 (10)	94 (10)
Pulse wave velocity, m/s	256	4.8 (0.5)	4.8 (0.5)	4.8 (0.5)
Heart rate recovery, bpm	252	65 (14)	**67 (13)**	**64 (14)***

Mean (SD) unless noted**.** Boldface indicates statistically significant difference between boys and girls (**p*<0.05, ***p*<0.001).
[a] Number of valid assessments for each variable
BMI, body mass index; CDC, Centers for Disease Control and Prevention; MVPA, moderate-to-vigorous physical activity; TPA, total physical activity; WHO, World Health Organization.

Appendix Table 2. ROC curve results for physical activity discriminating between favourable and unfavourable cardiovascular health indicators in boys

	AUC (95% CI)	*p*-value
TPA		
BMI	0.467 (0.351 to 0.584)	.64
Blood pressure	0.476 (0.349 t0 0.603)	.70
Resting heart rate	0.485 (0.342 to 0.627)	.84
Pulse wave velocity	0.580 (0.458 to 0.702)	.21
Heart rate recovery	0.558 (0.412 to 0.703)	.49
MVPA		
BMI	0.422 (0.295 to 0.548)	.26
Blood pressure	0.522 (0.399 to 0.645)	.72
Resting heart rate	0.563 (0.415 to 0.711)	.42
Pulse wave velocity	0.556 (0.433 to 0.680)	.38
Heart rate recovery	0.558 (0.400 to 0.715)	.49
Step count		
BMI	0.472 (0.347 to 0.597)	.69
Blood pressure	0.527 (0.413 to 0.641)	.66
Resting Heart rate	0.582 (0.444 to 0.719)	.30
Pulse wave velocity	0.602 (0.466 to 0.737)	.11
Heart rate recovery	0.529 (0.364 to 0.693)	.73

AUC, area under the curve; BMI, body mass index; MVPA, moderate-to-vigorous physical activity; ROC, receiver operating characteristic; TPA, total physical activity

CHAPTER 4: ASSOCIATIONS BETWEEN CAROTID ARTERY LONGITUDINAL WALL MOTION AND ARTERIAL STIFFNESS INDICATORS IN YOUNG CHILDREN

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Associations between carotid artery longitudinal wall motion and arterial stiffness indicators in young children

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HIGHLIGHTS

- Longitudinal arterial wall motion correlates with pulse wave velocity in children.
- Carotid artery longitudinal wall motion is not associated with local β-stiffness.
- There were no differences between boys and girls in longitudinal wall motion.

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ABSTRACT

Background and aims: Carotid artery longitudinal wall motion (CALM) is associated with established indicators of arterial stiffness in healthy adults and in adults with cardiovascular disease risk factors. CALM assessment may be more feasible for incorporation into routine clinical examination than traditional assessments of arterial stiffness; however, the relationship between CALM and arterial stiffness in children has not been established.
Methods: Data were collected from a subset of children participating in the Health Outcomes and Physical activity in Preschoolers study. CALM was characterized by segmental longitudinal wall displacement, velocity, and acceleration. Arterial stiffness was measured using whole-body pulse wave velocity (PWV) and carotid artery β-stiffness index. Associations between CALM and arterial stiffness and the influence of age, sex, and height on those associations were determined.
Results: One hundred and ninety-one children (ages 5.0–8.0 years) were included in the analyses. Systolic retrograde (r = -0.20, p = 0.01) and maximum (r = -0.15, p = 0.04) CALM displacements were weakly correlated with PWV while systolic anterograde and diastolic CALM displacements and wall velocities and accelerations were not correlated with PWV (r = -0.12 to −0.03, p = 0.10 to 0.64). There were no significant correlations between any CALM outcome and β-stiffness index (r = -0.12 to 0.10; $p > 0.05$). Associations were attenuated after adjusting for age, sex, and height.
Conclusions: Higher arterial stiffness, measured by PWV, but not β-stiffness, is weakly associated with less longitudinal movement of the common carotid artery in young, healthy children indicating CALM measurement is not a strong candidate for clinical assessment of arterial stiffness in children.

1. Introduction

Vascular health is a key factor linked to cardiovascular disease with aging [1], however, cardiovascular disease begins to develop in childhood [2], with arterial abnormalities observed in children as young as 1 year old [3]. Of the various vascular health indicators, arterial stiffness has emerged as an independent predictor of cardiovascular disease, thereby providing predictive value beyond traditional risk factors including the SCORE and the Framingham risk score [4–6].

Despite the prognostic value of arterial stiffness, its assessment has not been incorporated into routine clinical practice, in part, due to the technical difficulties associated with the existing arterial pressure-dependent arterial stiffness measurement techniques [6]. Preliminary studies suggest evaluation of the axial movement of the carotid artery in the longitudinal plane (i.e., towards and away from the heart) also provides additional vascular health information beyond that of current arterial stiffness measures and traditional cardiovascular disease risk factors [7–9] and can be obtained from standard B-mode ultrasound

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images. Carotid artery longitudinal wall motion (CALM) is one index of this axial movement that has been demonstrated to be altered in adults with cardiovascular disease [7,8,10] and cardiovascular disease risk factors [9,11] and is associated with established indicators of arterial stiffness in clinical adult populations and healthy adults [7,12].

The previously reported relationships between CALM and established indicators of arterial stiffness [7–9,12,13] have led to the hypothesis that the magnitude of CALM displacements are reflective of the intrinsic elasticity of the arterial wall, with more elastic arteries having greater wall displacement. The relationship between CALM and arterial stiffness in children, a population on the other end of the arterial stiffness spectrum [14], has not been explored. Children have less stiff arteries than adults [15]; however, arterial stiffness is greater in children with chronic health conditions [16–18] and a number of behaviours, including physical activity and diet, have been shown to impact arterial stiffness in otherwise healthy children [19]. Given the associations between arterial stiffness and CALM in older populations, it would stand to reason that a similar relationship may exist in children, who present with highly elastic central arteries prior to a progressive decline in vascular health with age [14].

The purpose of this study was to determine the relationship between CALM outcomes and established indicators of arterial stiffness in young children. Using data collected from 5-8-year-olds participating in the Health Outcomes and Physical activity in Preschoolers (HOPP) study [20], we assessed the associations between established arterial stiffness indicators (pulse wave velocity [PWV] and carotid artery β-stiffness index) and CALM outcomes, specifically CALM displacements during systole and diastole and velocity and acceleration during diastole. We hypothesized that CALM outcomes would be inversely associated with PWV and β-stiffness index in children.

2. Materials and methods

The HOPP Study was a longitudinal observational study with three repeated annual visits; details of the study have been published [20]. The acquisition of higher frame rate vascular ultrasound images required for the analysis of CALM (> 90 frames per second) was an addition to the original HOPP protocol (23 frames per second in original protocol); thus, this study includes a subset of participants during their final visit of the study (n = 191). The study was approved by the Hamilton Intergraded Research Ethics Board and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Written, informed consent was obtained from each child's legally authorized representative.

All measurements were done with the child in the supine position following at least 10-mins of rest. To assess CALM, we placed a 12 MHz linear ultrasound probe (Vivid-q, GE Medical Systems, Horten, Norway) on the skin over the left common carotid artery with an orientation to view the long axis of the artery. Cineloops of brightness mode ultrasound images were captured for at least two consecutive cardiac cycles at > 90 frames per second for robust speckle tracking analysis [21]. Images were analyzed offline using a custom speckle-tracking program (MATLAB, The MathWorks, Natick, MA, USA) [22] to detect wall motion in the axial (longitudinal) plane of the intima-media complex. CALM was characterized as the segmental longitudinal wall displacement during systole (anterograde and retrograde) and diastole (anterograde), the maximum displacement, as well as the wall velocity and acceleration during diastole. Systolic anterograde CALM was defined as the phase between the first systolic anterograde movement to the peak systolic anterograde displacement. Systolic retrograde CALM was defined as the phase between the peak systolic anterograde displacement and the peak retrograde displacement, and diastolic CALM was defined as the phase between the peak retrograde displacement and the local diastolic anterograde plateau (Fig. 1) [7]. To reduce speckle drift and motion artifact over time, caused by participants freely breathing during image acquisition, analyses were limited to one cardiac cycle at

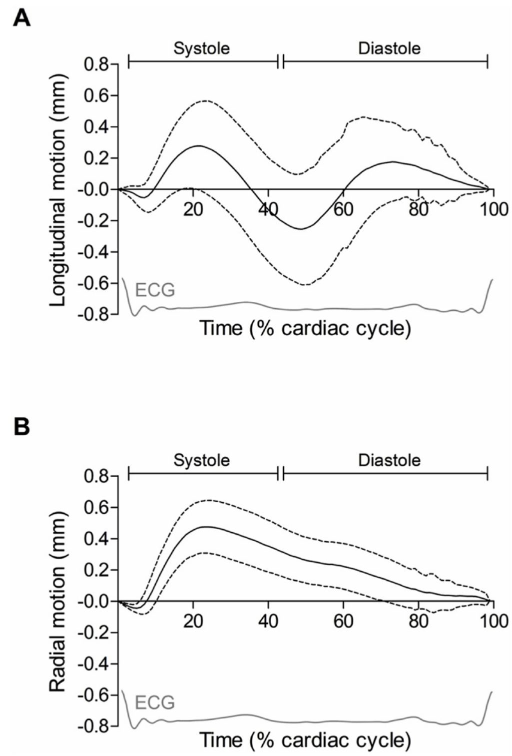

Fig. 1. Average CALM pattern and single-wall radial motion of the sample expressed as the displacement across a single cardiac cycle.
(A) Carotid artery longitudinal wall motion. Anterograde CALM is shown as the positive deflection during systole (i.e. in the direction of blood flow), retrograde CALM is the negative deflection during systole (i.e., toward the heart), and diastolic CALM is the anterograde displacement as the artery recoils back to its staring position. (B) Single-wall radial displacement of the carotid artery. The solid lines are the means and dashed lines are ± 1 SD. Approximate ECG shown in grey. CALM, carotid artery longitudinal wall motion; ECG, electrocardiogram.

a time and a linear regression was performed from the origin to the final speckle kernel position to remove linear bias from the signal (i.e., displacement was forced to return to the reference position). This is similar to the approach used with 2D speckle tracking echocardiography [23]. To determine wall velocities and acceleration, motion traces were digitally filtered (2nd order, dual pass Butterworth at an effective cut-off of 10 Hz) and differentiated to calculate maximum instantaneous diastolic velocity (MIDV) and acceleration (MIDA).

Following the acquisition of higher frame rate images, ultrasound images were collected at 23 frames per second for 10 consecutive heart cycles for determination of arterial stiffness. Simultaneously, a high-fidelity pressure transducer (tonometer model SPT-301, Millar Instruments Inc., Houston, TX) was placed on the skin over the right common carotid artery to obtain pressure waveforms. The carotid artery ultrasound images and pressures were used to calculate carotid artery β-stiffness index according to the equation [24]:

$$\beta\ Stiffness\ Index = \frac{\ln(P_s/P_d)}{(AD_{max} - AD_{min})/AD_{min}}$$

Where P_s and P_d are carotid artery systolic and diastolic blood pressure, respectively, and AD_{max} and AD_{min} are maximum and minimum carotid artery diameter, respectively. To determine AD_{min}

and AD_{max}, the frames corresponding with minimum and maximum artery diameters were extracted using DICOM editing software (Sante DICOM Editor, Santesoft Version 3.3.10) and stored in two separate files for diameter analysis. Semi-automated edge tracking software (Artery Measurement System (AMS), Version 2.0, Gothenburg, Sweden) [25] was used to detect the media-adventitia boundaries on the near and far wall for 100 points over a 1-cm section. The minimum and maximum diameters were averaged over the 10 consecutive beats to determine AD_{min} and AD_{max}. P_s and P_d were determined by calibrating the carotid pressure waveforms to discrete measures of brachial artery blood pressure, obtained immediately following in the right arm (Dinamap Pro 100, Critikon Inc, Tampa, FL, USA), as previously described [26].

Whole-body PWV was assessed from the carotid to dorsalis pedis arteries. Carotid artery pressure waveforms were acquired using the tonometer, as described above, and a photoplethysmograph probe (MLT1020PPG, ADInstruments, Colorado Springs, CO, USA) was placed on the foot to obtain pulse waveforms in the dorsalis pedis artery. The time delay between the arrival of the pulse at these two sites was determined by applying a 5–30 Hz bandpass filter to the signals to remove low frequencies and high frequency noise (LabChart7 Pro v7.3.4 ADInstruments) [27]. The remaining high frequency components are due to sharp inflections at the foot of the waveform [28], which were located at the minimum point of the filtered signal [27]. PWV was calculated as the average time delay over 20 consecutive heart cycles divided into the measured distance between the two sites using the subtraction method [(suprasternal notch to location of sensor on foot) – (suprasternal notch to location of tonometer on neck)] [24,29]. A faster PWV indicates increased arterial stiffness.

Pearson correlations were used to determine the associations between CALM outcomes and arterial stiffness (PWV and β-stiffness index). Linear regression was used to determine if these associations were consistent after adjustment for the child's age, sex, and height-for-age. In the initial model (model 1), each CALM outcome was a dependent variable and age, sex, and height-for-age (i.e., height z-score) were independent variables. In subsequent separate models, PWV (model 2), and β-stiffness index (model 3), were added as predictor variables. Independent t-tests were used to compare boys and girls for all variables. Data were analyzed in SPSS (PASW Statistics 18). Significance was set at $p < 0.05$.

3. Results

One hundred and ninety-one children between the ages of 5.0–8.0 years were included in the analyses. Participant characteristics are shown in Table 1 with descriptive results for CALM outcomes and arterial stiffness in Table 2. There were no differences between the boys and girls for participant characteristics, CALM outcomes, or arterial stiffness ($p > 0.05$). Correlation results showed systolic retrograde and maximum CALM displacements were weakly correlated with PWV; systolic anterograde and diastolic CALM displacement and wall velocities and accelerations were not correlated with PWV (Table 3). There were no significant correlations between any CALM outcome and carotid artery β-stiffness index (Table 3). After adjusting for age, sex, and height-for-age in linear regression analyses (Table 4), PWV was only a significant predictor of retrograde CALM displacement while β-stiffness index was not a significant predictor of any CALM outcome. There were no significant effects of sex or age on any CALM outcomes. Height-for-age was a significant predictor of retrograde CALM only.

Table 1
Participant characteristics.

	All n = 191	Girls n = 90	Boys n = 101
Age, y	6.5 (1.0)	6.6 (0.9)	6.4 (0.9)
Height[a], cm	120.2 (8.1)	120.2 (8.1)	120.1 (8.1)
Height z-score[b]	0.37 (0.95)	0.29 (0.92)	0.44 (0.97)
Weight[a], kg	22.7 (4.4)	22.6 (4.4)	22.7 (4.5)
Weight z-score[b]	0.13 (0.90)	0.08 (0.9)	0.16 (0.9)
BMI, kg/m^2	15.5 (1.5)	15.5 (1.5)	15.6 (1.4)
BMI z-score[b]	−0.09 (0.90)	−0.08 (0.9)	−0.10 (0.9)
Systolic blood pressure, mmHg	98 (7)	98 (6)	99 (7)
Diastolic blood pressure, mmHg	56 (5)	57 (5)	55 (6)

Data presented as mean (SD).
BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).
[a] Height and weight were collected using standard procedures [20].
[b] z-scores were determined from U.S. Centre for Disease Control and Prevention data [40].

Table 2
CALM outcome variables and arterial stiffness indictors.

	Mean (SD)	Minimum	Maximum
CALM			
Anterograde systolic CALM, mm	0.43 (0.24)	0.03	1.41
Retrograde systolic CALM, mm	0.73 (0.29)	0.18	1.71
Maximum CALM, mm	0.83 (0.29)	0.22	1.71
Diastolic CALM, mm	0.64 (0.28)	0.10	1.53
MIDV, mm/s	7.25 (3.01)	1.26	16.2
MIDA, mm/s [2]	197.6 (76.7)	54.9	446.7
Arterial stiffness			
PWV, m/s	4.94 (0.42)	3.84	6.22
β-stiffness index, AU	3.71 (0.84)	2.00	6.66

CALM, carotid artery longitudinal wall motion; PWV, pulse wave velocity; MIDV, maximum instantaneous diastolic velocity; MIDA, maximum instantaneous diastolic acceleration.

4. Discussion

We found that in a group of young, healthy children, retrograde systolic and maximum longitudinal displacement of the carotid artery wall (CALM) were only weakly associated with PWV. The strength of these associations were attenuated after adjusting for age, sex, and height. We did not find any associations between PWV and longitudinal wall velocity or acceleration, and carotid β-stiffness index was not related to any CALM outcome. While there are some associations between CALM and one index of arterial stiffness (PWV), these correlations are weak and not consistently observed.

The inverse associations between PWV and retrograde and maximum CALM displacement, while weak, are in line with our hypothesis, showing that young children with greater arterial stiffness have less longitudinal movement of the carotid artery wall. In a cohort of 30–45 year-old men (n = 292) as part of the Cardiovascular Risk in Young Finns Study, Taivainen et al. [12] found significant correlations between longitudinal wall motion displacements and PWV. In that study, similar to our findings, retrograde and maximum displacements, but not anterograde displacement, were associated with PWV; the strength of the correlations were also similar ($r = -0.18$ and -0.14). The correlations in the current paper and the Young Finns Study are not as strong as those previously reported in our laboratory by Au et al. between CALM displacements (anterograde, retrograde, maximum, and diastolic) and PWV (ρ = -0.19 to -0.42)[7]. The differences in correlation strength are likely related to population differences, with the current study and Taivainen et al. [12] including more narrow age-ranges of healthy individuals and Au et al. including adults from ages 20–83 years, with, and without, cardiovascular disease. Further, after adjusting for age, weight, and heart rate, the study by Au et al. found arterial stiffness was no longer a significant predictor of CALM [7], suggesting that the previously reported correlations between CALM and arterial stiffness may be related to these factors, in particular age, rather than stiffness, *per se*. CALM and arterial stiffness may undergo parallel changes with age and/or disease, but may not necessarily be directly related to each other in adults [7,9] or in children.

Table 3
Pearson correlations between CALM outcomes and arterial stiffness.

		Anterograde	Retrograde	Maximum	Diastolic	MIDV	MIDA
PWV	r	−0.03	**−0.20****	**−0.15***	−0.12	−0.08	−0.11
	p	0.64	0.005	0.04	0.10	0.27	0.15
β-stiffness Index	r	−0.12	0.03	0.01	0.10	0.06	0.03
	p	0.09	0.70	0.85	0.19	0.42	0.71

CALM, carotid artery longitudinal wall motion; MIDV, maximum instantaneous diastolic velocity; MIDA, maximum instantaneous diastolic acceleration; PWV, pulse wave velocity.
*, $p < 0.05$; **, $p < 0.01$.

Our finding that retrograde systolic and maximum CALM displacement are related to PWV but not β-stiffness index is surprising given that β-stiffness index and CALM are acquired locally at the same site – the common carotid artery. Studies which have assessed the association between CALM and both PWV and carotid artery elasticity, as measured by distensibility, have found associations with both variables [7,12], or with carotid distensibility only [9]. In this study, we chose to use β-stiffness index rather than distensibility as this measure is more reliable in young children [30] and takes into account blood pressure, which increases with age in children [31]. The methodological differences between our study and previous work [7,9,12], however, does not explain the conflicting findings as a post-hoc analysis showed that carotid artery distensibility was also not significantly correlated with CALM outcomes in this study (results not shown). The contrasting findings in the current study may relate to the overall more elastic nature of the carotid artery in children in comparison to adults [24], contributing to differing axial and radial wall movements early in life. While our understanding of the physiological cause of CALM is far from complete, evidence suggests the anterograde motion is primarily driven by blood flow and the retrograde motion is due to cardiac twist during contraction pulling the artery back towards the heart [32,33]. Thus, while the amount of carotid artery wall movement may be restricted by the radial stiffness of the carotid artery in adults, the more elastic carotid arteries of young children may not constrain this movement. The association between CALM displacement and PWV, but not β-stiffness index, could also be the result of differing elastic properties of arteries in the axial and radial planes. PWV, like β-stiffness, is considered a measure of radial stiffness [28]; however, PWV may be affected by arterial properties in multiple planes [34] and therefore more closely related to CALM.

Most evidence linking CALM and arterial stiffness has focused on the magnitude of displacements. Recently, we demonstrated associations between arterial stiffness and maximum instantaneous diastolic CALM velocity and acceleration of the carotid wall (i.e., MIDV and MIDA, respectively) that were similar in strength, if not stronger, than the associations observed for the magnitude of displacements [7]. It is unknown whether these outcomes offer independent information about the elastic nature of blood vessels, or if displacement is a surrogate for arterial tethering or connective tissue accumulation at the adventitia. Regardless, we did not find relationships with MIDV or MIDA and arterial stiffness, suggesting the speed at which the carotid artery wall is moving longitudinally is not related to arterial stiffness in young, healthy children.

While CALM and arterial stiffness may be related, CALM also provides independent information regarding cardiovascular disease presence and risk in adults. For example, Svedlund et al. found that CALM, but not carotid artery radial stiffness, predicted cardiovascular events over 1-year in individuals with coronary artery disease [35]. Likewise, CALM outcomes were shown to be attenuated to a greater extent than arterial stiffness in individuals with coronary artery disease [7] and periodontal disease [9]. CALM retrograde displacements were found to decrease as the number of cardiovascular disease risk factors increase [36] and were lower in adults with type 2 diabetes [11] and spinal cord injury [22]. Although not assessed in the current study it is possible that CALM could indicate the presence of cardiovascular alterations in children with chronic health conditions and perhaps be a more sensitive indictor of cardiovascular risk than arterial stiffness in these populations. Children at risk of developing cardiovascular disease, including those with asthma, type 1 diabetes, and juvenile idiopathic arthritis, have increased arterial stiffness [18,37,38] and future research should evaluate if CALM outcomes are also impacted. Should CALM provide predictive insight into cardiovascular health, it is a potentially more feasible method for assessment of arterial health than current techniques [8,9]. A number of challenges exist with the traditional arterial stiffness measurements, including the use of specialized equipment, such as pressor sensors, and technical skill. Obtaining and maintaining a high-fidelity arterial pressure signal, as required for both PWV and carotid artery stiffness measurement, can be particularly challenging in young children [17]. The ultrasound images required for CALM determination, on the other hand, can be acquired quickly using equipment and skills already available in many clinical and research settings.

Table 4
Multiple linear regression results for arterial stiffness as predictors of CALM outcomes after adjusting for age, sex, and height.

	Anterograde	Retrograde	Maximum	Diastolic	MIDV	MIDA
Model 1						
Age	−0.03 (0.02)	−0.02 (0.02)	−0.3 (0.02)	0.01 (0.02)	0.1 (0.2)	−5 (6)
Sex	0.05 (0.03)	0.05 (0.04)	0.05 (0.04)	−0.003 (0.04)	−0.4 (0.4)	7 (11)
Height z-score	0.03 (0.02)	**0.04 (0.02)***	0.04 (0.02)	0.02 (0.02)	0.3 (0.2)	9 (6)
Model 2[a]						
PWV	0.003 (0.04)	**−0.13 (0.05)****	−0.09 (0.05)	−0.09 (0.05)	−0.7 (0.5)	−17 (14)
Model 3[b]						
β-stiffness index	−0.03 (0.02)	0.01 (0.03)	0.01 (0.02)	0.03 (0.02)	0.2 (0.3)	3 (7)

Results reported as B(SE). B, unstandardized beta; CALM, carotid artery longitudinal wall motion; MIDV, maximum instantaneous diastolic velocity; MIDA, maximum instantaneous diastolic acceleration; PWV, pulse wave velocity.
*, $p < 0.05$; **, $p < 0.01$.
[a] Model 2 includes all variables from model 1 (age, sex, height z-score) and PWV.
[b] Model 3 includes all variables from model 1 (age, sex, height z-score) and β-stiffness index.

4.1. Limitations

There were limitations in our measurements primarily due to the nature of working with young children. We did not have the children hold their breath during the CALM assessments; however, we did adjust for breathing artifact using a technique similar to that used in echocardiography (i.e., drift compensation). Further, we did not use the gold-standard locations in our assessment of PWV – carotid to femoral. Instead, our measure of PWV was 'whole-body' (carotid to foot), including both central and peripheral arteries. We have demonstrated previously that whole-body PWV is reliable in young children [30] and it has been shown to have strong agreement with carotid-femoral PWV in adults [39].

4.2. Conclusions

Our findings suggest that higher arterial stiffness, measured by whole-body PWV, is associated with less longitudinal movement of the common carotid artery in prepubertal children. However, these associations were not strong, nor corroborated by associations with local artery stiffness, as measured by carotid artery β-stiffness. Thus, while arterial stiffness may have some influence on CALM, it does not explain a large proportion of the variance of CALM in young, healthy children.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

NAP and MJM conceptualized the current study; BWT and MJM conceived, designed, and supervised the HOPP study. NAP collected the data and NAP and JSA analyzed the data. NAP, JSA, and MJM interpreted the data. NAP drafted the initial manuscript. JSA, BWT, and MJM critically reviewed and revised the manuscript for important intellectual content. All authors approved the final manuscript and agree to be accountable for all aspects of the work.

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CHAPTER 5: DISCUSSION

5.1 Discussion overview

The overall purpose of this thesis was to explore the relationships between physical activity and indicators of cardiovascular health during early childhood. Specifically, we examined the effect of physical activity on trajectories of cardiovascular health indicators during early childhood (Chapter 2, Study 1), determined the threshold of physical activity associated with favourable cardiovascular health in preschool-aged children (Chapter 3, Study 2), and investigated a novel index of vascular health in young children (Chapter 4, Study 3). The main findings of these studies were that 1) physical activity has beneficial effects on cardiovascular fitness, autonomic function, blood pressure, and arterial stiffness in early childhood; 2) preschool-aged children should engage in higher levels of physical activity than the current recommendations suggest (at least 240 minutes of TPA, 80 minutes of MVPA, or 8700 steps/day) to avoid unfavorable cardiovascular health; 3) there is a weak, inverse correlation between PWV, but not carotid artery β-stiffness index, and the novel index of carotid artery longitudinal wall displacement in young, healthy children. This general discussion provides an integrated summary of findings of these studies and recommendations for future directions focusing on the effect of physical activity on cardiovascular health (including recommendations), key similarities and differences between boys and girls, and arterial stiffness during early childhood.

5.2 Physical activity and cardiovascular health in early childhood

Chapter 2 filled an important gap in the literature demonstrating that physical activity has beneficial effects on cardiovascular fitness, autonomic function, arterial stiffness, and blood pressure during early childhood. This was the first prospective, observational study using device-measured physical activity, together with these cardiovascular health indicators, to study children during early childhood. Building on the results of Chapter 2, we then set out to determine the amount of physical activity preschool-aged should engage in to promote cardiovascular health. To do this, we took an empirical approach to determine the amount of physical activity that distinguished between favourable and unfavourable cardiovascular health. Based on these analyses, we determined that the amount of physical activity recommended in current guidelines (at least 180 minutes/day of TPA (Ministry of Health, 2017; US Department of Health and Human Services, 2018), with some guidelines further recommending the 180 minutes of TPA include at least 60 minutes of MVPA (Okely et al., 2017; Tremblay et al., 2017; UK Chief Medical Officers, 2019; World Health Organization, 2019)) is not sufficient for preschool-aged children to avoid unfavourable cardiovascular health (Chapter 3). We found that higher targets of activity, at least 240 minutes (4 hours) of TPA or 80 minutes of MVPA, are required for favourable cardiovascular health, in particular favourable autonomic function and blood pressure. While it is not clear if these activity recommendations apply equally to both boys and girls, these are the first empirically-derived activity targets for preschool-aged children and represent an improvement on the current recommendations, which are based on average physical activity levels in preschool-aged children (Carson et al., 2017; Pate et al., 2019).

While activity recommendations for preschool-aged children include either a TPA recommendation alone (Ministry of Health, 2017; US Department of Health and Human Services, 2018), or TPA and MVPA recommendations (Okely et al., 2017; Tremblay et al., 2017; UK Chief Medical Officers, 2019; World Health Organization, 2019), activity recommendations for school-aged children and adults are primarily focused on MVPA (i.e. 60 minutes/day of MVPA for school-aged children (Tremblay et al., 2016; US Department of Health and Human Services, 2018; World Health Organization, 2010); 150 minutes/week of MVPA for adults (US Department of Health and Human Services, 2018; World Health Organization, 2010)) as MVPA has been associated with stronger and more consistent health benefits in these age groups (Piercy et al., 2018; Poitras et al., 2016). Our findings suggest that MVPA also has a more potent effect on cardiovascular health indicators than TPA during early childhood. More specifically, MVPA, but not TPA, significantly slowed the rate of arterial stiffening (PWV and carotid artery β-stiffness index) in both boys and girls (Chapter 2). Furthermore, MVPA, but not TPA, was significantly associated with both slowing down the increase in SBP over time (Chapter 2) and discriminating between normal and elevated blood pressure (Chapter 3) in girls. Likewise, the estimated effect of MVPA on HRR and treadmill time in Chapter 2 was 1.6 and 2 times greater, respectively, than the estimated effect of TPA. In the ROC curve analysis used in Chapter 3, specificity (the ability to detect true negatives) was higher for the threshold of MVPA that classified unfavourable HRR than TPA, meaning that children who are above the MVPA threshold are more likely to be correctly identified as having favourable HRR than when using the TPA threshold. Collectively, these results provide evidence that the intensity of physical activity matters for cardiovascular health during early childhood and that an intensity component should be added to those guidelines that currently do not currently have one (e.g. Physical Activity Guidelines for Americans (US Department of Health and Human Services, 2018)). These findings also suggest that, similar to school-aged children and adults (Tremblay et al., 2016; US Department of Health and Human Services, 2018; World Health Organization, 2010), it may be reasonable for physical activity guidelines for preschool-aged children to focus on MVPA. This requires further exploration as TPA may have differential effects on other important health and developmental outcomes in early childhood (e.g. motor and cognitive development).

It is important to note that while the results related to MVPA were pronounced, we did find positive effects of TPA on cardiovascular health (Chapters 2 & 3). Children who engaged in more activity at any intensity (i.e. TPA) had higher cardiovascular fitness, better autonomic function, and reduced arterial stiffness. However, in our studies, there were no indicators that were significantly associated with TPA and not MVPA (Chapters 2 & 3). Since TPA is composed of LPA and MVPA, it is possible that the associations we found between TPA and the cardiovascular health indicators are driven primarily by MVPA. While we chose to examine TPA and MVPA since these variables are the focus of physical activity guidelines for the early years, our analyses do not allow for conclusions on the effect of lower intensities of physical activity (e.g. LPA) on cardiovascular health. Studies on physical activity and cardiovascular fitness in preschool-aged children provide some evidence that the higher intensities of activity (MVPA and VPA) are the key drivers for cardiovascular fitness, as lower intensity activity (LPA) was not associated with field-based measures of cardiovascular fitness in 4-6 year-olds (Bürgi et al., 2011; Leppänen et al., 2017). In order to further tease out the effects of physical activity intensity, mathematical modeling is increasingly being used to estimate the effect of replacing one intensity of activity with another. In a large study of 3-18-year-olds (n=19 502) using data from the International Children's Accelerometry Database, substituting sedentary time with MVPA, but not LPA, was estimated to lower SBP in boys and girls, and DBP in girls (Wijndaele et al., 2019). Similarly, in 9-10-year-olds (n=169), it was predicted that reallocating MVPA to LPA would result in a decrease in cardiovascular fitness (Fairclough et al., 2017). To address these remaining gaps in our knowledge, the contributions of various intensities of physical activity to cardiovascular health during early childhood should continue to be explored in future research.

5.3 Similarities and differences between boys and girls

In line with the literature, we found that both TPA and MVPA were higher in boys than girls. In Chapter 2, TPA was approximately 25 minutes/day higher and MVPA was 15 minutes/day higher in boys compared to girls. In Chapter 3, (4-year-olds only) TPA was approximately 20 minutes/day higher in the boys; MVPA was approximately 13 minutes higher. This difference in MVPA between boys and girls is slightly higher than the 10 minutes/day reported in a recent review in a similar age group which transformed the mean MVPA from 33 studies using 7 different cut-points to the same cut-points (Pate) we used in Chapters 2 and 3 (Ravagnani et al., 2017).

Previous studies examining physical activity and cardiovascular health typically included both boys and girls and often controlled for sex; however, many did not conduct either stratified analyses or determine effect modification through interactions (Pate et al., 2019). In Chapter 2, we added sex interactions to all models to determine if the effect of physical activity on cardiovascular health indicators was moderated by sex and/or gender. In Chapter 3, we conducted a stratified analysis to determine the thresholds of physical activity associated with cardiovascular health separately for boys and girls. For the most part, results from Chapter 2 showed that engaging in physical activity has similar benefits for boys and girls: both boys and girls who engaged in greater amount of physical activity had longer time to exhaustion on a maximal treadmill test, faster HRR following exercise, and lower arterial stiffness during early childhood.

Despite the observed similarities in boys and girls, we found physical activity, specifically MVPA, had a favourable effect on SBP (Chapter 2) and was able to discriminate elevated blood pressure (Chapter 3) in only girls. One previous study that examined the relationships between physical activity, measured by pedometer (steps/day), and SBP and DBP found unfavourable associations in both preschool-aged boys and girls (Kolpakov et al., 2011); however, these unfavourable associations may be due to not adjusting for important confounders like age and height (Rosner et al., 2008). Other studies have found null associations between device-measured physical activity and blood pressure during early childhood but did not determine if the associations were different between boys and girls (Klesges et al., 1990; Vale et al., 2015). In 3- to 6-year-olds, there were no significant correlations between TPA, measured on 1 day using accelerometry, and SBP or DBP (Klesges et al., 1990) nor was there a greater likelihood of having elevated blood pressure from not engaging in at least 60 minutes of MVPA (Vale et al., 2015); notable, neither study examined if there were differential effects for boys and girls. Given the effect of physical activity on blood pressure over time in Chapter 2 was only observed upon including the sex interaction, our findings highlight the importance of conducting analyses to examine the influence of sex and/or gender, even during early childhood. It is unclear, however, why we found relationships between physical activity and blood pressure in only the girls. In contrast to our findings, in school-aged children, favourable effects of physical activity on blood pressure in prospective studies were observed more often in boys than girls (Skrede et al., 2019). While most cross-sectional studies find associations between device-measured physical activity and blood pressure in school-aged children (Poitras et al., 2016), only about half of prospective observational studies have reported favourable effects between physical activity and blood pressure (Poitras et al., 2016; Skrede et al., 2019). Thus, our contrasting findings between boys and girls add to the evidence of inconsistent relationships between physical activity and blood pressure in preschool (Bell et al., 2018; Carson et al., 2017) and school-aged children (Poitras et al., 2016; Skrede et al., 2019). Continued follow-up of this cohort will provide an opportunity to determine if a relationship between physical activity and blood pressure in boys emerges in the school-aged years and to determine if it is related to physical activity in early childhood.

We found the observed positive effects of MVPA on cardiovascular fitness, measured by treadmill time, were consistent between boys and girls in early childhood (Chapter 2), which is similar to the positive associations observed between MVPA and VPA and the number of shuttle run laps completed in both 3-5-year-old boys and girls (Bürgi et al., 2011; Fang et al., 2017; Leppänen et al., 2017, 2016). Together, these findings provide strong evidence that for both boys and girls, those who are more active have higher cardiovascular fitness during early childhood. The effect of physical activity on HRR, an indicator of fitness (Daanen et al., 2012) and measure of autonomic function (Lahiri et al., 2008), also did not vary between boys and girls in the current study (Chapter 2). This is similar to the findings of Kühne *et al.* (2016) who found an association between MVPA and a field assessment of HRR in both 3-6-year-old boys and girls. However, despite the similar effect of physical activity on HRR (Chapter 2), Chapter 3 revealed that TPA and MVPA discriminated unfavourable HRR only in girls. As discussed in Chapter 3 (Study 2), the higher physical activity levels in boys compared to girls may have resulted in few boys in the current cohort falling below a level of physical activity that would result in unfavourable HRR. Alternatively, the selection of unfavourable (low) HRR using a sample-specific cut-off 1 SD below the sex-specific mean may have resulted in a cut-off that did not reflect unfavourable HRR in boys. Since boys had a higher average HRR than girls (67 vs. 64 bpm), the cut-off for unfavourable HRR was higher in boys (<54 bpm) than girls (<49 bpm). We utilized a sex-specific cut-off as we found HRR was approximately 5 bpm higher in boys than girls after adjusting for age, height, and physical activity (Chapter 2). Similarly, Singh *et al.* (2008) found a difference in HRR of 5 bpm between 5-18-year-old boys and girls even with adjustment for BMI, baseline heart rate, age, and fitness (time to exhaustion). Thus, while a sex-specific approach seemed appropriate in the current analysis (Chapter 3), it may have resulted in a cut-off that was not low enough to reflect unfavourable HRR in boys, who, on average, have higher parasympathetic reactivity than girls.

We found that the effect of physical activity on arterial stiffness, measured by both PWV and carotid artery β-stiffness index, was not different between boys and girls (Chapter 2). In slightly older children (6-8-year-olds) Haapala *et al.* (2017) found that accelerometry-measured MPA and VPA were inversely (favourably) associated with arterial stiffness, assessed by pulse contour analysis; however, when the analysis was stratified by sex, the association between physical activity and arterial stiffness was attenuated and no longer significant in boys (p=0.06). This is likely due to the smaller number of boys (n=57) than girls (n=79) in the study (Haapala *et al.*, 2017), rather than evidence that physical activity has differential effects in pre-pubertal boys and girls. We show, in a larger sample with a balanced number of boys and girls and repeated measures, that the protective effect of physical activity on arterial stiffness is similar for boys and girls during early childhood.

5.4 Arterial stiffness during early childhood

Studies consistently show that arterial stiffness increases across the lifespan (Avolio et al., 1983; Diaz et al., 2018; Kucharska-Newton et al., 2019; Reusz et al., 2010); however, it was not clear if arterial stiffening begins during early childhood. In Chapter 2, we showed for the first time using repeated measures, that arterial stiffness (measured by carotid-to-dorsalis pedis PWV and carotid artery β-stiffness index) increases in children under the age of 8 over a 3-year period. One previous cross-sectional study found that arterial stiffness was not different across age 3-8 years (Hidvégi et al., 2012). That study, by Hidvégi *et al.*, estimated PWV using a brachial pulse wave analysis technique; however, in adults this technique has been found to reflect upper limb PWV, not the gold standard carotid-to-femoral PWV (Trachet et al., 2010). Our measure of carotid-to-dorsalis pedis PWV incorporates the central arteries and we also directly measured stiffness in the carotid artery (β-stiffness index). In our study, both of these indices showed an increase in arterial stiffness over time, even with adjustment for height, sex, and physical activity.

Theories on vascular aging propose that arterial stiffness progressively increases, with the rate of change varying between individuals due in part to lifestyle factors, including physical activity (Laurent et al., 2019; Nilsson et al., 2009). However, few studies in adults or children have tested this hypothesis with longitudinal designs and repeated measures of arterial stiffness to determine if physical activity slows the progression of arterial stiffness. In Chapter 2, we report, for the first time in children using device-measured physical activity, that increased MVPA slows the progression of arterial stiffening. Previous studies in adolescents found mixed results between self-reported physical activity and arterial stiffness over time. Mikola *et al.* (2017) did not find an effect of self-reported leisure time physical activity on carotid artery distensibility from age 11 to 19 years old across 5 repeated time points; however, this analysis combined data from intervention and control participants enrolled in a diet and lifestyle randomized control trial. In a longitudinal observation study of adolescents, Chen *et al.* (2012) found an association between decreased self-reported physical activity (activity engagement rated from not at all [1] to very often [5]) over 3 years and an increase in carotid-to-radial PWV. In our prospective, observational study, children engaging in greater amounts of device-measured MVPA had a slower rate of change of both PWV and carotid artery β-stiffness index. These findings provide additional support for early and supernormal vascular aging theories, which posit that trajectories of arterial health are affected by physical activity (Figure 5-1) (Laurent et al., 2019; Nilsson et al., 2009). Future studies should determine if arterial stiffness continues to increase at a slower rate in children who engage in higher amounts of MVPA through the school years and into adulthood. The increase in arterial stiffness with aging is attributed to changes in the properties of the artery wall (e.g. fragmentation of elastin, increased collagen, and proliferation of smooth muscle cells) and an increase sympathetic activity resulting in greater vasomotor tone (Chirinos et al., 2019; Kucharska-Newton et al., 2019; Tanaka, 2019; Zieman et al., 2005). The proposed mechanisms for the favourable impact of physical activity on the vascular system are complex and multifactorial, including both structural and neuro-hormonal adaptations (Green, Spence, Halliwill, Cable, & Thijssen, 2011), and are based on evidence from both acute and chronic exercise training studies in adult humans and in animal models. An acute bout of aerobic exercise using large muscle groups causes transient increases in heart rate and pulse pressure, which results in

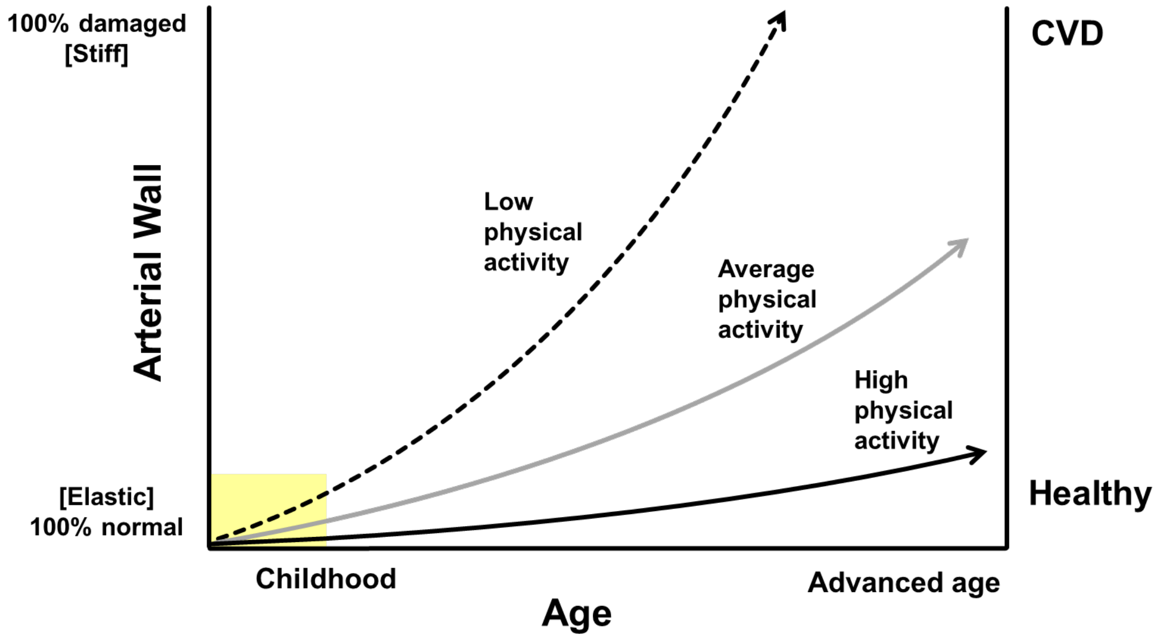

Figure 5-1. Hypothesized progression in arterial stiffness across the lifespan for individuals engaging in high (black), average (grey), and low (dashed) amounts of physical activity. Shaded area represents the progression in arterial stiffness according to different levels of physical activity during early childhood, based on the findings from Chapter 2 in this thesis. CVD, cardiovascular disease. [Adapted from Nilsson et al. (2009), *Hypertension* and Laurent et al. (2019), *Hypertension*]

increases in circumferential stress and shear stress in the artery wall. When repeated over time, these stresses may result in chronic adaptations in the artery, including structural changes to the artery wall (e.g. in the quantity and alignment of elastin and collagen) (Green et al., 2011). In children, chronic high levels of habitual physical activity may preserve elastic proprieties (e.g. less fragmentation of elastin) resulting in a slower increase in artery stiffness over time. Physical activity may also decrease sympathetic drive and circulating catecholamines, thereby reducing the tone of the artery and lowering stiffness (Green et al., 2011; Tanaka, 2019; Zieman et al., 2005). There is additional evidence from animal models that exercise training causes remodeling in the cardiorespiratory centers in the brainstem, thereby reducing sympathetic, and increasing parasympathetic, activity (Green et al., 2011; Thijssen et al., 2010). It is also hypothesized that decreased stiffness in barosensitive areas (e.g. carotid arteries) may increase parasympathetic outflow (Green et al., 2011). These mechanisms, while not exhaustive, may explain the favourable effects we found between physical activity and arterial stiffening, but also the beneficial effects of physical activity on HRR and resting heart rate (i.e. increased parasympathetic and decreased sympathetic activity), and blood pressure (i.e. lower peripheral resistance due to reduced sympathetic tone (Pedersen & Saltin, 2015)). The greater stimuli (e.g. higher pressures and sheer stress) from higher intensity activity (MPVA) (Green, Hopman, Padilla, Laughlin, & Thijssen, 2017) may explain the stronger associations found in the current studies between MVPA and cardiovascular health indicators in comparison to TPA, and specifically for MVPA attenuating the age-associated increases in arterial stiffness.

We found the progressive increase in arterial stiffness can be slowed through increased engagement in MVPA (Chapter 2), but there was not a threshold of physical activity that cross-sectionally discriminated between favourable and unfavourable arterial stiffness in preschoolers (Chapter 3). There were several methodological differences between the studies contained in this thesis which may explain these contrasting findings. Chapter 2 was a repeated measures analysis, including 3 annual time points with participants enrolling in the study at age 3, 4, or 5; outcome (PWV) and predictor (physical activity) variables were continuous. Chapter 3, in comparison, was a cross-sectional analysis of participants at age 4. While physical activity was a continuous variable, the outcome variable (PWV) was dichotomous (favourable or unfavourable). Taking into account these differences, the findings could be interpreted that there may not yet be an effect of physical activity on arterial stiffening at age 4 (Chapter 3) or that the repeated measures design used in Chapter 2 allowed us to detect changes in arterial stiffening over time that were not apparent in a cross-sectional analysis. Alternatively, creating the dichotomous variable of 'favourable' and 'unfavourable' PWV required using a sample-specific cut-off (>1SD from the mean), which may not be an accurate cut-off point to define unfavourable arterial stiffness. Further, the ROC analysis used in Chapter 3 does not allow for control of variables, and while we performed analyses stratified by sex, not controlling for other variables like height and age, which were controlled for in Chapter 2, may have affected the results.

A key direction of the systematic review conducted as part of the Physical Activity Guidelines for Americans was the need to identify cardiometabolic indicators that are sensitive enough to detect change in young children, who for the most part have healthy cardiovascular systems (Pate et al., 2019). Our findings from Chapter 2 support the sensitivity of arterial stiffness to detect change due to an exposure (i.e., physical activity) over time; further, the assessments using carotid-to-dorsalis pedis PWV and carotid artery β-stiffness index mirrored each other, providing more support that arterial stiffness is measurable and sensitive to change in this age group. Despite the sensitivity of these measures, both require specialized equipment, trained personnel, and the acquisition of a high-quality carotid pulse wave signal, which can be challenging in young children (Savant et al., 2014). Collectively, these issues limit the assessment of arterial stiffness from widespread use outside of research-based environments. In Chapter 4, we explored if a novel index, CALM, could be used as a more feasible, yet valid, indicator of arterial stiffness in young children. CALM is acquired using only B-mode ultrasound in a similar way to carotid artery intima media thickness, which is routinely assessed by sonographers, thus addressing the limitations of the current techniques. However, our findings suggest that CALM is not a strong indicator of arterial stiffness during early childhood. We found weak, inverse associations between retrograde and maximal CALM displacements and PWV, and no significant associations with carotid artery β-stiffness index (Chapter 4). This was the first published measurement of CALM in children and, as expected, CALM displacements (anterograde, retrograde, and maximal) were larger compared to values reported in adults (Au et al., 2017; Zahnd et al., 2012). In a companion paper to Chapter 4 (Study 3), we found that retrograde, diastolic, and maximal displacements increased over 12 months in this sample of children (Au, Proudfoot, Timmons, & MacDonald, 2019). This suggests that CALM displacements may increase in early childhood before beginning a progressive decline either later in childhood or adulthood. This pattern contrasts with our findings that arterial stiffness progressively increases in early childhood (Chapter 2) and continues to increase through adolescence and adulthood (Avolio et al., 1983; Diaz et al., 2018; Kucharska-Newton et al., 2019; Reusz et al., 2010). Thus, the elastic properties of the artery wall may not linearly and/or directly impact CALM displacements in early childhood. Together, the weak association between PWV and CALM and the contrasting pattern of CALM with age suggest that CALM should not be used as an alternative measure of arterial stiffness in young, healthy children. Although we found similar effects of time and MVPA on both PWV and carotid artery β-stiffness index (Chapter 2), carotid-to-dorsalis pedis PWV is the preferred measure of arterial stiffness in young children as this most closely matches the gold-standard measurement of arterial stiffness in adults (carotid-to-femoral PWV). A simple modification that could allow for more widespread use of PWV in young children is to determine PWV from ventricular depolarization (R-spike on ECG) to dorsalis pedis artery

(Currie et al., 2010), which eliminates the need to obtain and maintain a carotid pulse signal.

5.5 Conclusions

A life course approach to cardiovascular disease prevention is endorsed by a number of organizations, including the American Heart Association; however, while physical activity has been shown to be one of the best preventative measures for cardiovascular disease, there was previously little evidence to support that physical activity engagement could begin to prevent cardiovascular disease in early childhood. The findings in this thesis fill this gap in knowledge and demonstrate that the protective effects of physical activity on cardiovascular health begin in early childhood. Specifically, physical activity has beneficial effects on cardiovascular fitness, autonomic function, blood pressure, and arterial stiffness in early childhood, and the amount of physical activity recommended in current guidelines are insufficient for preschool-aged children to maintain favourable cardiovascular health. Based on the first empirically-derived physical activity targets for preschool-aged children, as determined in this thesis, preschool-aged children should engage in at least 4 hours of TPA or 80 minutes of MVPA per day to avoid unfavourable cardiovascular health. Further, the findings in this thesis highlight that MVPA has more pronounced effects on cardiovascular health than TPA during early childhood. Thus, while the interplay between physical activity intensity and volume on cardiovascular health during early childhood remains an area for further investigation, it may be reasonable for physical activity recommendations to focus on MVPA for health promotion. Overall, our findings suggest that physical activity has similar cardiovascular health benefits for boys and girls; however, there were some notable differences, particularly the lack of association between physical activity and blood pressure in boys, and the lack of physical activity threshold that discriminated between favourable and unfavourable cardiovascular health indicators for the boys. Future studies are needed to determine if and when an association emerges between physical activity and blood pressure in this cohort of boys, and to evaluate if the physical activity targets determined in this thesis are appropriate for preschool-aged boys. Finally, we found that arterial stiffness progressively increases during early childhood, and report, for the first time in children using device-measured physical activity, that this progressive increase can be slowed through engaging in higher levels of MVPA. This provides support for theories on vascular aging and demonstrates that the prevention of cardiovascular disease should begin early in life. Future research is required to determine if these favourable trajectories associated with higher levels of physical activity extend to later childhood and ultimately reduce cardiovascular disease risk in adulthood.

CHAPTER 6: REFERENCES

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Associations between carotid artery longitudinal wall motion and arterial stiffness indicators in young children

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