ARTERIAL STIFFNESS IN HEALTHY PRESCHOOL CHILDREN

ARTERIAL STIFFNESS DURING THE EARLY YEARS: RELATIONSHIP WITH ADIPOSITY AND PHYSICAL ACTIVITY

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ABSTRACT

Arterial stiffness is a natural and inevitable process for an ageing artery. In adults and school-aged children, increased stiffness of the central arteries is associated with cardiovascular disease (CVD) and CVD risk factors. Arterial stiffness, and its relationship with adiposity and physical activity (PA), has not been studied in preschool-aged children (3-5 years) despite the high prevalence of obesity and inactivity in this age group. Ninety-eight healthy preschoolers (4.4 ± 0.9 years; 50% boys) participated in this thesis, completing baseline and follow up assessments 12.5 ± 1.1 months apart. Whole-body PWV (carotid to dorsalis pedis; m/s) was used to assess arterial stiffness, body mass index percentile (BMI%ile) was a surrogate measure of adiposity, and PA levels (total, TPA; moderate-to-vigorous, MVPA) were quantified objectively with accelerometers and expressed as a percent of wear time. In our cohort, PWV increased significantly from baseline (4.3 m/s) to follow up (4.8 m/s; p< .001). PWV also tracked fair-to-moderately well (κ=0.25, r=0.37) with no sex differences (χ^2=.485, p=.785). Girls had a higher BMI%ile than boys, and the prevalence of overweight/obese preschoolers increased from 18.8% to 21.3% over the 1-year period. Boys were more active than girls and engaged in more MVPA. 75% of preschoolers at baseline, and 70% at follow up, met the current PA guidelines (3-hrs of TPA/day). PWV was not related to BMI%ile or PA at baseline; however, it was weakly related to TPA (r=-0.28, p=.013) and MVPA (r=-0.25, p=.024) at follow up. Furthermore, longitudinal and cross-sectional regression models of

sex, age, BMI%ile and TPA or MVPA could not predict PWV. Our findings indicate that adiposity and PA do not influence arterial stiffness in healthy 3 to 5 year old children. Nevertheless, maintaining a healthy body composition and engaging in regular PA has other health benefits and should be encouraged.

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"I can do all things through Christ who strengthens me"

~Philippians 4:13

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LIST OF ABBREVIATIONS

BIA   Bioelectrical impedance analysis

BF%   Body fat percentage

BMI   Body mass index

BMI%ile   BMI percentile

CAR   Carotid

CDC   Centers for Disease Control

CVD   Cardiovascular disease

DP   Dorsalis pedis

ECG   Electrocardiogram

MVPA   Moderate to vigorous physical activity

PA   Physical activity

PPG   Photoplethysmograph

PTT   Pulse Transit Time

PWV   Pulse wave velocity

SBP   Systolic blood pressure

TFM   Total fat mass

TPA   Total physical activity

WC   Waist Circumference

WT   Wear time

CHAPTER 1: LITERATURE REVIEW

1.1 INRODUCTION

Arterial stiffness is a progressive phenomenon whereby an artery becomes more rigid over time due to changes in its structural composition and elastic properties. Although the process is natural and inevitable for an ageing artery, it is accelerated by and associated with the development of cardiovascular disease (CVD) and CVD risk factors. It is well established that increased arterial stiffness has serious cardiovascular outcomes in adults (Arnett et al. 1994), and that ramifications are also seen in the health of school-aged children (Sakuragi et al. 2009). Although cardiovascular events do not manifest in the early years, atherosclerosis – the underlying cause of heart attacks and strokes – initiates in as early as the third year of life (Newman et al. 1991) and progresses 'silently' throughout childhood. Increased arterial stiffness may be unrelated to the cardiovascular health of 3 to 5 year old children, or it may be affected by and/or affecting atherosclerotic progression. While young children are generally presumed to be healthy, studies have reported high prevalences of obesity (Canning et al. 2004, Shields 2006) and inactivity (Pate et al. 2008), both of which are cardiovascular risk factors in adults (Thompson et al. 2003) and school-aged children (Kavey et al. 2003). Unfortunately, arterial stiffness and its relationship with adiposity and physical activity levels have yet to be studied in preschool-aged children (3-5 years); the aim of this thesis is to fill this current gap in the literature.

1.2 ARTERIAL STIFFNESS

Arterial stiffness is a term used to describe the rigidity of an artery (Mackenzie et al. 2002) or its resistance to deformation (Cavalcante et al. 2011). With every heartbeat, an artery adapts to increases in intra-arterial pressure by expanding and recoiling. The ability of an artery to expand with increases in pressure is partially determined by its structural composition, which differs along the arterial tree. Large arteries proximal to the heart have a high elastin to collagen ratio, with the aorta being the largest and most elastic (Oliver & Webb 2003). The high elastin content enables these central arteries to effectively cushion the pressure generated by ventricular contraction and ejection. Conversely, arteries further from the heart are relatively smaller in size and more muscular in nature (Laurent et al. 2006). The walls of these peripheral arteries have less elastin and are rich in collagen fibers and smooth muscle, as their primary function is to control blood flow distribution (Nichols & O'Rourke 2005). Nevertheless, arterial stiffness is more than just a term used to describe the structural differences between central and peripheral arteries. More importantly, arterial stiffness describes an ongoing and progressive phenomenon in which arteries become more rigid and resistant to deformation with ageing.

1.2.1 Factors Associated with Arterial Stiffness

Various factors contribute to increases in arterial stiffness, but ageing is perhaps the most apparent of them all; as individuals age so do their arteries. Age-related

endothelial damage is known to induce thickening and thus stiffening of the arterial wall (Lee & Oh 2010). Uniquely in central arteries, ongoing pressure-induced mechanical stress causes elastin fibers in the arterial wall to fray and fracture (Nichols 2005, Lee & Oh 2010). This structural degeneration of elastin, along with increases in collagen, results in increased central arterial stiffness. Although arterial ageing is a natural and inevitable process, it is accelerated with the development of CVD and CVD risk factors (Lee & Oh 2010). Accelerated stiffness of the ascending aorta and carotid arteries are of particular interest due to the clinical implications of a stiff pressure-buffering system. The aorta not only experiences more than a doubling of collagen content between the ages of 20 and 70 years (Nichols & O'Rourke 2005), but it is also prone to atherosclerosis (Oliver & Webb 2003, Laurent et al. 2006). As the aorta and other central arteries become more rigid, the left ventricle is forced to work harder, generating greater end-systolic pressure (Zieman et al. 2005). Elevated systolic pressure in turn leads to left ventricular hypertrophy (Lorell & Carabello 2000) and potential alteration of coronary artery perfusion (Chae et al. 1999), both of which are associated with adverse cardiovascular events.

1.2.2 Arterial Stiffness and Cardiovascular Disease

It is well established that increased stiffness of the central arteries is closely associated with CVD (Arnett et al. 1994), a leading cause of death worldwide (WHO 2013). Central arterial stiffness is not only linked with CVD risk factors

such as obesity (Ferreira et al. 2005), hypercholesterolemia (Wilkinson et al. 2002) and hypertension (Laurent et al. 2001), but it is also an independent risk factor for atherosclerosis (Wada et al. 1994, van Popele et al. 2001), coronary artery disease (Hirai et al. 1989, Weber et al. 2004) and stroke (Laurent et al. 2003). While cardiovascular outcomes manifest in adulthood, health implications of increased stiffness are already evident in childhood. Arterial stiffness is associated with subclinical atherosclerosis in school-aged children living with obesity (Celik 2011, Pandit et al. 2011), diabetes (Urbina et al. 2010, Wadwa et al. 2010), and end-stage renal disease (Covic et al. 2006). Furthermore, arterial stiffness is negatively correlated with cardiorespiratory fitness in healthy 10-year old children (Sakuragi et al. 2009). Evidently, increased stiffness is related to diminished cardiovascular health in both adults and school-aged children; however, it remains unclear at what age this relationship is established. Arterial stiffness and its ramifications have yet to be studied in preschool-aged children. However, with pathological indicators demonstrating that atherosclerosis can initiate in the early years (Newman et al. 1991), it may be that increased stiffness is associated with accelerated atherosclerotic progression in children as young as 3 to 5 years old. If so, there is potential for pediatricians to identify children at risk of future CVD earlier in life. Earlier identification would, in turn, allow for earlier implementation of preventative and/or interceptive measures.

1.2.3 Measuring Arterial Stiffness: Pulse Wave Velocity

As an artery stiffens, its ability to buffer pressure generated by ventricular contraction decreases, while the speed at which it conducts pressure waves increases (Cavalcante et al. 2011). Consequently, the speed of a pressure wave traveling between two arterial sites is a reflection of regional stiffness. This association is the theoretical basis of the arterial stiffness measure known as pulse wave velocity (PWV). PWV is expressed in meters/second and can be defined by the Moens-Korteweg equation (Nichols & O'Rourke 2005):

$$\text{Equation 1} \qquad PWV = \sqrt{\frac{hE}{2R\rho}}$$

Where h is the arterial wall thickness, E is Young's modulus of elasticity, R is the arterial radius and ρ is the blood density. However, a more practical and common measure of PWV involves dividing the distance between arterial sites by the transit time of the pressure wave (O'Rourke et al. 2002):

$$\text{Equation 2} \qquad PWV = \frac{Distance}{\Delta Time}$$

PWV can be measured non-invasively, making it ideal for use in children. Briefly, distance is estimated across the surface of the body with an anthropometric measuring tape, and transit time is determined from digitalized pressure waves obtained with transcutaneous transducers. Several superficial arteries have been established as suitable and standardized measurement sites for the assessment

of central and peripheral arterial stiffness. Central PWV is measured from the carotid to the femoral artery, while peripheral PWV can be measured in the upper (carotid to radial) or lower (femoral to dorsalis pedis) body (Laurent et al. 2006). A higher PWV is indicative of increased arterial stiffness (Cavalcante et al 2011). Due to the structural heterogeneity of the arterial tree, PWV differs between arterial segments, with values in healthy middle-aged adults increasing from 4-5 m/s in the ascending aorta to 5-6 m/s in the abdominal aorta and 8-9 m/s in the iliac and femoral arteries (Laurent et al. 2006). Furthermore, central arteries experience greater age-related increases in stiffness than do peripheral arteries, with PWV increasing up to 15 m/s in central arteries of older individuals (O'Rourke & Mancia 1999). Since the aorta is the best surrogate of coronary health, central PWV is considered the gold standard measurement of arterial stiffness (Laurent et al. 2006). Central PWV has been shown to be an independent predictor of cardiovascular morbidity and mortality in clinical populations (Blacher et al. 1999, Boutouyrie 2002, Meaume et al. 2001) and in the general population (Najjar et al. 2008, Mattace-Raso et al. 2006). These findings have led to the establishment of a clinical threshold of 12 m/s for central PWV, above which risk of cardiovascular events is considered elevated (Mancia et al. 2007). Furthermore, central PWV is a valid (Asmar et al. 1995) and reliable measure (Wilkinson et al. 1998) with acceptable coefficients of variation in both adults (3.2% carotid to femoral, 5.0% femoral to foot; Liang et al. 1998) and children (3.5%, ECG to foot; Currie et al. 2010).

1.3 ADIPOSITY

Deemed a worldwide epidemic, childhood obesity has been on the rise both nationally and internationally (Deckelbaum & Williams 2001, De Onis et al. 2010). In Canada alone, from 1981 to 1996, the prevalence of overweight 7 to 13 year olds increased from 15% to 28.7% in boys and 23.6% in girls. Over this 15-year period, obesity rates more than doubled from 5% to 13.5% in boys and 11.8% in girls (Tremblay & Willms 2000). In a younger cohort of 2 to 11 year olds, the 1998-1999 National Longitudinal Survey of Children and Youth reported that 19% of boys and 17% of girls were obese (Statistics Canada 2002). As for preschool children, Canning and colleagues (2004) found that more than 25% of 3.5 to 5.5 year olds were overweight or obese in a province-wide study. Similarly, the 2004 Canadian Community Health Survey indicated that 21.5% of 2 to 5 year olds were overweight (15.2%) or obese (6.3%) (Shields 2006). This is slightly lower than the prevalence of overweight or obese 2 to 5 year olds in the United States, which increased from 22% to 26.2% between 1999 and 2004 (Ogden et al. 2006). Evidently, increased adiposity is as much an issue for preschool-aged children as it is for school-aged children. Furthermore, 3 to 5 year old children who are obese are nearly eight times more likely than their healthy-weight peers to be obese as adults (Whitaker et al. 1997). Another study that followed 2 to 5 year old children for an average of 19 years, reported that 83% of obese preschoolers became obese adults (Freedman et al. 2005). The prevalence of overweight and obesity in children and its tracking into adulthood is of great

concern due to the associated health risks.

1.3.1 Adiposity and Arterial Stiffness

In both adults and school-aged children, surrogate measures of adiposity consistently show positive and moderate correlations with arterial stiffness. While there are several measures that can be used to assess stiffness, only studies using PWV are referenced. In both younger (20 to 40 years) and older (41 to 70 years) adults, central PWV significantly correlates with body mass index (BMI) and waist circumference (WC). Although the relationships among younger adults (BMI, r= 0.37; WC, r= 0.38) are stronger than those in older adults (BMI, r= 0.18; WC, r= 0.19), both groups maintain significant correlations independent of age, sex, ethnicity and systolic blood pressure (SBP) (Wildman et al. 2003). Similar associations between adiposity and stiffness are reported in children. In a large community-based study of school-aged children, central PWV positively correlated with BMI (r= 0.34), WC (r= 0.32) and body fat percent (BF%, r= 0.32), with all three measures also relating to arterial stiffness independently of age, sex, SBP, mean arterial pressure and heart rate (Sakuragi et al. 2009). Moreover, other studies have reported accelerated central stiffness in overweight and obese children relative to their normal-weight peers (Celik 2011, Pandit et al. 2011). Evidently, children with increased adiposity are at risk for accelerated arterial stiffness. Additionally, comorbidities associated with adiposity in adults (i.e. high blood pressure, dyslipidemia and type 2 diabetes) also manifest in pediatric

populations (Deckelbaum & Williams 2001) and track into adulthood (Lobstein et al. 2004). Consequently, overweight and obese children have a high risk of developing CVD. In the Bogalusa Heart Study, approximately 60% of overweight 5 to 10 year olds had one CVD risk factor, and more than 20% had at least two risk factors (Freedman et al. 1999). While the relationship between adiposity, arterial stiffness and CVD is complex and not fully understood, it appears to be mediated by inflammation (Berg & Scherer 2005). Adipose tissue has been demonstrated to directly contribute to systemic inflammation. Since hypercholesterolemia was once believed to be the pathogenic basis for atherosclerosis, increased inflammation was presumed to be a response to atherosclerotic progression. However, it is now understood that increased systemic inflammation actually mediates multiple pathogenic mechanisms associated with atherosclerosis, CVD and related comorbidities (Berg & Scherer 2005). Consequently, increased adiposity in children is associated with concurrent cardiovascular risk factors (Freedman et al. 2001) and is predictive of future morbidity and mortality (Must & Strauss 1999). Despite the rising obesity rates among 3 to 5 year olds, no studies to date have examined the relationship between adiposity and arterial stiffness in healthy preschool children.

1.3.2 Measuring Adiposity: BMI Percentile

The body mass index (BMI) is a measure of an individual's weight relative to their height squared (BMI = kg/m^2). Although BMI is not a direct measure of adiposity

and cannot distinguish between fat and lean mass, it has been validated in children against more direct measures (Pietrobelli et al. 1998, Mei et al. 2002, Eisenmann et al. 2004). Dual-energy X-ray absorptiometry (DXA) is a full-body low dose X-ray scan that differentiates between bone mineral, lean mass, and fat mass (Goran et al. 1998). Known to accurately measure regional and total body composition, DXA is commonly used as a reference method to validate anthropometric-based measures. In 3 to 8 year old children, BMI strongly correlates with DXA total fat mass (TFM, r= 0.85) and BF% (r= 0.75) (Eisenmann et al. 2004). These correlations are also strong in both 3 to 5 year old boys (TFM, r= 0.87; BF%, r= 0.80) and girls (TFM, r= 0.75; BF%, r= 0.78) (Mei et al. 2002). Despite the availability of more direct measures of adiposity, BMI is a widely used surrogate because of its simplicity, feasibility, and validity. Moreover, BMI percentile (BMI%ile) is the recommended measure in routine clinical practice for screening overweight and obesity in adults and children (Mei et al. 2002, Barlow 2007), as it accounts for age- and sex-based differences in body composition. During the early years, children experience an adiposity rebound, whereby BMI increases from birth to age 1 year, declines gradually until about age 6 years, and continues to increase once again after reaching a nadir (Rolland-Cachera et al. 1984). With regards to sex-differences, preschool girls have less lean mass and more fat mass than boys (Taylor et al. 2008). In this thesis, overweight and obese is defined according to the Centers for Disease Control (CDC) BMI cut-points. The CDC cut-points for children ages 2 to 20 years are as follows: underweight

(≤15th %ile), normal-weight (between the 16th and 84th %ile), overweight (between the 85th and 94th %ile) and obese (≥95th %ile) (Kuczmarski et al. 2002). These cut-points are based on sex-specific growth curves, which were developed from data from five nationally representative surveys conducted in the United States between 1963 and 1994 (Shields & Tremblay 2010). CDC BMI cut-points for defining obesity has moderate sensitivity (75%) and high specificity (96%) in school-aged children. This means that 75% of obese children are correctly classified as obese (BMI ≥95th %ile), and 96% of non-obese children are correctly classified as non-obese (BMI <95th %ile) (Boeke et al. 2013).

1.4 PHYSICAL ACTIVITY

Physical activity (PA) is defined as any body movement generated by skeletal muscles that results in energy expenditure above resting levels (Caspersen et al. 1985). In young children, play is considered to be a form of PA as it has components of vigorous intensity (Pellegrini & Smith 1998). In the early years, most of children's PA is in the form of unstructured play rather than structured activities (Tucker 2008). Currently, PA guidelines recommend that 3 to 4 year old children accumulate at least 3-hrs of PA at any intensity spread throughout the day, with progression towards at least 1-hr of energetic play by 5 years of age (Tremblay et al. 2012). In accordance to PA guidelines for school-aged children (Tremblay et al. 2011), energetic play is equivalent to moderate-to-vigorous PA (MVPA). In a nationally representative sample, 84% of Canadian 3 to 4 year olds

met the recommended 3-hrs of daily TPA, while only 14% of 5 year olds met the recommended 1-hr of daily MVPA (Colley et al. 2013). Furthermore, in a sample of Ontario preschoolers, ~12% of wear time (WT) was spent in MVPA (Obeid et al. 2011); however, other studies have reported lower proportions (<3%, Pate et al. 2008; 3.4%, Fisher et al. 2005; 9%, Colley et al. 2013). Discrepancies between studies can partly be attributed to methodology differences in measuring PA. To account for these differences, Bornstein and colleagues (2011) conducted a meta-analysis of PA levels among preschoolers using only accelerometer-derived data. Findings from the meta-analysis revealed that preschoolers engaged in MVPA an average of 42.8 min/day (5.5% of WT) with values ranging from 40 to 100 min/day depending on how MVPA was defined. Several studies have reported that among preschool children, boys are more active than girls and spend more time engaging in higher intensity activities (Finn et al. 2002, Fisher et al. 2005, Pate et al. 2008, Pfeiffer et al. 2009). Furthermore, Trost and colleagues (2003) found that overweight boys, but not girls, were less active than their non-overweight peers, while Tremblay & Willms (2003) showed support for the intuitive link between a child's PA levels and being overweight or obese. Developing an active lifestyle in the early years is particularly important, as PA is typically at its highest during those years, and progressively declines from about 6 years of age onwards (Malina 1996). The benefits of PA on multiple health measures (adiposity, cardiometabolic health, bone and skeletal health, psychosocial health, motor skill development, cognitive development) have been

reviewed in preschool children (Timmons et al. 2007, Timmons et al. 2012a) but are beyond the scope of this thesis. Only the relationship between PA and arterial stiffness (i.e. cardiovascular health) is addressed in the section to follow.

1.4.1 Physical Activity and Arterial Stiffness

It is well established that PA protects against and has the potential to treat CVD and related risk factors (Thompson et al. 2003). In adults, regular PA is associated with smaller increases in age-related arterial stiffness, and with improvements in arterial stiffness (Seals et al. 2008). There are both direct and indirect mechanisms in which PA can influence arterial stiffness. Directly, PA is thought to reduce arterial stiffness by increasing nitric oxide availability and improving endothelial function (DeSouza et al. 2000, Hambrecht et al. 2003, Fernhall & Agiovlasitis 2008). Indirectly, regular PA can protect against arterial stiffness by improving body composition and reducing adiposity (Tremblay et al. 1990, Klesges et al. 1995). Furthermore, PA can help treat comorbidities of adiposity such as elevated blood pressure, dyslipidemia, insulin resistance and glucose intolerance (Thompson et al. 2003). Not only do these disorders manifest in pediatric populations, but they have also shown significant correlations with atherosclerotic progression, even in young children (Must & Strauss 1999, Deckelbaum & Williams 2001, Ebbeling et al. 2002). Consequently, there has been a growing interest among researchers and clinicians in promoting PA in preschool children (Fitzgibbon et al. 2005, Bluford et al. 2007, Trost et al. 2008);

however, benefits of PA on the cardiovascular health of this young population has yet to be determined.

1.4.2 Measuring Physical Activity: Accelerometry

Accelerometers are small, lightweight and robust devices used to measure acceleration of body movement as a function of time (Chen & Bassett 2005). These devices measure PA duration, intensity and frequency patterns objectively, making them more appealing than self-reports, direct observations or pedometers (Pate et al. 2010). Furthermore, accelerometers can record acceleration in the longitudinal (up and down), anteroposterior (forwards and backwards) and mediolateral (side to side) axes (Cliff et al. 2009); however, this thesis focuses on analysis of vertical acceleration. Since accelerometers can only accurately measure movement of the body segment they are attached to, they are typically worn on an elastic belt over the right hip (Pate et al. 2004, Toschke et al. 2007). The hip is chosen because the trunk generates the greatest physical activity-related energy expenditure. A limitation of the hip placement is that the accelerometer will not measure non–weight-bearing activities such as cycling, movement of the upper limbs, or added energy expenditure from carrying a load (Cliff et al. 2009). For accurate assessment of habitual PA, 7 days of monitoring with 10 hrs of wear time per day was found to maximize reliability (r= 0.80); however, a minimum of 3 days was sufficient (Penpraze et al. 2006). PA does not differ between weekdays and weekends in preschool children (Jackson et al.

2003, Obeid et al. 2011). Accelerometers produce unit-less digital signals called *counts*, which are averaged over preset sampling intervals known as *epochs* (Chen & Bassett 2005). For counts to be meaningful, cut-points corresponding to PA intensities must be established by calibrating accelerometers against measures of energy expenditure or direct observation. This thesis uses cut-points validated for the ActiGraph accelerometer in healthy 3 to 5 year old children (Pate et al. 2006). Pate and colleagues measured energy expenditure during unstructured play and structured walking and running using indirect calorimetry and 15-sec epochs. Moderate-intensity activity corresponding to a VO_2 of 20 mL/kg/min, and differentiating between slow and brisk walking, was defined as 420 counts/15-sec. Vigorous activity corresponding to a VO_2 of 30 mL/kg/min, and differentiating between brisk walking and jogging, was defined as 840 counts/15-sec (Pate et al. 2006). It is important to note that cut-points used to distinguish between PA intensities are device-, epoch- and population-specific. Epoch lengths can vary from 3-sec to 60-sec; however, longer epoch-lengths often underestimate higher intensity activity (Vale et al. 2009, Obeid et al. 2011), which is more likely to combine with lower intensity activity and be misclassified (Cliff et al. 2009). This is particularly true of young children since their activity is short, sporadic, and intermittent in nature (Bailey et al. 1995, Obeid et al. 2011). Furthermore, compared to 3-sec epochs, 15-, 30- and 60-sec epochs miss 2.9 min (5.1%), 9.0 min (15.4%), and 16.7 min (26.8%) of preschool children's MVPA, respectively (Obeid et al. 2011). Furthermore, while PA variables can be

expressed in absolute terms (min/day), expressing them in relative terms (%WT) accounts for differences in accelerometer wear time.

1.5 STUDY RATIONALE

Increased adiposity and inactivity are associated with increased arterial stiffness, and in turn an increased risk of developing CVD and CVD risk factors. However, these relationships have only been established in adults and school-aged children. It is unknown if adiposity and physical activity influence the arterial stiffness of preschool-aged children in a similar manner.

1.5.1 Objectives

To fill the current gap in pediatric cardiovascular research, this thesis project will assess the natural progression of arterial stiffness in a group of healthy 3 to 5 year old children. The primary objective of this thesis is to track PWV over a 1-year period and to examine potential sex differences in tracking behaviour. The secondary objective is to examine whether BMI%ile and PA (TPA and MVPA) measures are able to predict arterial stiffness in preschool children.

1.5.2 Hypotheses

Arterial stiffness, adiposity and physical activity levels were assessed in preschool children to test the hypotheses that (i) PWV will increase and track strongly over a 1-year period, with no sex differences, and (ii) higher BMI%ile and lower PA levels are predictive of increased stiffness.

CHAPTER 2: METHODS

2.1 PARTICIPANTS

Ninety-eight healthy preschool children (ages 3-5 years) from the Health Outcomes and Physical activity in Preschoolers (HOPP) study (Timmons et al. 2012b) participated in this thesis (Table 1). Participants were recruited from local daycares, preschools and recreation facilities, and with the support of the Ontario Early Years Centers, the Hamilton Wentworth Catholic District School Board and the Halton Public School Board. Parents or guardians completed a medical questionnaire and provided written informed consent prior to their child's participation. Approval was granted by the Hamilton Health Sciences and McMaster University Faculty of Health Sciences Research Ethics Board.

Table 1. Participant Characteristics

	All Preschoolers	**Boys**	**Girls**
N	98	49	49
Age (years)	4.4 ± 0.9	4.5 ± 0.9	4.3 ± 0.9
Height (cm)	106.2 ± 7.8	108.1 ± 8.3	104.3 ± 6.9
Weight (kg)	17.9 ± 3.1	18.6 ± 3.6	17.2 ± 2.3

Data: Mean ± SD; Age: chronological (date of vascular testing – date of birth); boys were taller and weighed more than girls ($p < .05$).

2.2 STUDY DESIGN

This thesis was observational and longitudinal in nature. Participants completed the study protocol (Figure 1) at baseline (Year 1) and 12.5 ± 1.1 months later at follow up (Year 2). Testing occurred between August 2010 and January 2013. The protocol below is that of the HOPP study, with emphasis placed on measures used in this thesis.

Visit 1
Anthropometric measurements, (Fitness & motor skills)

7 Consecutive Days
Physical activity monitoring, (3 day nutrition log)

Visit 2
Vascular Assessment: PWV, (HR, carotid IMT, BP)
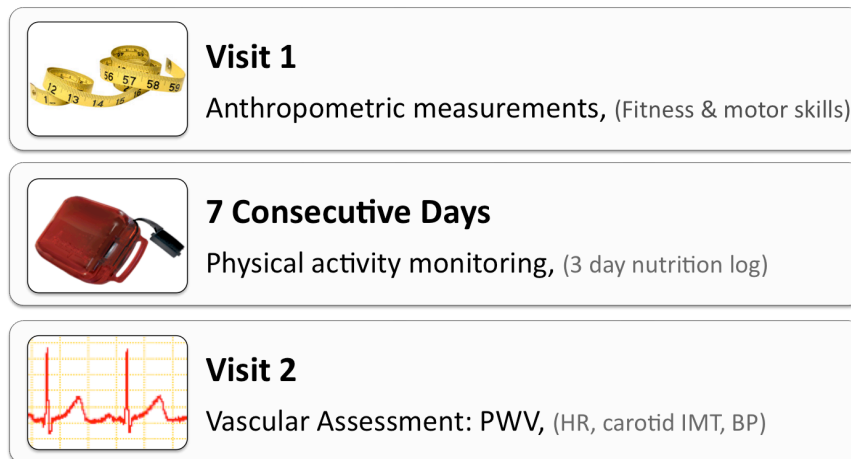
Figure 1. Study protocol

Each year participants completed two testing sessions scheduled at least one week apart (Year 1: 14 ± 8 days; Year 2: 20 ± 6 days). Participants first visited the Child Health & Exercise Medicine Program laboratory at Chedoke Hospital. During Visit 1, body composition measurements were obtained and the child was fitted with an accelerometer. The accelerometer recorded the child's physical activity for the next 7 consecutive days. During Visit 2, participants visited the Vascular Dynamics Laboratory at McMaster University where the accelerometer was returned and arterial stiffness was measured.

2.3 PULSE WAVE VELOCITY

As previously discussed, central PWV (carotid to femoral) is the gold standard measurement of arterial stiffness; however, in adaption to our young study population the more feasible measure of whole-body PWV (carotid to dorsalis pedis) was acquired. Previous pilot work has demonstrated the reliability of whole-body PWV in healthy 2 to 6 year old children (Currie et al. 2010). PWV was expressed in meters/ second according to Equation 2:

$$\text{Equation 2} \qquad PWV = \frac{\text{Distance}}{\Delta\text{Time}}$$

Where *Distance* was an estimate of the arterial path length, and *ΔTime* was the pulse (or pressure wave) transit time (PTT). Despite efforts from consensus papers (Laurent et al. 2006, Van Bortel et al. 2012) there remain several methods of measuring distance and PTT. The following sections describe the main instruments, techniques, and methods used for PWV analysis in this thesis. Prior to data collection, participants rested for 10 minutes in supine position in a temperature-controlled room (Year 1: 23.1°C, 44% RH; Year 2: 23.1°C, 40% RH). Additionally, a single-lead electrocardiogram (ECG) was used to record the heart's electrical activity throughout the session. Electrodes were positioned over the child's lower ribs, with the positive on the left side, and the negative and ground on the right side.

2.3.1 Applanation Tonometry

Applanation tonometry is a common technique used for the non-invasive acquisition of pressure waves for PWV analysis. A tonometer (Mikro-Tip Catheter Transducer, model SPT-301, Millar Instruments Inc., Houston, TX) is a handheld pressure transducer with a micromanometer probe tip (Davies and Struthers 2003). The tonometer was held over the strongest detectable pulse at the right common carotid artery. A slight hold-down pressure was applied, such that the artery was flattened against underlying structures and the intra-arterial pressure was transmitted to the tonometer tip (Mackenzie et al. 2002). The tonometer was connected to an interface unit (Transducer Control Unit, model TCB-600, Millar Instruments Inc., Houston, TX) that amplified, optimized and calibrated (1 V = 100 mmHg) the analogue signal before it was converted to a digital signal by the data acquisition system (PowerLab, model ML870, ADInstruments Inc., Colorado Springs, CO). The final output was a pressure waveform that was viewed and stored simultaneously with the ECG signal and analyzed on compatible computer software (LabChart 7, ADInstruments Inc., Colorado Springs, CO). In accordance with recommendations that data acquisition span at least one respiratory cycle (Van Bortel et al. 2002, Van Bortel et al. 2012), a minimum of 20 carotid signals was collected.

2.3.2 Infrared Photoplethysmography

Pulsatile blood flow can also be detected using an infrared photoplethysmograph (PPG) sensor (IR Plethysomograph, model MLT1020PPG, ADInstruments,

Colorado Springs, CO). While applanation tonometry detects intra-arterial pressure, photoplethysmography uses a built-in infrared light transmitter and photoelectric receiver to detect changes in blood volume. A Velcro strap was used to position the PPG sensor over the strongest detectable pulse at the dorsalis pedis (DP) artery of the participant's right foot – this allowed for simultaneous signal acquisition from the carotid and DP arteries. By connecting the PPG sensor directly into the data acquisition system (PowerLab, model ML870, ADInstruments Inc., Colorado Springs, CO), digital pressure waveforms from the DP were produced in the same LabChart file as the ECG and carotid pressure via tonometry signals.

2.3.3 Transit Time

Once pressure waveforms were acquired at the carotid and DP arteries using the aforementioned techniques, they were analyzed to determine the transit time. PTT is the visible time delay between an upstream (carotid) and downstream (DP) waveform, when the two are acquired simultaneously. Unfortunately, performing tonometry and photoplethysmography simultaneously was not always feasible, for instance when a child was fearful or simply uncooperative. In such cases the carotid and DP signals were collected sequentially. Regardless of how signals were acquired, PTT was calculated using Equation 3, as it relies on the time delay between ventricular depolarization, or ECG R-spike, and the arrival of the pressure waves at the carotid (ECG-CAR) and DP (ECG-DP) arteries.

Equation 3   PTT = (ECG-DP) – (ECG-CAR)

The forefront of a waveform was used to indicate the arrival of a pressure wave, and was identified by the *foot*, or minimum point at end diastole just before the systolic rise (McDonald 1968). Since the sharp inflection of the foot is comprised of high frequency components (Nichols and O'Rourke 2005), low and high frequency noise was eliminated. This was achieved by applying a digital band-pass filter to the signal (Munakata et al. 2003), with low and high frequency cut-offs of 5 Hz and 30 Hz, respectively. The minimum point of the filtered signal (Figure 2) corresponded to the foot of the waveform, and the time at which it occurred was identified accurately and objectively by the LabChart software.
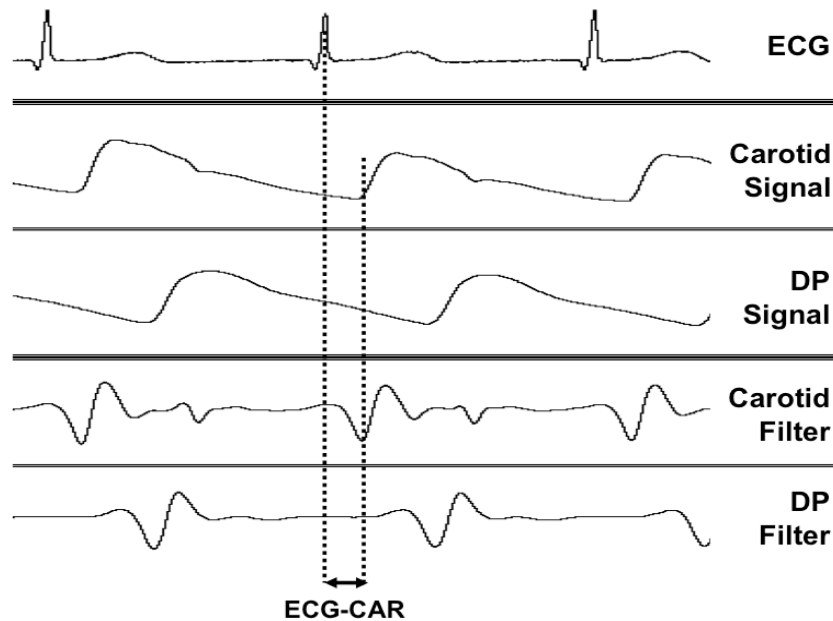
Figure 2. The use of digital band-pass filters to determine pulse transit time. The ECG-CAR transit time was determined as the time delay between ventricular depolarization (ECG R-spike) and the minimum point of the carotid filter. The same process was used to determine the ECG-DP transit time. PTT was then calculated using Equation 3, and expressed in seconds.

2.3.4 Distance

Distance was measured along the surface of the body with an anthropometric measuring tape. The subtraction method was used to estimate the arterial path length, as it accounts for the pressure waves traveling to the carotid and DP arteries simultaneously, and in opposite directions. Briefly, distance was measured from the carotid artery to the suprasternal notch (CAR-STN) and from the suprasternal notch to the DP artery (STN-DP), and calculated as follows:

Equation 4   Distance = (STN-DP) – (CAR-STN).

Prior to the commencement of the HOPP study, Weber and colleagues demonstrated that of various distance estimates, the subtraction method most closely agreed with the real distance measured invasively (Weber et al. 2009). More recently, Huybrechts and colleagues found that 80% of the total distance was the most accurate estimate of the real distance measured with magnetic resonance imaging (Huybrechts et al. 2011). The latest expert consensus, released after the initiation of data collection for this thesis, has since adopted this estimate as the new standard for daily practice (Van Bortel et al. 2012). It is important to note that both studies compared measurements specific to central PWV (carotid to femoral) in adults. There is currently no data to suggest that either estimate of distance is more appropriate for whole-body PWV in young children.

2.4 ADIPOSITY

Participants completed standard anthropometric measurements in light clothing and without shoes. Height was measured to the nearest 0.1 cm using a digital stadiometer. Weight was measured to the nearest 0.1 kg using a digital scale (BWB-800, Tanita Corporation, Japan). All measurements were taken in duplicate and averaged. Body mass index (BMI) was calculated as kg/m^2, and BMI percentile (BMI%ile) was calculated using sex- and age-based data from the Centers for Disease Control (CDC) (Kuczmarski et al. 2002). Participants were classified as overweight or obese if BMI%ile was between the 85th and 94th %ile or ≥95th %ile, respectively. Since BMI%ile is not a direct measure of adiposity, it was compared against waist circumference (WC) and body fat percent (BF%), two other body composition measurements collected in the HOPP study. WC was measured with an anthropometric measuring tape at three sites: 4cm above the naval, midway between the lower ribs and iliac crest, and at the iliac crest. BF% was determined from bioelectrical impedance analysis (BIA; RJL systems 101A, Miami, FL) and the following equation, where lean body mass was first estimated using total body water and a prediction equation validated in 4 to 6 year old children (Goran et al. 1993).

$$\text{Equation 5} \qquad BF\% = \frac{(\text{Body Weight} - \text{Lean Body Mass})}{\text{Body Weight}} \times 100$$

2.5 PHYSICAL ACTIVITY

Physical activity was quantified objectively using triaxial accelerometers (ActiGraph, models GT3X and GT3X+, Fort Walton Beach, FL). Although both the GT3X and GT3X+ models are similar in design and dimension (GT3X, 1.50"x 1.44"x 0.70" and 27 g; GT3X+, 1.81"x 1.30"x 0.59" and 19 g), the newer GT3X+ model is water resistant, withstanding submersion at a maximum depth of 1 meter for up to 30 minutes (ActiGraph 2012). The two models also show strong agreement and can be used interchangeably within a study (Robusto & Trost 2012). Accelerometers were attached to elastic belts and worn over the right hip for 7 consecutive days. Participants were instructed to put the accelerometer on each morning, and to take it off before any aquatic activities (e.g. swimming, bathing) and before bed. Parents or guardians were given a logbook to record times of wear and removal throughout the day, along with reasons for removal. Accelerometers were preset to record activity counts averaged over 3-sec epochs. For this thesis, only acceleration in the longitudinal plane was analyzed. The variables of interest were total physical activity (TPA) and moderate-to-vigorous physical activity (MVPA). Once accelerometers were returned, the data was uploaded to a Visual Basic for Applications (VBA) data reduction program in Microsoft Excel. The data was cleaned such that activity counts during non-wear times, as reported in the logbook, were deleted. Remaining counts were used to determine TPA and MVPA using cut-points validated for healthy 3 to 5 year old children (Figure 3; Pate et al. 2006).
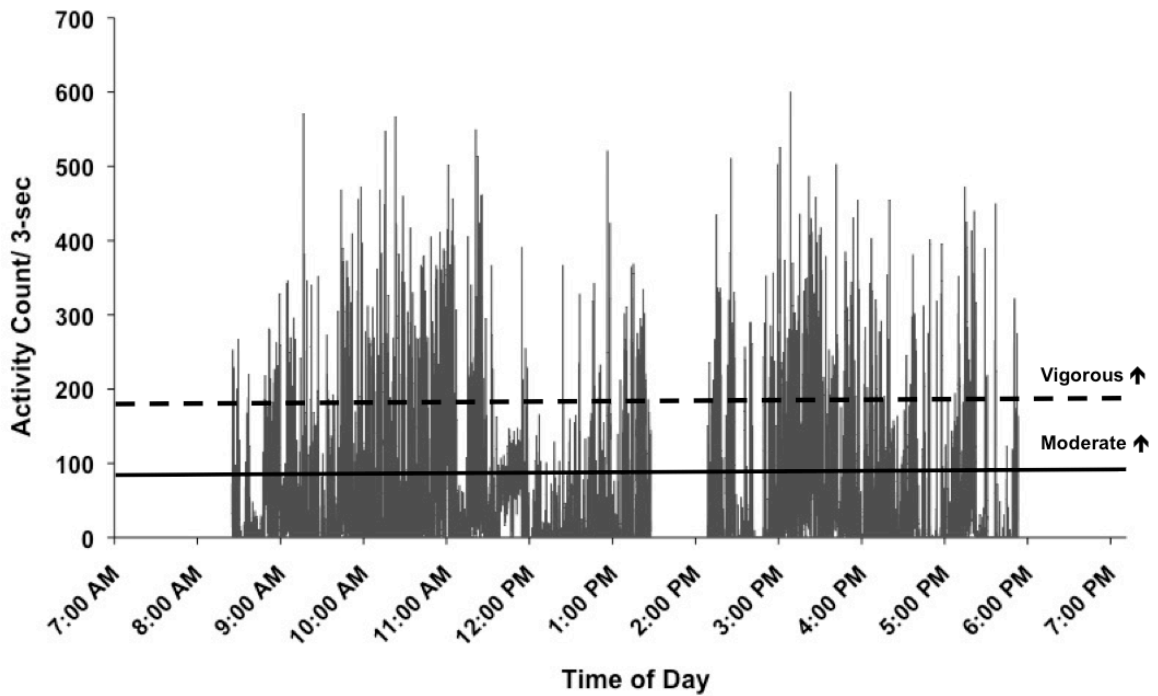
Figure 3. Sample accelerometer output.
Accelerometer activity count per 3-sec epoch is on the y-axis, while time of day is on the x-axis. Cut-points were modified from those established by Pate et al. (2006). The solid line (84 counts/3-sec) differentiates between light and moderate intensity PA, while the dashed line (168 counts/3-sec) differentiates between moderate and vigorous intensity PA.

To accommodate for Pate and colleagues using 15-sec epochs and this thesis using 3-sec epochs, we divided the cut-points by five. Therefore, activity counts were classified as TPA if ≥8 counts/3-sec and MVPA if ≥84 counts/3-sec. TPA and MVPA were reported in relative terms as a percent of total wear time to account for differences in wear time. Furthermore, participants were only included in the analyses if, at both baseline and follow-up, they wore the accelerometer for at least 3 days with a minimum wear time of 10 hrs/day.

2.6 STATISTICAL ANALYSES

Prior to analysis, data were screened for normality using the Shapiro-Wilk test and visual inspection of histogram plots. In the case of non-normal distributions, non-parametric tests were used where appropriate. Statistical analyses were performed using SPSS Statistics for Mac (SPSS Inc., Version 20.0, Chicago, IL), and significance was set at $p \leq 0.05$. Results are expressed as mean ± SD, unless otherwise noted.

2.6.1 Participant and Variable Characteristics

Participant and variable characteristics were analyzed using independent (sex differences) and dependent (year differences) t-tests. Relationships between variable were assessed using Pearson's correlations (r), and in the case of non-normally distributed variables Kendall Tau's (τ) correlations were used.

2.6.2 Primary Objective Analyses

To assess how PWV tracked from baseline to follow up, Spearman rank-order correlations and Kappa statistics were used. Tracking in this thesis refers to how well children maintained their relative rank within the group after a 1-year period. Kappa statistics differed from Spearman rank-order correlations in that children were not simply ranked according to ascending PWV values, but they were further categorized into tertiles (N/3; low, middle, high). Kappa statistics determined the strength of Year 1 and Year 2 categorization agreement. These tests were performed on the complete cohort of preschool children, as well as for

boys and girls separately. To determine sex differences in tracking behaviour, a chi-square tested the null hypothesis that Kappa agreement distributions were similar between boys and girls. Agreement groups were as follows: *Same* indicated the same Kappa tertile rank (low, middle, high) at both years, *Higher* indicated a higher rank at Year 2 (low → middle, or middle → high), and *Lower* indicated a lower rank at Year 2 (middle → low, or high → middle). Spearman correlations were interpreted as weak (0.1-0.3), moderate (0.3-0.5), or strong (> 0.5) (Cohen 1988). Kappa statistics were interpreted as poor (0.00-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), or almost perfect (0.81-1.00) (Landis & Koch 1977).

2.6.3 Secondary Objective Analyses

To test the strength of BMI (%ile) and PA (%WT) in predicting PWV, multiple regression analyses were performed on various models. For each model, the Enter method was used, and the following variables were included: sex, chronological age at baseline, BMI%ile, and one of TPA or MVPA. Various tests were used to ensure that assumptions of a linear model were met. The Durban-Watson test was used to check the independence of the residuals, while the variance inflation factor (VIF) and tolerance statistic were used to check for multicollinearity in the data. Furthermore, plots of the standardized predicted (y-axis) and standardized residual (x-axis) values were used to examine linearity and homoscedasticity. Lastly, visual inspection of histograms and normal probability plots (P-P Plots) verified that residuals were normally distributed.

Regression analyses were run using longitudinal as well as cross-sectional data.

2.6.4 Supplemental Analyses

Supplemental analyses were performed on select preschoolers who demonstrated extreme increases or decreases in PWV from baseline to follow up. This was done to maximize the likelihood of identifying between group differences that may be contributing to increased arterial stiffness in some preschoolers and decreased arterial stiffness in others. Three graphs were created to display the ΔPWV for each participant – one for the complete study cohort, one for boys, and one for girls. Participants on either extreme of the graph were represented with darker bars. Those with the greatest increases in PWV comprised the *PWV Up* group, while those with the greatest decreases in PWV comprised the *PWV Down* group. Independent t-tests were used to compare chronological age at baseline, PWV, BMI%ile, and PA levels between the two groups. The odds ratio (OR) was then used to assess whether being overweight/ obese (Exposure 1: Adiposity) or failing to meet PA guidelines (Exposure 2: Guidelines) influenced a child's risk of increased arterial stiffness (Outcome: PWV Increased). For a given exposure, the OR is a ratio of two probabilities, the probability of an event occurring (PWV Up) divided by the probability of an event not occurring (PWV Down). The OR was interpreted as follows: exposure does not affect outcome (OR=1), exposure increases likelihood of outcome (OR>1), or exposure decreases likelihood of outcome (OR<1). This analysis was performed for both exposures at Year 1 and Year 2.

CHAPTER 3: RESULTS

3.1 VARIABLE CHARACTERISTICS

The following sections explore changes in PWV (3.1.1), BMI%ile (3.1.2), PA (3.1.3), and the relationship between all three variables (3.1.4). Where appropriate, potential sex differences were examined. All ninety-eight preschool children in this thesis had complete vascular data at baseline and follow up; however, only eighty children also had complete adiposity and PA data sets.

3.1.1 Longitudinal Changes in PWV

For the complete cohort of preschool children (All, N=98), as well as for each sex separately (Boys, N=49; Girls, N=49), PWV increased significantly over a relatively short period of 12.5 ± 1.1 months (Figure 4, p< .001). No sex differences were observed in either the increase in PWV (ΔPWV, p= .372) or in absolute values at Year 1 (p= .798) and Year 2 (p= .275).
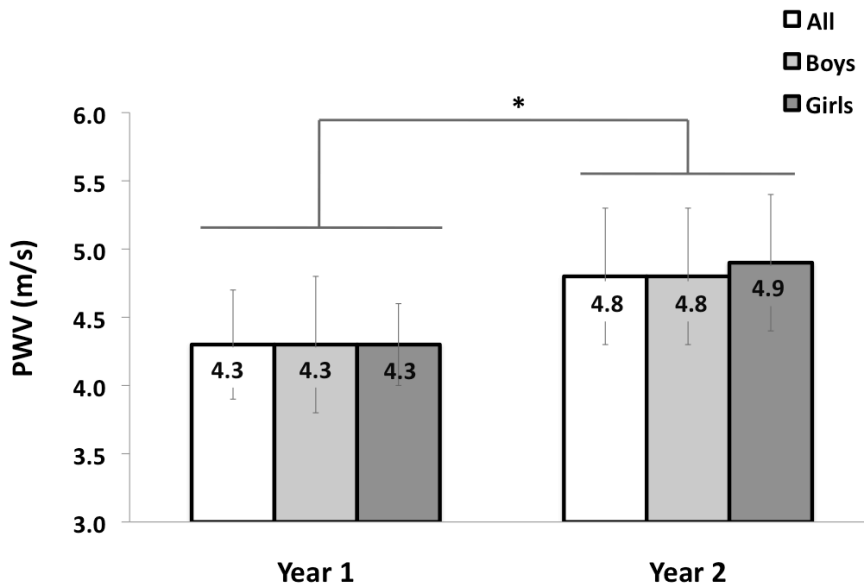
Figure 4. Increases in PWV after a 1-year period

3.1.2 BMI Percentile

The correlation coefficients between BMI%ile, waist circumference (WC), and body fat percent (BF%) are reported in Table 2. WC and BF% are two other body composition measurements collected in the HOPP study that can be used as indices for adiposity.

TABLE 2. Bivariate Correlations Between BMI%ile and Other Body Fat Variables

		BMI%ile	WC 4cm	WC mid	WC iliac	BIA BF%
BMI%ile	*Year 1*	1	.493**	.509**	.509**	.297**
	Year 2		.523**	.536**	.571**	.371**
WC 4cm	*Year 1*		1	.893**	.789**	.008
	Year 2			.893**	.816**	.118
WC mid	*Year 1*			1	.841**	.030
	Year 2				.879**	.157*
WC iliac	*Year 1*				1	.079
	Year 2					.200**
BIA BF%	*Year 1*					1
	Year 2					

Kendall's Tau Correlations; WC: waist circumference; BIA: bioelectrical impedance analysis; BF%: body fat percent; Significance (2 tailed): (*) $p < .05$; (**) $p < .01$

BMI%ile was strongly correlated with all three WC measures (4cm, mid, iliac) at Year 1 and Year 2. More importantly, BMI%ile was the measure that most strongly correlated with BF% (Year 1, τ= .297; Year 2, τ= .371). These relationships in our cohort enabled us to confidently use BMI%ile in our analyses as an indicator of adiposity. The average BMI%ile for this study population is

presented in Table 3, along with a breakdown of the number of children in each weight classification.

Table 3. Adiposity Variables at Year 1 and Year 2

Variable	Year	All (N=80)	Boys (N=42)	Girls (N=38)	P value
BMI (kg/m^2)	Year 1	15.9 ± 1.0	15.8 ± 1.0	16.0 ± 1.1	.382
	Year 2	15.9 ± 1.3	15.7 ± 1.4	16.1 ± 1.2	.223
BMI%ile	Year 1	58 ± 25	52 ± 26	63 ± 22	.044
	Year 2	58 ± 24	51 ± 25	65 ± 21	.008
Healthy, Overweight, Obese (N)	Year 1	65, 11, 4	36, 3, 3	29, 8, 1	-
	Year 2	63, 12, 5	36, 3, 3	27, 9, 2	-

Girls had a significantly higher BMI%ile than boys at both baseline (p= .044) and follow up (p= .008). Moreover, 21% of girls were classified as being overweight at Year 1 compared to only 7% of boys. This was similar at Year 2 with a slight increase in the percent of overweight girls (24%) but no change in the percent of overweight boys. On the other hand, obesity was consistently higher in boys (Years 1 and 2: 7%) than in girls (Year 1: 3%; Year 2: 5%). Moreover, while the average BMI%ile for the group remained the same from baseline to follow, changes (ΔBMI%ile = Year 2 BMI%ile – Year 1 BMI%ile) were evident for individual participants. After 1 year, 47.5% of preschoolers had a lower BMI%ile, 7.5% had the same BMI%ile, and 45% had a higher BMI%ile (Figure 5).
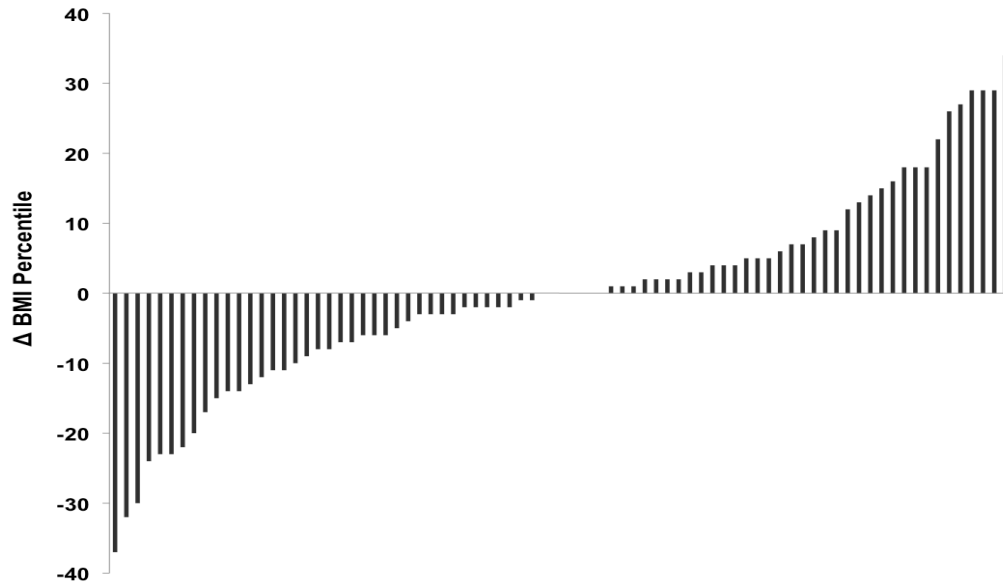
Figure 5. Individual ΔBMI%ile for all preschoolers (N=80)

3.1.3 Physical Activity

Table 4. PA Variables at Year 1 and Year 2

Variable	Year	All (N=80)	Boys (N=42)	Girls (N=38)	P value
WT (hr/day)	Year 1	12 ± 1	12 ± 1	12 ± 1	.426
	Year 2	12 ± 1	12 ± 1	12 ± 1	.387
TPA (%WT)	Year 1	37 ± 5	38 ± 4	35 ± 4	.003
	Year 2	36 ± 4	38 ± 4	35 ± 4	.002
MVPA (%WT)	Year 1	13 ± 3	14 ± 3	12 ± 3	.001
	Year 2	14 ± 3	15 ± 3	13 ± 3	.001

With regards to characterizing the accelerometer data from Year 1 and Year 2 (Table 4), both boys and girls wore the device for approximately 12 hours per day. Of those hours, boys were physically active (TPA) for an average of 4.5 hours, while girls were physically active for 4.1 hours. The 20-min difference

between sexes was statistically significant. Sex differences were also apparent in the percentage of preschool children meeting the Canadian Physical Activity Guidelines for the Early Years (Tremblay et al. 2012). At baseline, 83% of boys and 66% of girls met the recommended 3 hours of daily PA at any intensity (Cohort Average, 75%). The difference between sexes was even greater at follow up with 81% of boys but only 58% of girls meeting the recommendation (Cohort Average, 70%). Boys also spent more time engaging in higher intensity activities (close to 2 hours of MVPA) than did girls (1.5 hours). Lastly, group averages for TPA and MVPA did not change over the 1-year period. Nevertheless, from baseline to follow up, TPA (%WT) decreased in 49% of preschoolers, stayed the same in about 3% of preschoolers, and increased in 48% of preschoolers (Figure 6, Appendix C). Similarly, MVPA (%WT) decreased in 39% of preschoolers, stayed the same in 2% of preschoolers, and increased in 59% of preschoolers (Figure 7, Appendix C).

3.1.4 Correlations Between Variables

Bivariate correlations assessed the relationship between chronological age, anthropometric, physical activity, and vascular measures. At Year 1 (Table 5), BMI%ile was moderately and significantly correlated with weight (т= .369). MVPA, but not TPA, was weakly correlated with age (т= .200). MVPA was also significantly correlated with height (r= .315) and weight (т= .231); however, these relationships were no longer significant when age was accounted for (partial

correlation data not shown). More importantly, there was no relationship between PWV and any of the variables.

TABLE 5. Bivariate Correlations Between Year 1 Variables

	Age	1-Height	1-Weight	1-BMI%ile	1-TPA	1-MVPA	1-PWV
Age	1	.633**	.515**	.052	.119	.200*	.072
1-Height		1	.755**	.124	.196	.315**	.156
1-Weight			1	.369**	.153*	.231**	.111
1-BMI%ile				1	.030	-.037	.054
1-TPA					1	.875**	-.081
1-MVPA						1	.007
1-PWV							1

Age at baseline (yrs), Height (cm), Weight (kg), TPA and MVPA (%WT), PWV (m/s); Non-normal data: Age, Weight, BMI%ile; Significance (2 tailed): (*)$p < .05$, (**)$p < .01$

At Year 2 (Table 6), BMI%ile continued to be moderately correlated with weight (τ = .399). The relationships between MVPA and age, height, and weight, however, were weaker and no longer significant. The relationship between PWV and all other variables also remained weak; nevertheless, the negative correlations between PWV and TPA ($r = -.277$, $p = .013$) and MVPA ($r = -.253$, $p = .024$) were statistically significant.

TABLE 6. Bivariate Correlations Between Year 2 Variables

	Age	2-Height	2-Weight	2-BMI%ile	2-TPA	2-MVPA	2-PWV
Age	1	.574**	.489**	.047	-.021	.083	-.123
2-Height		1	.753**	.147	.022	.191	-.021
2-Weight			1	.399**	.019	.092	-.014
2-BMI%ile				1	-.030	-.092	.013
2-TPA					1	.824**	-.277*
2-MVPA						1	-.253*
2-PWV							1

Age at baseline (yrs), Height (cm), Weight (kg), TPA and MVPA (%WT), PWV (m/s); Non-normal data: Age, Weight, BMI%ile; Significance (2 tailed): (*)$p < .05$, (**)$p < .01$

Lastly, correlations between how variables changed from baseline to follow up were examined (Table 7, Appendix D). The only significant correlation of interest was between Δ-BMI%ile and Δ-Weight (τ= .509), whereby a greater change in weight from Year 1 to Year 2 was strongly related to a greater change in BMI%ile. As for Δ-PWV, it was not related to age at baseline, nor was it related to changes in anthropometric or physical activity measures.

3.2 PRIMARY OBJECTIVE

To track PWV in healthy preschool children (3-5 years) over a 1-year period, where tracking is defined as maintaining a relative rank within a group over time, and to examine potential sex differences in PWV tracking behaviour.

3.2.1 Tracking PWV

For all three groups, PWV tracking strength was moderate according to the Spearman correlations, and fair as per the Kappa statistics (Table 8).

TABLE 8. Tracking PWV from Year 1 to Year 2

		Spearman Correlations			Kappa Statistics		
	N	r	P value	Strength	κ	P value	Strength
All	98	.373	.000	Moderate	.250	.000	Fair
Boys	49	.341	.016	Moderate	.265	.009	Fair
Girls	49	.401	.004	Moderate	.326	.001	Fair

3.2.2 Sex Differences in Tracking Behaviour

Despite the same strength classification between sexes, there was a trend for PWV to track slightly stronger in girls than in boys. To examine this statistically, Spearman correlation coefficients were not compared directly. Instead, a chi-square test compared the observed and expected distributions of boys and girls in each Kappa agreement group (Same, Higher, Lower) (Table 9, Appendix E). The chi-square test revealed no sex differences in PWV tracking behaviour (χ^2= .485, p= .785).

3.3 SECONDARY OBJECTIVE

To determine whether adiposity and physical activity levels can be used to predict PWV, and thus arterial stiffness, in preschool children.

3.3.1 Predicting PWV with Longitudinal data

Multiple regression (Enter method) was used to assess whether Year 1 and Δ BMI%ile and PA measures were able to predict Year 2 PWV (Table 10).

TABLE 10. Predicting Year 2 PWV from Year 1 and Delta Variables

	R	R^2	$R^2_{adjusted}$	$\beta_{Unstandardized}$	$\beta_{Standardized}$	P value
Model 1	**.183**	**.034**	**-.018**			**.627**
Constant				5.594		.000
Sex				.000	.000	.998
Age				-.084	-.142	.225
1-BMI%ile				.000	-.022	.850
1-TPA				-.010	-.091	.456
Model 2	**.171**	**.029**	**-.023**			**.689**
Constant				5.367		.000
Sex				-.012	-.012	.924
Age				-.081	-.138	.248
1-BMI%ile				-.001	-.032	.785
1-MVPA				-.010	-.058	.644
Model 3	**.253**	**.064**	**.014**			**.283**
Constant				5.327		.000
Sex				-.015	-.015	.896
Age				-.111	-.190	.104
ΔBMI%ile				.002	.050	.655
ΔTPA				-.023	-.193	.095
Model 4	**.247**	**.061**	**.011**			**.310**
Constant				5.337		.000
Sex				-.020	-.020	.861
Age				-.107	-.183	.115
ΔBMI%ile				.002	.044	.697
ΔMVPA				-.035	-.183	.111

All four models incorporated sex and chronological age at baseline as predictors. Year 1 measures of BMI%ile and PA (TPA & MVPA) were the other predictors in models 1 and 2, while the changes in those measures from baseline to follow up comprised models 3 and 4. Since TPA quantifies all activity levels, including moderate and vigorous intensities, separate models were created for TPA (models 1 and 3) and MVPA (models 2 and 4) to avoid issues of multicollinearity. Evidently, none of the longitudinal models using baseline and delta variables were able to predict PWV at follow up (p> .05). Models 1 and 2 accounted for 3.4% and 2.9% of the variability in Year 2 PWV, respectively. Models 3 and 4 were only slightly better at explaining the variance with R^2 = 6.4% and 6.1%, respectively. Furthermore, in all four models the adjusted R^2 is even lower, indicating that the models explain less of the PWV variance when the number of predictors is accounted for.

3.3.2 Predicting PWV with Cross-sectional data

Multiple regression (Enter method) was also performed using cross-sectional data. Two models assessed whether Year 1 BMI%ile and PA measures were able to predict Year 1 PWV (Table 11). The analysis was repeated with the follow up data to examine the strength of Year 2 BMI%ile and PA measures in predicting Year 2 PWV (Table 12, Appendix E).

TABLE 11. Predicting Year 1 PWV from Year 1 Variables

	R	R^2	$R^2_{adjusted}$	$\beta_{Unstandardized}$	$\beta_{Standardized}$	P value
Model 1	.173	.030	-.022			.678
Constant				4.382		.000
Sex				.096	.125	.323
Age				.034	.078	.505
1-BMI%ile				.001	.068	.566
1-TPA				-.012	-.137	.265
Model 2	.123	.015	-.037			.884
Constant				4.085		.000
Sex				.071	.092	.469
Age				.033	.075	.534
1-BMI%ile				.001	.054	.652
1-MVPA				-.005	-.042	.739

All models incorporated sex and chronological age at baseline. TPA was the PA predictor in model 1, and MVPA was the predictor in model 2. At baseline, BMI%ile and TPA (Table 11, model 1) explained only 3.0% of the variance in Year 1 PWV, while BMI%ile and MVPA (Table 11, model 2) explained 1.5% of the variance. At follow up, BMI%ile and TPA (Table 12, model 1) explained 11.1% of the variance in Year 2 PWV, while BMI%ile and MVPA (Table 12, model 2) explained 8.5% of the variance. Similar to the longitudinal models, none of the cross-sectional models were significant (p> .05).

3.4 SUPPLEMENTAL ANALYSES

This section examined how PWV changed for each individual child (3.4.1). Preschoolers who demonstrated extreme increases and decreases in PWV over the 1-year period were selected for further statistical analyses (3.4.2 and 3.4.3).

3.4.1 Individual ΔPWV Responses

Each participant's ΔPWV from baseline to follow up is displayed in Figure 8. Similar changes were observed for each sex separately (Figures 9 and 10; Appendix F). Dark bars highlight the 20 participants with the greatest changes in PWV. Highlighted individuals with a positive ΔPWV, or the *PWV Up* group, had the greatest increases in arterial stiffness. Conversely, highlighted individuals with a negative ΔPWV, or the *PWV Down* group, had the greatest reductions in arterial stiffness.
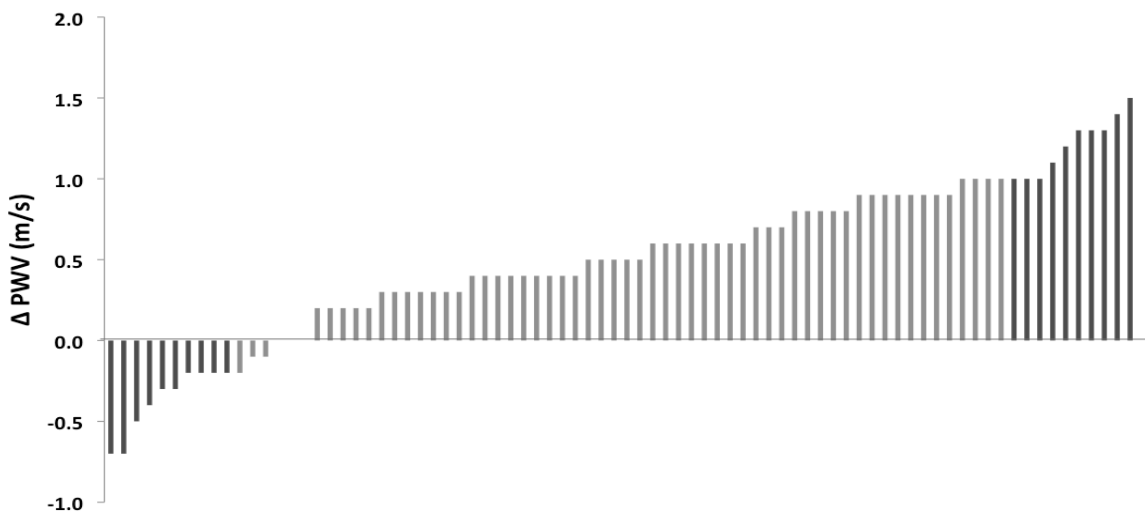
Figure 8. Individual ΔPWV for all preschoolers (N=80)
Dark bars represent the extremes: 10 Up, 10 Down.

3.4.2 Comparing Extreme Responders

T-tests compared the *PWV Up* and *PWV Down* groups for their age at baseline, as well as for the following Year 1, Year 2 and Δ variables: PWV, BMI%ile, TPA and MVPA (Table 13, Appendix F). Of these variables, Year 1 PWV, Year 2 PWV, and ΔPWV were the only variables that differed significantly between the two groups. Only the combined cohort is displayed in Figure 11, as boys and girls did not differ and displayed the same interaction as seen in the figure below.
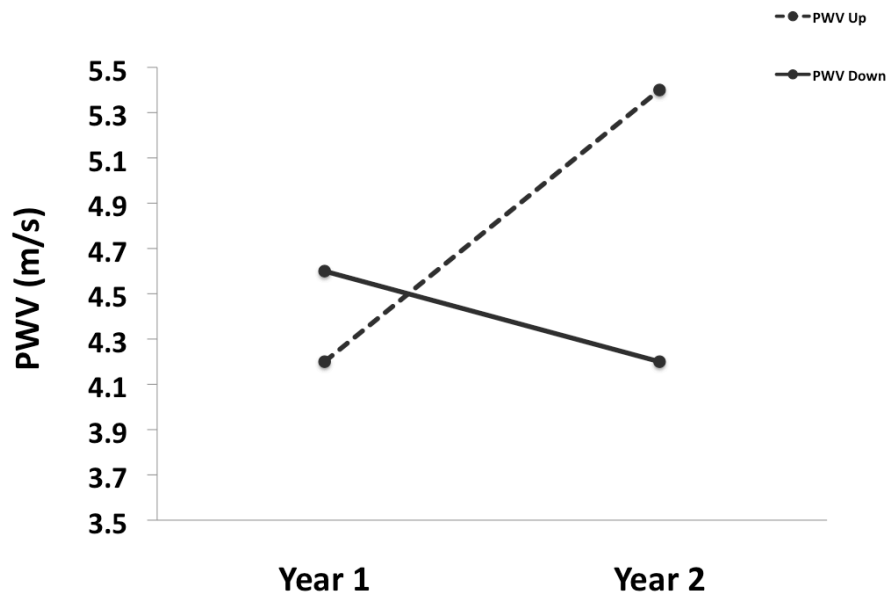
Figure 11. PWV differences between Extreme Groups

Preschoolers in the *PWV Up* group had a lower baseline PWV (4.2 m/s) than those in the *PWV Down* group (4.6 m/s). Over the 1-year period, PWV increased an average of 1.2 m/s in the *PWV Up* group but decreased by only 0.4 m/s in the

PWV Down group. These differences could not be attributed to BMI%ile, TPA, or MVPA as these variables did not differ between the two groups (p> .05).

3.4.3 Assessing Odds Ratios

Continuing with the same groups, *PWV Up* and *PWV Down*, the odds ratio (OR) was used to assess whether being overweight/obese (Exposure 1: Adiposity) or failing to meet PA guidelines (Exposure 2: Guidelines) influenced a child's risk of increased arterial stiffness (Outcome: PWV Increased). This analysis was performed for both exposures at Year 1 (Table 14) and Year 2 (Table 15). For Year 1 exposures, the OR was 1.78 for Adiposity (95% CI, 0.36 to 8.85) and 1.00 for Guidelines (95% CI: 0.28 to 3.54).

TABLE 14. Year 1 Odds Ratios

		95% CI	
	Odds Ratio	**Lower**	**Upper**
1-Adiposity	**1.78**	**0.36**	**8.85**
	PWV Increased	PWV Decreased	Total
Overweight/Obese	13	2	15
Healthy	51	14	65
Total	64	16	80
1-Guidelines	**1.00**	**0.28**	**3.54**
	PWV Increased	PWV Decreased	Total
No	16	4	20
Yes	48	12	60
Total	64	16	80

Adiposity (BMI%ile classifications): Healthy (< 85%ile), Overweight (between 85%ile and 95%ile), Obese (≥ 95%ile); Guidelines: Met PA guidelines (3 hours/day)

For Year 2 exposures, the OR was 5.00 for Adiposity (95% CI: 0.61 to 40.91) and 2.12 for Guidelines (95% CI: 0.54 to 8.24).

TABLE 15. Year 2 Odds Ratios

		95% CI	
	Odds Ratio	**Lower**	**Upper**
2-Adiposity	**5.00**	**.61**	**40.91**
	PWV Increased	PWV Decreased	Total
Overweight/Obese	16	1	17
Healthy	48	15	63
Total	64	16	80
2-Guidelines	**2.12**	**.54**	**8.24**
	PWV Increased	PWV Decreased	Total
No	21	3	24
Yes	43	13	56
Total	64	16	80

Adiposity (BMI%ile classifications): Healthy (< 85%ile), Overweight (between 85%ile and 95%ile), Obese (≥ 95%ile); Guidelines: Met PA guidelines (3 hours/day)

The OD for both exposures – overweight/obesity and failure to meet PA guidelines – was higher at follow up (Year 2) than at baseline (Year 1). Nevertheless, neither exposure significantly influenced a child's risk of increased arterial stiffness, at baseline or follow up, as indicated by the wide confidence intervals spanning 1.

CHAPTER 4: DISCUSSION

4.1 ARTERIAL STIFFNESS IN PRESCHOOL CHILDREN

This thesis was the first to establish normative whole-body PWV (carotid to DP) values for healthy 3 to 5 year old children, and to assess age-related increases in arterial stiffness in the early years. With an understanding of 'normal' progression of arterial stiffness in healthy preschoolers, researchers can identify how much, if at all, clinical preschool populations differ. Any differences would indicate disease-accelerated arterial stiffness rather than simply age-related arterial stiffness. Furthermore, by tracking whole-body PWV over a 1-year period, this thesis was the first to examine its stability as a measure of arterial stiffness in this young population.

4.1.1 Longitudinal Changes in PWV

In our cohort of preschool children, average whole-body PWV was 4.3 m/s at baseline (ages 3-5 years) and 4.8 m/s at follow up (ages 4-6 years). This 0.5 m/s increase over a 1-year period was significant and similar between sexes. No reference values currently exist as whole-body PWV is not commonly measured in previous literature. It was first used by our group in a pilot study that tested the reliability of non-invasive vascular measures in twenty preschool children (ages 2-6 years). In the pilot study, PWV was 3.5 m/s; however, it was measured from ECG R-spike to the DP artery (Currie et al. 2010). Since carotid to femoral PWV has conclusively proven to be an independent predictor of CVD in adults, we modified the measure for this thesis to include the carotid artery. In this way, the

new definition of whole-body PWV (carotid to DP) more accurately reflects whole-body arterial stiffness, since it combines central (carotid to femoral) and peripheral (femoral to DP) segments. Unfortunately, we are aware of only two other studies assessing the same measure, which the authors term foot PWV. Both studies used data from the Georgia Cardiovascular Twin Study, and reported PWV values near 7.0 m/s in adolescents (mean age: 17.6 years) (Zhu et al. 2007, Zhu et al. 2008). Regarding the 0.5 m/s increase in arterial stiffness from baseline to follow up, no studies have reported year-to-year changes in whole-body PWV. Nevertheless, annual progression of central PWV has been calculated in adults. Central PWV increased 0.15 m/s per year in hypertensive adults and 0.08 m/s per year in normotensive adults (Benetos et al. 2002). In 20 to 40 year olds, Wildman et al. (2005) reported annual changes in central PWV ranging from -0.15 m/s per year to 0.19 m/s per year, with decreases and increases in central PWV strongly associating with weight loss and weight gain, respectively. Therefore, it would appear that the magnitude of ΔPWV seen in this thesis is higher than what has been reported by other studies; however, no concrete conclusions can be drawn from comparisons made between whole-body PWV in preschoolers and central PWV in adults. Furthermore, this thesis presumes that age-related arterial stiffness is responsible for the 0.5 m/s increase in whole-body PWV, but it is plausible that other factors also contributed to this increase. Arterial stiffness is not only influenced by structural changes in the arterial wall, but also by changes in heart rate (Lantelme et al. 2002), blood

pressure and smooth muscle tone (Mottram et al. 1999), all of which are influenced by sympathetic neuronal activity and/or endothelial function. Heart rate and blood pressure are not reported in this thesis; however, their inclusion could elucidate as to which physiological factors contributed to the longitudinal change in whole-body PWV seen in this cohort of preschool children.

4.1.2 Tracking PWV

Tracking is defined as the maintenance of a relative rank or position within a group over time, and describes the stability of a characteristic (Malina 1996). It is often times used to understand the predictability of chronic disease risk factors or growth parameters (Twisk et al. 1994). A stable characteristic – one that tracks well – demonstrates a positive relationship over time such that early measures are predictive of values later in life (Twisk et al. 1994). In our study population, PWV tracked moderately well according to the Spearman rank-order correlation (r= .373) and fair according to the Kappa Statistic (κ= .250). Cohen's kappa likely produced a lower tracking coefficient because it is not based on changes in individual rankings over time, but rather on movements between tertile groups. In this way, any change from one tertile group to another is weighted equally regardless of the magnitude of that change (Twisk et al. 1994). Between the two analyses, Spearman's correlation was most consistent with values reported for the tracking of SBP (r= 0.38-0.43) in preschool-aged children (De Swiet et al. 1992, Chen & Wang 2008). Furthermore, PWV and SBP, both indices of

cardiovascular health, appear to track slightly weaker than BMI (r= 0.46-0.53) in the early years (Monyeki et al. 2008). Nevertheless, the moderate tracking strength of PWV suggests that it is a stable and predictable measure in healthy 3 to 5 year old children. This finding established the foundation for our secondary objective, in which we examined the potential role of BMI%ile and PA levels in predicting PWV.

4.2 ARTERIAL STIFFNESS, ADIPOSITY & PHYSICAL ACTIVITY

Although arterial stiffness is an inevitable process, increased adiposity and physical inactivity are known CVD risk factors associated with atherosclerotic progression (World Heart Federation 2013); however, evidence for this is limited to adults and school-aged children. While adiposity and PA levels have been previously examined in preschool children, this thesis is the first to assess their relationship with arterial stiffness, an index of cardiovascular health. Using multiple regression analyses, this thesis examined the potential for BMI%ile, TPA and MVPA to predict whole-body PWV in healthy 3 to 5 year olds. Furthermore, in supplemental analyses, this thesis was the first to compare the adiposity and activity levels of preschool children who demonstrated extreme changes in PWV, and to assess whether being overweight/obese (BMI ≥ 85th %ile) or failing to meet PA guidelines (TPA < 3 hrs/day) influenced a child's risk of increased PWV. In this thesis, the prevalence of overweight/obese preschoolers (18.8% at baseline, 21.3% at follow up) was comparable to a nationally representative

sample of Canadian preschool children (21.3%; Shields 2006), while compliance with PA guidelines (75% at baseline, 70% at follow up) was lower (84%; Colley et al. 2013).

4.2.1 Predicting PWV

All longitudinal regression models with sex, baseline age, and either Year 1 or delta BMI%ile and PA (TPA or MVPA; %WT) did not significantly predict Year 2 PWV. While our models could not account for the majority of the variance in PWV, the delta models explained twice as much of the variance than the Year 1 models (R^2, 6.1% and 6.4% vs. 2.9% and 3.4%). This suggests that perhaps changes in BMI%ile and PA levels over time are more important than baseline levels in understanding the trajectory of PWV in preschool children. With regards to the cross-sectional models, which used Year 1 and Year 2 data to predict PWV at the corresponding year, they too were not significant. Differences, however, were noted between cross-sectional models. Year 1 models explained only 1.5% and 3.0% of the variance in Year 1 PWV, while Year 2 models explained 8.5% and 11.1% of the variance in Year 2 PWV. Thus, it would appear that the relationship between BMI%ile and PA with PWV was stronger at follow up than at baseline. Correlation between variables both supplement and support these findings. At baseline, PWV did not correlate with BMI%ile (r= 0.05), TPA (r= -0.08), or MVPA (r= 0.01); however, it weakly but significantly correlated with TPA (r= -0.28, p< .05) and MVPA (-0.25, p< .05) at follow up. These correlations help

to explain the differences between cross-sectional models, and why none of the regression models significantly predicted PWV. Together, multiple regression and correlation analyses seem to suggest that BMI%ile does not influence PWV in the early years, and that PA influences PWV in 4 to 6 years olds but not in 3 to 5 year olds. While similar relationships have been examined in 10-year old children, conflicting data has been reported and so cannot aid in the interpretation of our findings. In one study, ECG to femoral PWV was not related to BMI (r= -0.01) but was significantly related to PA measured using a 24-hr recall questionnaire (r= -0.26, p< .05) (Schack-Nielsen et al. 2005). In another study, central PWV correlated more strongly with BMI (r= 0.34, p< .001) than with PA measured using pedometer counts (r= -0.08, p= .046) (Sakuragi et al. 2009). Evidently, the literature is inconclusive regarding the relationship between arterial stiffness, adiposity and physical activity in school-aged children. Different measures of PWV and PA make inter-study comparisons difficult. Nevertheless, in this thesis, BMI and PA levels were poor predictors of whole-body PWV in healthy 3 to 5 year old children.

4.2.2 Supplemental Analyses

When preschool children showing the greatest increases in PWV (PWV Up) were compared against those showing the greatest decreases in PWV (PWV Down), no significant differences were found between the groups with regards to age, BMI%ile, TPA or MVPA. These findings suggest that our measures of adiposity

and PA cannot be used to explain why arterial stiffness increases in some preschoolers and decreases in others. This was evident with the regression analyses in the complete cohort, and now also with this analysis in preschoolers representing extreme changes in PWV. Regarding the risk assessment of being overweight/obese or failing to meet PA guidelines, at Year 1 the OR was 1.78 for Adiposity (95% CI, 0.36 to 8.85) and 1.00 for Guidelines (95% CI: 0.28 to 3.54). This suggests that overweight/obese preschoolers are 1.78 times more likely to have a higher PWV than their healthy-weight peers, while failing to meet PA guidelines does not affect increases in PWV. However, the OR must be interpreted in light of its 95% confidence interval (CI). Recall, an exposure increases the odds of an outcome if the OR> 1, and decreases its odds if the OR< 1; therefore a 95% CI spanning 1 indicates that the relationship between adiposity (exposure) and PWV increasing (outcome) can be in either direction, and is likely not significant. Similar results were seen for the Year 2 exposures. The OR was 5.00 for Adiposity (95% CI: 0.61 to 40.91), and 2.12 for the Guidelines (95% CI: 0.54 to 8.24). Although both exposures had an OR> 1, the wide confidence intervals indicate a high amount of variability within our data, and a poor estimate (with 95% confidence) of where the true effect lies. Therefore, being overweight/obese and not meeting PA guidelines do not appear to influence a child's risk of increased PWV, and thus increased arterial stiffness, relative to their healthy-weight and active peers. These results are consistent with those from our correlation and regression analyses.

4.3 STRENGTHS & LIMITATIONS

This thesis was the first to assess the longitudinal changes in arterial stiffness in a cohort of healthy preschool children. It was also the first to examine the influence of adiposity and PA on arterial stiffness in the early years. Strengths of this thesis include the large sample size and the objectively measured levels of PA by means of accelerometry. On the other hand, measures of arterial stiffness and adiposity pose some limitations. Whole-body PWV (carotid to DP) is not common in the literature, has no available reference values, and its clinical value has yet to be established. Furthermore, possible confounders of PWV include cardiorespiratory fitness, birth weight, heart rate and blood pressure, all of which are not reported in this thesis. Consequently, we cannot conclude that the longitudinal increase in PWV observed in this thesis was due to an age-related increase in arterial stiffness. With regards to adiposity, BMI is not a direct measure of body fat, but simply a ratio of weight to height. Nevertheless, its use allows for consistency in the literature, as it is a widely used surrogate for adiposity due to its simplicity and feasibility.

4.4 FUTURE CONSIDERATIONS

Little is known about changes in arterial stiffness during the early years, and more research in a preschool population is needed. Despite our negative findings, the relationship between arterial stiffness, adiposity and PA are worth re-examining in a larger cohort of the HOPP study (400 participants). A larger

sample size would allow for sex- and age-specific analyses, as this thesis was limited to only sex-based comparisons. Furthermore, longitudinal changes and tracking of whole-body PWV should be examined over all 3 years of the HOPP study to better understand annual changes of arterial stiffness in this young population. Moreover, since accelerometers can be used to measure sedentary behaviour and characterize PA patterns, it would be interesting to examine whether these variables are better predictors of PWV. Lastly, it would be valuable to determine if PWV correlates more strongly with other surrogate measures of adiposity (i.e. WC, DXA-BF%, BIA-BF%) than it does with BMI.

4.5 CONCLUSIONS

In summary, arterial stiffness may already be increasing in healthy 3 to 5 year old children. Whole-body PWV, an index of arterial stiffness, tracks fair-to-moderately well over a 1-year period in this young population. Furthermore, this thesis found no association between arterial stiffness, and measures of adiposity and physical activity in preschool-aged children. Nevertheless, maintaining a healthy body composition and engaging in regular physical activity are associated with other health benefits in the early years; therefore, preschool children should be encouraged to lead healthy and active lifestyles.

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APPENDIX A: MEDICAL QUESTIONNAIRE

HOPP MEDICAL QUESTIONNAIRE   Study ID _____   Year _____

DOB (dd-mmm-yyyy): _____

Does your child have any of the following conditions (circle each of the appropriate):

a) Heart disease   g) Allergies
b) High blood pressure   h) Diabetes
c) Loss of consciousness   i) Epilepsy
d) Asthma   j) Motor delay
e) Intestinal disease   k) Developmental delay/issues _____
f) Surgery or fractures   l) Others: _____
                                  m) None

Present health:

a) Good   b) Complaints: _____

When thinking of prior exercise involvement, has your child experienced (circle the appropriate):

a) An inability to keep up with other children
b) Chest pain
c) Fainting
d) Dizziness
e) Irregular heart beat
f) Wheezing
g) Other: _____
h) None of the above

Is your child currently taking any medications? If yes, how frequently?

Medication	Frequency
_____	_____
_____	_____
_____	_____

☐ My child is not currently taking any medications

Has your child taken any medications in the past month? If yes, how frequently?

Medication	Frequency
_____	_____
_____	_____
_____	_____

☐ My child has not taken any medications in the past month

HOPP Year 1 & 2, Oct 2011, Version 2

HOPP MEDICAL QUESTIONNAIRE   Study ID _____   Year _____

Has your physician ever suggested that your child restrict their levels of physical activity?

☐   Yes   ☐   No

Do you know of any medical reason that would prevent your child from participating in physical activity?

☐   Yes. Please specify: _____   ☐   No

Year 2:

Over the past year, have there been any environmental changes that have affected this child?

☐   Yes. Please specify: _____   ☐   No

Have there been any health or medical issues that are concerning to you?

☐   Yes. Please specify: _____   ☐   No

APPENDIX B: PARENT/GUARDIAN CONSENT FORM

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PARENT CONSENT FORM

Title of Study: _Health Outcomes and Physical activity in Preschoolers: The HOPP Study_

Principal Investigator: _Brian W. Timmons (PhD), Children's Exercise & Nutrition Centre_

Co-Investigators: _Steven Bray (PhD), Department of Kinesiology, McMaster University_
Maureen MacDonald (PhD), Department of Kinesiology, McMaster University
John Cairney (PhD), Department of Family Medicine, McMaster University

Study Sponsor: _Canadian Institutes of Health Research_

___

INTRODUCTION

Your child is being invited to participate in a research study conducted by Dr. Brian Timmons because they are 3 to 5 years of age. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate. Feel free to discuss it with your family and take your time to make your decision.

WHY IS THIS RESEARCH BEING DONE?

We believe that exercise and physical activity are good for young children and that play is an important part of child development. But we do not know how much physical activity is required for good health. The need to recommend appropriate levels of physical activity has never been greater because more children are becoming overweight and opportunities for preschoolers to be physically active are being replaced by sedentary activities. The best way to understand the relationships between physical activity and health is to follow the same children over time (called longitudinal research) with regular assessments of physical activity and health.

WHAT IS THE PURPOSE OF THIS STUDY?

The main purpose of this study is to determine how physical activity is related to health in preschoolers. We also hope to learn about nutrition habits in preschoolers and barriers parents face to getting their preschooler active. We will make these assessments once per year for 3 years to understand how the relationships between these important variables change or stay the same during the early years.

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WHAT WILL MY CHILD'S RESPONSIBILITIES BE IF THEY TAKE PART IN THE STUDY?

If you and your child volunteer to participate in this study, we will ask you to do the following things:

• Make 2 visits to our laboratories separated by about 8 days. At the first visit, your child will have some testing done at the Children's Exercise and Nutrition Centre (Chedoke Hospital). At the second visit, testing will be done in the Department of Kinesiology, which is at McMaster University. During each visit we will perform different tests on your child.

• At the Children's Exercise & Nutrition Centre, we will record your child's weight and height, determine their percent body fat, and measure their waist circumference. They will pedal our special bicycle for as fast as they can for about 10 sec. They will then walk on a treadmill for as long as they can to determine fitness while we record their heart rate during and shortly after the exercise test. We will have you fill out some questionnaires at this visit to tell us about your child's health and issues about their physical activity. When it is time to leave, we will put a small pager-like device, called an accelerometer, on the waist of your child. This little box (about the size of a matchbox) will record their physical activity over the next 8 days. We will explain how to care for it during this time. This visit will take about 1.5 hours.

• At the Department of Kinesiology, we will measure the health of the main blood vessel in your child's neck. This test requires your child to lie on a bed for about 15 min for the measurements to be taken. We will then assess your child's motor skills by having them do some running, hopping, and ball-throwing tasks, and this takes about 40 min. We will have you fill out some more questionnaires at this visit to tell us about your child's health and issues about their well-being. This visit will take about 1.5 hours.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

There are no unusual risks or discomforts associated with your child's participation in this study. Your child may feel a little tired after the exercise tests, but this feeling won't last long. If your child already rides a tricycle or a bicycle, the bike test is no different than what they might experience while riding their own machine as fast as they can. Your child may not be used to walking on a treadmill so we will ensure they can safely do this task. To measure body composition we will use a special machine that estimates how much water is in your child's body by sending a small electrical current through your child's body. The current is so small that they will not feel it at all and it will not hurt them whatsoever. To measure blood vessels, we will use the same ultrasound machine that doctors use to take a picture of a baby during a pregnancy. We will ask you about your confidence in providing your child opportunities to be physically active. You can answer these questions to the extent you feel comfortable doing so. Any information collected from you and your child will become anonymous to ensure confidentiality.

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HOW MANY PEOPLE WILL BE IN THIS STUDY?

We are asking boys and girls aged 3 to 5 years from the Hamilton and surrounding communities to participate. We plan to test 400 children once per year for 3 years in a row. Your participation is voluntary.

WHAT ARE THE POSSIBLE BENEFITS FOR MY CHILD AND/OR FOR SOCIETY?

We cannot promise any personal benefits to you or your child from their participation in this study. You will learn how several health characteristics of your child (body fat, blood vessel health, and physical fitness) compares with other children their age. You will also learn how these things change over time and whether these markers of health are associated with how much physical activity or play they do. By participating in this study, you will be contributing to knowledge that will be used to develop physical activity guidelines for Canadian preschoolers.

WHAT INFORMATION WILL BE KEPT PRIVATE?

All of your information will be stored in filing cabinets under the supervision of Dr. Brian Timmons for 25 years. We will supervise access to your child's information by other people in our group, only if necessary. Your child will be assigned a subject number, and this number will be used to identify them. Records identifying your child will be kept confidential. If the results of the study are published, their identity will remain confidential.

CAN PARTICIPATION IN THE STUDY END EARLY?

If your child volunteers to be in this study, you or your child may withdraw at any time with no prejudice. The investigator may withdraw your child from this research if circumstances arise which warrant doing so.

WILL MY CHILD BE PAID TO PARTICIPATE IN THIS STUDY?

Your child will not be paid to participate in this study, but we will provide them a gift package including various trinkets and a certificate of participation each year of the study. We will also reimburse you for any parking expenses and provide you an annual "*physical activity report card*". You will be able to make regular visits to our website for updates on the HOPP study.

IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CALL?

If you have any questions about the research now or later, please contact Nicole Proudfoot at 905-521-2100, ext 77217 or proudfna@mcmaster.ca.

If you have any questions regarding your rights as a research participant, you may contact the Office of the Chair of the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board at 905-521-2100, ext. 42013.

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CONSENT STATEMENT

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction and to the satisfaction of my child. I agree to allow my child to participate in this study entitled: *"Health Outcomes and Physical activity in Preschoolers: The HOPP Study"*. I understand that I will receive a signed copy of this form.

_____
Name of Participant (child's name)

_____
Printed Name of Legally Authorized Representative

_____   _____
Signature of Legally Authorized Representative   Date

Consent form administered and explained in person by:

_____
Printed Name of Person Obtaining Consent

_____   _____
Signature of Person Obtaining Consent   Date

SIGNATURE OF INVESTIGATOR:

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent for their child to participate in this research study.

_____
Printed Name and title

_____   _____
Signature of Investigator   Date

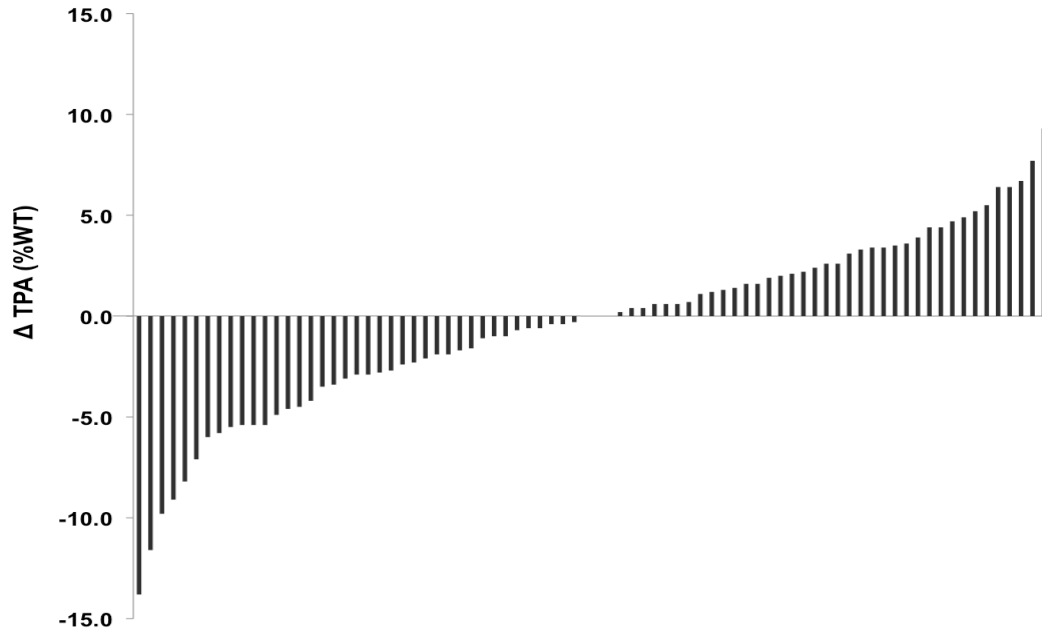## APPENDIX C: VARIABLE CHARACTERISTICS

Figure 6. Individual ΔTPA for all preschoolers (N=80)
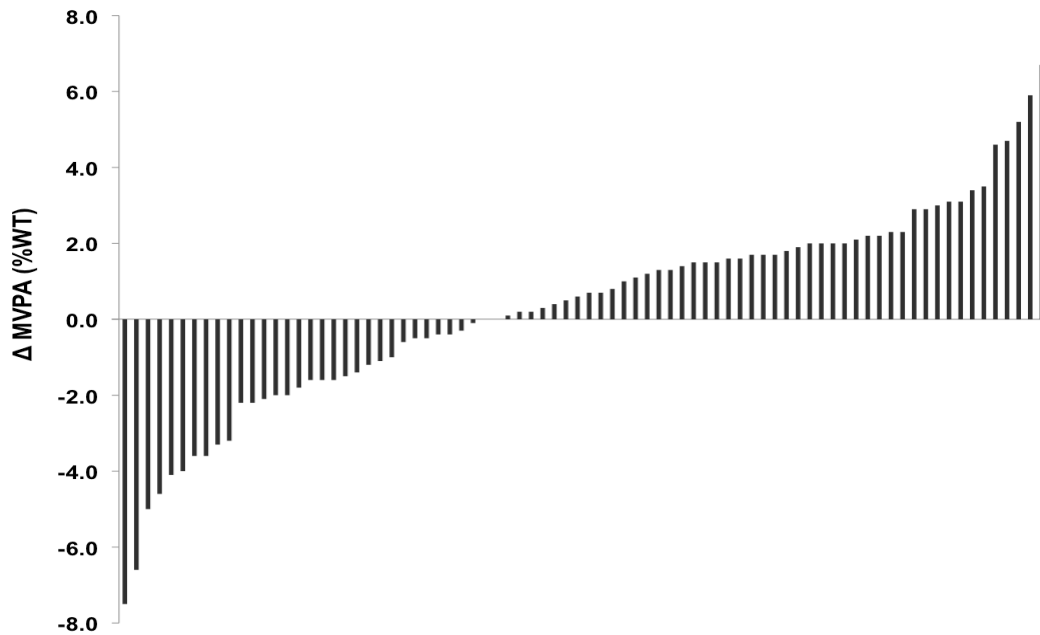

Figure 7. Individual ΔMVPA for all preschoolers (N=80)

APPENDIX D: VARIABLE CORRELATIONS

TABLE 7. Bivariate Correlations Between Delta (Year 2 – Year 1) Variables

	Age	Δ-Height	Δ-Weight	Δ-BMI%ile	Δ-TPA	Δ-MVPA	Δ-PWV
Age	1	-.250**	.138	-.004	-.136	-.129	-.150
Δ-Height		1	.257**	-.050	.082	.094	.117
Δ-Weight			1	.509**	-.042	-.088	-.021
Δ-BMI%ile				1	-.015	-.049	.058
Δ-TPA					1	.855**	-.088
Δ-MVPA						1	-.095
Δ-PWV							1

Age at baseline (yrs), Height (cm), Weight (kg), TPA and MVPA (%WT), PWV (m/s); Non-normal data: Age, Height, Weight; Significance (2 tailed): (*)$p < .05$, (**)$p < .01$

APPENDIX E: TRACKING AND PREDICTING PWV

TABLE 9. Sex Differences in Kappa Tertile Agreement

	Kappa Tertile Agreement			
	Same	**Higher**	**Lower**	**Total**
Sex				
Boys	25	14	10	49
Girls	27	11	11	49
Total	52	25	21	98

Pearson Chi-Square: χ^2 (Df=2, N=98) = .485, p= .785

TABLE 12. Predicting Year 2 PWV from Year 2 Variables

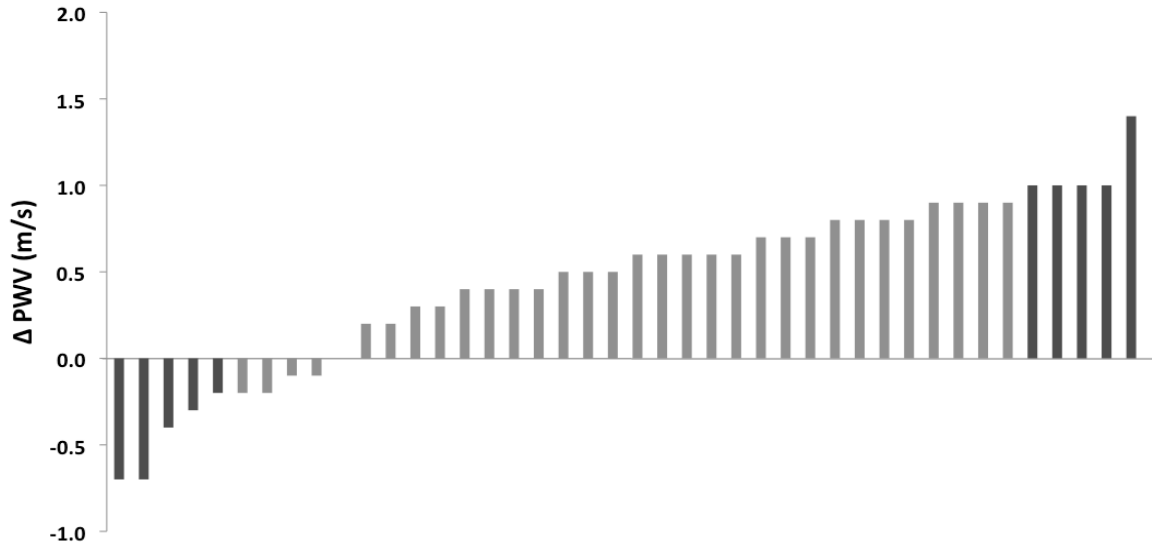|	R	R^2	$R^2_{adjusted}$	$\beta_{Unstandardized}$	$\beta_{Standardized}$	P value
Model 1	.334	.111	.064			.062
Constant				6.483		.000
Sex				.091	.089	.471
Age				-.108	-.183	.104
2-BMI%ile				.000	.012	.920
2-TPA				-.037	-.313	.009
Model 2	.291	.085	.036			.150
Constant				5.758		.000
Sex				.066	.064	.604
Age				-.079	-.135	.236
2-BMI%ile				.000	-.017	.888
2-MVPA				-.049	-.263	.030

APPENDIX F: SUPPLEMENTAL ANALYSES

Figure 9. Individual ΔPWV for boys (N=42)
Dark bars represent the extremes: 5 Up, 5 Down.
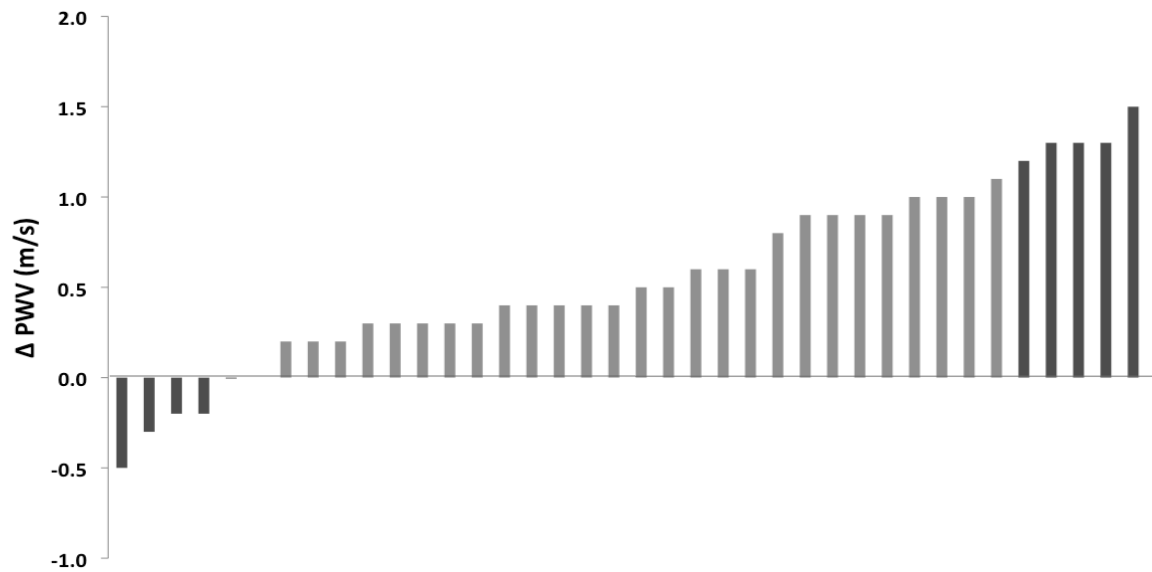

Figure 10. Individual ΔPWV for girls (N=38)
Dark bars represent the extremes: 5 Up, 5 Down.

TABLE 13. Comparing Adiposity and PA Variables of Extreme Responders

		PWV Up	PWV Down	P value
Age (years)				
	All	4.1 ± .8	4.9 ± .9	.068
	Boys	4.3 ± .8	5.5 ± .3	.023*
	Girls	4.0 ± 1.0	4.3 ± .8	.602
PWV (m/s)				
Year 1	All	4.2 ± .2	4.6 ± .4	.008*
	Boys	4.2 ± .1	4.7 ± .3	.008*
	Girls	4.2 ± .3	4.7 ± .3	.041*
Year 2	All	5.4 ± .3	4.2 ± .4	.000**
	Boys	5.2 ± .3	4.2 ± .4	.001**
	Girls	5.6 ± .2	4.4 ± .4	.000**
Delta	All	1.2 ± .2	-0.4 ± .2	.000**
	Boys	1.1 ± .2	-0.5 ± .2	.000**
	Girls	1.3 ± .1	-0.2 ± .2	.000**
BMI Percentile (%)				
Year 1	All	59 ± 25	58 ± 23	.948
	Boys	42 ± 28	65 ± 23	.191
	Girls	74 ± 11	57 ± 24	.200
Year 2	All	54 ± 27	53 ± 24	.923
	Boys	39 ± 29	61 ± 23	.231
	Girls	69 ± 18	58 ± 19	.403
Delta	All	-5 ± 10	-5 ± 12	.934
	Boys	-3 ± 4	-4 ± 7	.675
	Girls	-5 ± 13	1 ± 8	.377
TPA (%WT)				
Year 1	All	36.2 ± 4.8	37.4 ± 4.1	.557
	Boys	39.3 ± 5.2	40.0 ± 2.2	.766
	Girls	34.4 ± 5.8	34.7 ± 3.8	.921
Year 2	All	35.8 ± 4.7	37.1 ± 5.4	.558
	Boys	38.0 ± 4.5	39.8 ± 2.5	.448
	Girls	35.0 ± 4.9	33.6 ± 5.3	.675
Delta	All	-0.5 ± 3.1	-0.3 ± 4.4	.922
	Boys	-1.3 ± 7.7	-0.2 ± 4.0	.792
	Girls	-0.7 ± 2.9	-1.1 ± 4.6	.498

MVPA (%WT)

Year 1	All	12.8 ± 3.0	14.5 ± 2.9	.204
	Boys	14.6 ± 3.8	16.1 ± 2.4	.468
	Girls	11.2 ± 2.8	11.9 ± 2.3	.677
Year 2	All	12.9 ± 2.9	14.6 ± 3.1	.211
	Boys	14.9 ± 2.8	16.1 ± 1.5	.427
	Girls	11.7 ± 1.2	12.3 ± 2.5	.677
Delta	All	0.1 ± 2.3	0.1 ± 2.2	.992
	Boys	0.3 ± 4.2	0.0 ± 1.7	.871
	Girls	0.5 ± 2.3	0.4 ± 3.0	.927

Averages are Mean ± SD; Age at baseline; Significance: (*) $p < .05$, (**) $p < .001$