DYSGLYCEMIA AND CAROTID INTIMA-MEDIA THICKNESS IN CHILDREN AND YOUTH WITH OBESITY

INVESTIGATING THE RELATIONSHIP OF DYSGLYCEMIA AND MEASURES OF CAROTID INTIMA-MEDIA THICKNESS IN CHILDREN AND YOUTH WITH OBESITY ENROLLED IN WEIGHT MANAGEMENT

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TITLE: Investigating the relationship of dysglycemia and measures of carotid intima-media thickness in children and youth with obesity enrolled in weight management

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ABSTRACT

INTRODUCTION: The prevalence of obesity in Canadian adults is high and is mirrored in the pediatric population. The collective increase of childhood obesity over the last three decades has been associated with a corresponding rise in the deterioration in cardiometabolic health. Abnormally high measures of blood glucose are indicative of impaired glucose metabolism that is related to the development of adverse adult conditions such as type 2 diabetes mellitus (T2DM) and/or cardiovascular disease (CVD). Prediabetes is a transient state of abnormally elevated blood glucose levels that, in adults, has comparable cardiovascular risk to diagnosed diabetes. Previous studies have demonstrated that children with prediabetes can progress to T2DM over a short period of time. Given that up to 21% of children with obesity have abnormal glucose parameters, it is critical to manage risk at early stages of life in this population in order to prevent adverse adult outcomes. **PURPOSE**: The purpose of this study was to evaluate if there is a contributing role of prediabetes on vascular structure, assessed via carotid intima-media thickness (cIMT), in children and youth with obesity. Given the high prevalence of adverse health indicators, we aimed to characterize the relationships between these clinical measures and early structural changes associated with atherosclerosis progression. As protocols used to measure cIMT in pediatric studies vary, an additional aim of this project was to assess comparability of cIMT protocols reported in the literature. **HYPOTHESIS**: It is hypothesized that children with obesity and prediabetes will have greater mean cIMT compared to normal glycemic controls and that mean cIMT will be significantly different across varying cIMT methodologies. **METHODS**: We recruited

165 participants (ages 5-17y) into this cross-sectional study, a sub-study of the CANadian Pediatric Weight Management Registry (CANPWR). This study assessed cIMT via B-mode ultrasonography in children and youth at time of entry into a weight management program. Clinical measures were obtained via prospective clinical chart review and were evaluated to identify potential relationships between modifiable and non-modifiable cardiovascular health indicators and measures of cIMT, using univariable and multivariable regression analyses. To compare methodologies, we assessed differences of mean cIMT (mm) using an intra-class correlation and a Bland-Altman plot. **RESULTS**: Fasting plasma glucose was related to mean cIMT (mm) in univariable analyses ($p=0.047$), but no measure of glycemia was independently correlated to arterial thickness. Segment-specific thickening contributed to significant differences in mean cIMT (mm) between methodologies that included all arterial segments (far and near wall of the common carotid artery, carotid bulb, and internal carotid artery; 12-segment) compared to those limited to the far wall of the common carotid artery (2-segment) ($p<0.001$). Intra-class correlation between these cIMT methodologies indicated moderate resemblance (ICC: 0.65, 95%CI: 0.55 to 0.73), however, a significant difference between means (mean difference: 0.012mm, 95%CI: -0.016 to -0.009, $p<0.001$) and a positive proportional bias ($r=0.19$, 95%CI: 0.06 to 0.31, $p=0.003$) were present. Furthermore, correlates of mean cIMT were inconsistent between varying methodologies commonly used to assess cIMT in children. **CONCLUSIONS**: In this study, dysglycemia did not predict on arterial thickness in children and youth with obesity. By comparing determinants and absolute

differences between measures of cIMT, this study emphasizes the heterogeneity between the two methodologies and suggests that they should not be used interchangeably.

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CHAPTER 1: LITERATURE REVIEW

Prevalence of Obesity. Although a condition once associated with affluence, the prevalence of obesity has increased dramatically in both developed and developing countries (Caballero, 2007; Tran, Nair, Kuhle, Ohinmaa & Veugelers, 2013; Lau et al., 2007). In Canada, the prevalence has risen over the last several decades and is now stabilizing (Shields, 2005; Roberts, Shields, Groh, Aziz & Gilbert, 2012)**.** Despite this, the prevalence of severe obesity continues to increase in both children and adults (Roberts et al., 2012; Twells, Gregory, Reddigan, & Midodzi, 2014). It is projected that by 2019, 21% of Canadian adults will be obese and that half of Canadian provinces will have a greater proportion of adults with overweight or obesity than with normal weight (Twells et al., 2014). As the single most significant contributor to poor health (Lau et al., 2007), 1 in 10 premature deaths in Canada is attributable to obesity and obesity-related health issues (Katzmarzyk & Ardern, 2004). In 2010, the economic burden of obesity and its associated conditions amounted to $6 billion (from direct and indirect costs) among Canadian adults, and represented 4.1% of Canada's total health care budget (Anis et al. 2010). Although the central viewpoint surrounding the cause of obesity derives from an imbalance of energy intake and expenditure, obesity manifests as a product of environmental, social and genetic interactions (Caballero, 2007; Lau et al., 2007). With no signs of improvement in the future (Manuel et al., 2014), obesity is not simply a "cosmetic or body image issue" (Lau et al., 2007), but rather a complex, multifactorial disease that is affecting the global population at an earlier age (Caballero, 2007; Shields, 2005; Anis et al., 2010). As the world's most prevalent nutritional problem (Lau et al.,

2007), childhood obesity has been declared a global epidemic within the last decade (Caballero, 2007). Childhood obesity is a major health challenge (Tran et al., 2013) with potential lifelong consequences for both the individual and society as a whole (Singh, Mulder, Twisk, Van Mechelen, & Chinapaw, 2008; Morrison, Friedman & Gray-McGuire, 2007; Freedman, Khan, Dietz, Srinivasan & Berenson, 2001).

Significance of Childhood Obesity. Within the last 30 years, obesity rates among Canadian children have tripled (Roberts, Shields, de Groh, Aziz, & Gilbert, 2012). Currently, nearly one third (31.5%) of Canadian children are classified as overweight or obese (Roberts et al., 2012). The consequences of obesity during childhood range from psychological to physiological in nature, with impacts to almost all health systems. Children with obesity have consistently reported low self-esteem, poor body image, high prevalence of depression and low overall quality of life (Williams, Wake, Hesketh, Maher, & Waters, 2005; Schwimmer, Burwinkle, & Varni, 2003). Children with obesity are also at risk for developing adverse physiological and metabolic conditions such as impaired glucose tolerance (IGT), insulin resistance, and hyperlipidemia (Must & Strauss, 1999; Dietz, 1998). Indeed, the National Health and Nutritional Examination Survey (NHANES) from 1999-2012 (n=8579), a cross-sectional analysis of children in America, demonstrated that up to 28% of children and youth with and obesity had at least one abnormal fasting lipid value (total cholesterol, LDL, HDL or triglycerides) (Skinner, Perrin, Moss, & Skelton, 2015). As the prevalence of many cardiometabolic or cardiovascular risk factors (CVRFs) increase with degree of obesity (Skinner at al.,

2015), it is critical to manage risk at early stages of life in children with overweight or obesity to prevent adverse adult outcomes. The impact of adiposity during childhood is severe, as Weiss and colleagues (2004) reported that each half unit increase in BMI z-score was associated with approximately 50% higher risk of developing metabolic syndrome.

Presently, the standard practice of treating children with obesity in Canada lies predominantly within outpatient weight management programs (Ball, Ambler, & Chanoine, 2011). Guidelines for the management of childhood obesity involve comprehensive family-centered lifestyle and dietary interventions, combining behaviour modification (activity and dietary) with cognitive behavioural therapy (Lau et al., 2007). A 2015 meta-analysis of behavioural interventions reported that treatments/programs targeted toward behaviour lifestyle interventions modestly reduce BMI (or BMI z-scores) in children that are overweight or obese (Peirson et al., 2015). However, response to interventions vary widely at this time and attrition rates in these programs are high (Zeller et al., 2004).

Adult Consequences of Childhood Obesity. Individually or combined, obesity and obesity-related conditions during childhood predispose individuals to higher risks of developing chronic conditions in adulthood, such as diabetes and cardiovascular disease (CVD) (Morrison et al., 2007; Must & Strauss, 1999; Dietz, 1998). Body mass index (BMI) during adolescence is strongly correlated to BMI in adulthood ($r=0.75$, $p<0.0001$,

Steinberger, Moran, Hong, Jacobs, & Sinaiko, 2001) and longitudinal studies have reported that up to 77% of children with obesity remain obese as adults (Morrison et al., 2007; Freedman et al., 2001). As a period marked with dynamic physiological changes, obesity during childhood increases the risk of overall mortality and chronic conditions (Must, 2003; Juonala et al., 2011; Juonala et al., 2010). Tirosh and colleagues (2011) reported that elevated BMI during adolescence is a significant predictor of incident diabetes and adult cardiovascular events in a group of 37,674 male youth. In this study, every unit increase in BMI (kg/m^2) corresponded with an increased risk of incident diabetes or cardiovascular disease of 9.8% (HR: 1.10, 95%CI: 1.08-1.12) and 12% (HR:1.12, 95%CI: 1.067-1.18), respectively. Similarly, metabolic syndrome during childhood was related to a 14.6-fold increased risk of a cardiac event (myocardial infarction, coronary artery bypass graft, angioplasty, or stroke) within 25 years (OR: 14.7, $p<0.0001$) (Morrison et al., 2007). These studies demonstrate that obesity and its related conditions increase the risk of adult CVD, and suggest that atherosclerosis progression might be accelerated in the overweight/obese pediatric population (Daniels, 2009). Although previous reports suggest that obesity-related disease risk diminishes when weight in adulthood is normalized (Juonala et al., 2011), Tirosh and colleagues (2015) demonstrated that the detrimental effects of excess weight in childhood persist regardless of adult weight. Notably, individuals who were obese during adolescence had a 7.65-fold higher risk of angiographic coronary artery disease as compared to their lean counterparts during adolescence (HR:7.65, 95% CI: 3.93-14.88). Importantly, this risk remained after adjustment for BMI in adulthood, family history of CVD, and other traditional CVRFs

(HR: 6.85, 95%CI: 3.30-14.21 (Tirosh et al., 2011). Collectively, these studies highlight the importance of preventing and managing obesity and its related conditions in childhood rather than waiting until adulthood to intervene.

Dysglycemia and Prediabetes in Children and Youth with Obesity. Once classified as "juvenile-" and "adult-onset diabetes", diabetes mellitus was previously characterized by the age of symptom-onset rather than the ability to produce and respond to insulin. Although outdated, this classification sheds light to previous etiology that type 2 diabetes mellitus (T2DM) was a chronic disease specific to adults. With an alarming increase in the prevalence of childhood obesity, the prevalence of T2DM in children has emerged as a growing problem (Rosenbloom, Joe, Young, & Winter, 1999). It is estimated that up to 45% of children diagnosed with diabetes have T2DM. As a comorbidity of obesity, adolescents with T2DM have higher risk of premature health complications and mortality (Rhodes, Prosser, Hoerger, Lieu, Ludwig, & Laffel, 2012) and are expected to lose up to 15 years of life with chronic complications by age 40 (Rhodes et al., 2012).

As an intermediate stage of dysglycemia, prediabetes is a transient state of abnormally elevated blood glucose levels that increase the risk of developing type 2 diabetes. Similar to diagnostic criteria in adults, prediabetes can be classified based on impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and/or elevated glycated hemoglobin (as measured by fasting plasma glucose, 2hr oral glucose tolerance tests (OGTT), and HbA1c, respectively) (Canadian Diabetes Association, 2013) (see **Table 1**). Although

there are limited studies in the pediatric age group, children and youth with prediabetes typically present with obesity (Weiss et al., 2003; Cali & Caprio, 2008a; Haemer et al., 2014) and up to 21% of children and adolescents with obesity present with IGT (Morrison, Tarnopolsky, Yusuf, Atkinson & Yusuf, 2012). Given that up to 31% of children with obesity had a glucose value in the abnormal range (≥5.6mmol/l) in a population survey of 1838 children and youth (12-19y), the increasing prevalence of prediabetes was associated with greater degrees of obesity (Skinner et al., 2015). Evidently, Cali et al. (2008a) reported that progression from normal glycemia to IGT and IGT to diabetes was related to significant increases in weight, which is consistent with the independent effect of weight gain on glycemic status in children with obesity reported by Weiss and colleagues (2005). In their study of 117 children and adolescents with obesity, Weiss and colleagues (2005) demonstrated that 32.3% of those with impaired glucose tolerance progressed to type 2 diabetes mellitus after 2 years (Weiss et al., 2005). Not surprisingly, subjects who reverted back to NGT from IGT observed a reduction in adiposity as measured by BMI z-score. Although not all individuals with prediabetes progress to T2DM (Amed et al., 2010), adult studies have shown a 100% predictive progression to T2DM over 5 years in individuals presenting with combined IFG and elevated HbA1c (Heianza et al., 2012). Youth with prediabetes exhibit a similar high risk of progressing to type 2 diabetes if left without intervention (Twigg, Kamp, Davis, Neylon, & Flack.,2007; Cali & Caprio, 2008).

Cardiovascular Risk Associated with Prediabetes. Consistent with progression to type 2 diabetes, risk for developing CVD is increased across blood glucose values in the prediabetic range. As an established CV risk equivalent (DeFronzo & Abdul-Ghani, 2011; Levitan, Song, Ford, & Liu, 2004), individuals with prediabetes (defined by either IGT, IFG or both), have reported similar risk of macrovascular complications as those with type 2 diabetes (DECODE Study Group, 2001). Previous studies have reported a 2.5-fold increased relative risk for CVD mortality associated with IFG (Barr et al., 2007). Indeed, in a 2010 systematic review summary of 8 studies (n=52,994) (Ford, Zhao, & Li, 2010), individuals with IGT had an overall 18% increased risk of CVD compared to those with normal glucose tolerance (NGT) (RR: 1.18, 95%CI: 1.09 to 1.28). Likewise, the Diabetes Epidemiology: Collaborative analysis of Diagnostic Criteria in Europe (DECODE) study group followed 20,707 individuals without diabetes and estimated up to 34% increased CVD risk in those with IGT (RR:1.34, 95%CI: 1.14 to 1.57). With an overall 20% increased risk of CVD in those with IGT (n=53,512; RR: 1.20, 95%CI: 1.07-1.34) (Ford et al., 2010), some reports suggest that 2h PG is a better marker than FPG to predict CVD risk and CVD mortality (Marini et al., 2012; DECODE Study Group, 2001; Coutinho, Gerstein, Wang, & Yusuf, 1999).

Screening and Treatment of Prediabetes in Children and Youth with Obesity. According to the Canadian Diabetes Association (CDA) 2013 Guidelines, screening for T2DM is recommended as early as 10 years of age in high risk pediatric populations (e.g. obese children) (Canadian Diabetes Association, 2013). However, up to 8% of Canadian

children diagnosed with T2DM were <10 years of age (Amed et al., 2010). Unfortunately, current recommendations supporting the screening of prediabetes and type 2 diabetes in children with overweight and obesity is reliant on expert opinions, with principles derived from adult care and limited small scale studies (class B evidence) (Haemer et al., 2014). The Pediatric Endocrine Society emphasizes that diagnostic criteria are based on long-term health outcomes from the adult population and have yet to be validated in the pediatric age group (Kapadia & Zeitler, 2012). In Canada, fasting plasma glucose (FPG) is the primary tool used for dysglycemia screening in children because of its high sensitivity to detect diabetes, although its sensitivity for prediabetes is poor (Morrison et al., 2012). While the CDA (2013) specifically recommends against using glycated hemoglobin (HbA1c) for diabetes diagnosis in children, such recommendations are not consistent with American guidelines. In a recent review of prediabetes management in American pediatric weight management programs, HbA1c was the most widely used screening tool for diabetes, with nearly 11% of clinicians relying solely on HbA1c at initial assessment (Haemer et al., 2014); 50% of clinicians used both HbA1c and fasting plasma glucose. Despite its high sensitivity to detect prediabetes, OGTTs were used in less than 36% of clinicians and preferences not to include the test's high cost, high day-to-day variability, and inconvenience (Canadian Diabetes Association, 2013; Haemer et al., 2014).

Acknowledging the limitations of OGTTs, this diagnostic tool has been shown to be more sensitive in detecting dysglycemia in children and youth with obesity (Morrison et al.,

2012) and is more strongly correlated to CVD outcomes compared to FPG in adults (Santaguida et al., 2005; DECODE Study Group, 2001; De Vegt et al, 1999). In the pediatric population, Morrison and colleagues (2012) reported that 68% of prediabetes diagnoses is missed when relying solely on impaired fasting glucose. With an increased risk of developing T2DM among obese children (Freedman et al., 2007), a major goal of obesity and prediabetes management is to prevent the progression of prediabetes to T2DM (and from normal glucose regulation to prediabetes) (Haemer et al., 2014). Presently, the CDA recommends a family-oriented interdisciplinary healthcare approach for treatment of dysglycemia, emphasizing healthy dietary and physical activity habits, with routine monitoring to ensure an HbA1c of <7.0% (Canadian Diabetes Association, 2013).

It is important to note that although dysglycemia can be measured in multiple ways, each diagnostic test has distinct pathophysiologic etiology. Indeed, although IFG and IGT (as measured by fasting plasma glucose and 2hr OGTT, respectively) present with peripheral insulin resistance, adults with IGT have marked resistance specific to muscle and limited or mild hepatic insulin resistance whereas those with IFG have moderate to severe insulin resistance in the liver and little to no insulin resistance in the muscle (Abdul-Ghani, Jenkinson, Richardson, Tripathy & DeFronzo, 2006a; Abdul-Ghani et al, 2006b). These unique patterns also been demonstrated in the pediatric cohort. Notably, youth with obesity and IGT have demonstrated higher concentrations of intramyocellular lipid content with limited hepatic insulin resistance (Weiss et al., 2003) as well as reduced first

and second phase glucose sensitivity compared to youth with IFG (Cali & Caprio, 2008), indicating that pathophysiological changes are detectable even with limited length of exposure to hyperglycemia.

Prediabetes Criteria: American Diabetes Association vs. Canadian Diabetes Association. The American Diabetes Association (2010) and Canadian Diabetes Association (2013) cutoffs for prediabetes differ (**Table 1**). Historically, diagnostic cut-offs for diabetes were based on risk of diabetes-specific complications reported from epidemiological studies, such as diabetic retinopathy and proteinuria (Gavin, Alberti, Davidson, & DeFronzo, 1997). Prediabetes cut-offs were defined according to longitudinal studies revealed that the threshold for increased all-cause mortality was FPG ≥6.1mmol/L or 2hr plasma glucose ≥7.8 mmol/L (Sorkin, Muller, Fleg, & Andres, 2005). In 2003, the ADA revised its original classification criteria for impaired fasting glucose from 6.1mmol/L to 5.6mmol/L which has greater sensitivity and specificity to predict diabetes over a 5-year period as reported in the Atherosclerosis Risk in Communities (ARIC) and Mauritus studies (Shaw et al., 2000). The ARIC study demonstrated that FPG ≥5.6 mmol/L was sensitive enough to predict 70% of incident diabetes (Schmidt et al., 2005). Despite the review of ADA (2003) revision, the WHO refrained from lowering glycemic thresholds for prediabetes classification in order to capture individuals with worse cardiovascular risk profiles and who would benefit most from prevention strategies (Gavin et al., 1997). Although CDA (2013) FPG screening criteria has greater sensitivity to detect IGT (81.8% vs. 43.2%) (but lower specificity (37.7% vs. 70.2%), (Morrison et

al., 2012), the ADA cut-offs captures a greater proportion of individuals at-risk of developing diabetes. Given that Tirosh and colleagues (2011) reported that risk of incident diabetes progression in male Israeli adolescents increased with FPG ≥4.8mmol/L (within normal glycemia range), these suggest a continuum of risk for adverse clinical and metabolic outcomes exist in FPG levels ≤6.1mmol/L.

In addition to differences in IFG definition, the CDA 2013 Guidelines do not recommend the use of HbA1c for diabetes diagnosis in children. As a measure of 3-month glycemic status, HbA1c is commonly used to monitor glycemic status in clinical practice and individuals with elevated HbA1c are at increased risk of macrovascular complications (Sarwar et al., 2010; Selvin et al., 2010). In the ARIC Study (n=11,092), the risk of coronary heart disease in individuals without diabetes increased incrementally by 23% and 78% for HbA1c values between 5.5-5.9% and 6.0-6.4%, respectively (HR: 1.23, 95%CI: 1.07-1.41; HR: 1.78, 95%CI:1.48-2.15) (Selvin et al., 2010). Similarly, a meta-analysis of 9 studies (n=49,099) reported a 20% increased risk of CVD for every 1% increase in HbA1c (Emerging Risk Factors Collaboration, 2010). As glycemic values within the prediabetic range measured by all three tests have been reported as independent risk factors for CVD in adults (DeFronzo and Abdul-Ghani, 2011; Selvin et al., 2010), individuals with prediabetes are recommended to seek aggressive treatment to reduce existing CVRFs.

Table 1. Definitions of Prediabetes: American Diabetes Association (2010) vs. Canadian Diabetes Association (2013).

Prediabetes Definition	ADA, 2010	CDA, 2013	Test
Impaired Fasting Glucose (IFG)	5.6-6.9 mmol/L	6.1-6.9 mmol/L	Fasting Plasma Glucose (FPG)
Impaired Glucose Tolerance (IGT)	7.8-11.0 mmol/L		2h Oral Glucose Tolerance Test (OGTT)
Prediabetes	5.7-6.4%	6.0-6.4%	HbA1c

Cardiovascular disease and Atherosclerosis Progression. Although the prevalence of cardiovascular-related death has declined with advances in medicine, its presence among the Canadian population remains high (Anis et al., 2010; Manuel et al., 2014; Statistics Canada, 2011). Currently, over 1.3 million Canadians are affected by cardiovascular disease (CVD) (Dai, Bancej, Bienek, Walsh, Stewart, & Wielgosz, 2009) and CVD is responsible for nearly 30% of Canadian deaths (Katzmarzyk & Ardern, 2004; Statistics Canada, 2011). With the financial burden of over $20.9 billion each year (Theriault, Stonebridge, & Browarski, 2010), CVD is one of the costliest diseases in Canada. Although there are many CVRFs described in the literature, over 90% of CVD risk can be explained by 9 risk factors: smoking, lipids, hypertension, diabetes, obesity, diet, physical activity, alcohol consumption and psychosocial factors (Yusuf et al., 2004). However, despite the projected decline in CVRFs in the Canadian population, the prevalence of obesity and diabetes are expected to rise (Manuel et al., 2014), which highlight the importance of early prevention and management of risk.

Physiologically, atherosclerosis is a process of artery wall thickening that begins early in life and gradually progresses across the life course (Berliner et al., 1995; Lusis, 2000; Bland, Skordaalaki, Emery, 1986; Napoli et al., 1997). Although there are many contributors to this process, atherosclerosis is initiated by adherence and accumulation of white blood cells (WBC) to the endothelium. Subsequent migration of WBCs and low-density lipoprotein (LDL) into the sub-endothelial space and macrophage activation promotes a localized inflammatory state (Berliner et al., 1995; Lusis, 2000; Bland et al., 1986). Over time, successive cycles of immune responses promote formation of a fatty streak to ultimately create a lesion. As the lesion matures to form an atheroma and smooth muscle cells migrate towards the intima layer and proliferate, the media layer thickens. Over time, this thickening can protrude into the luminal space, thereby narrowing the space for blood flow (Berliner et al., 1995; Lusis, 2000; Bland et al., 1986) (**Figure 1**).

Atherosclerosis in Children. Pathologic studies have identified lesions and fatty streaks in the aorta, coronary, and carotid arteries of children and adolescents (Holman, McGill, Strong, & Geer, 1958; Bland et al., 1986). Findings from these studies show that the atherosclerotic process begins in the fetus (focal lipid accumulation) (Stary, 2000). Although such lesions do not disrupt vessel structure, as foam cell accumulation progresses over childhood, as many as 15% of adolescents, ages 16-19 years, have detectable pre-atheromas or atheromas in their coronary arteries (Napoli et al., 1997). Given that atheromas typically form during the third decade of life, obese children may

be at increased risk of premature disease manifestation and accelerated progression (Freedman et al., 2001; Haemer et al., 2014). This is particularly concerning as adults who were overweight or obese in childhood have higher prevalence of unfavourable cardiovascular risk factors and angiographic CVD in adulthood (Freedman et al., 2001), compared to those who were normal weight (Tirosh et al., 2011).

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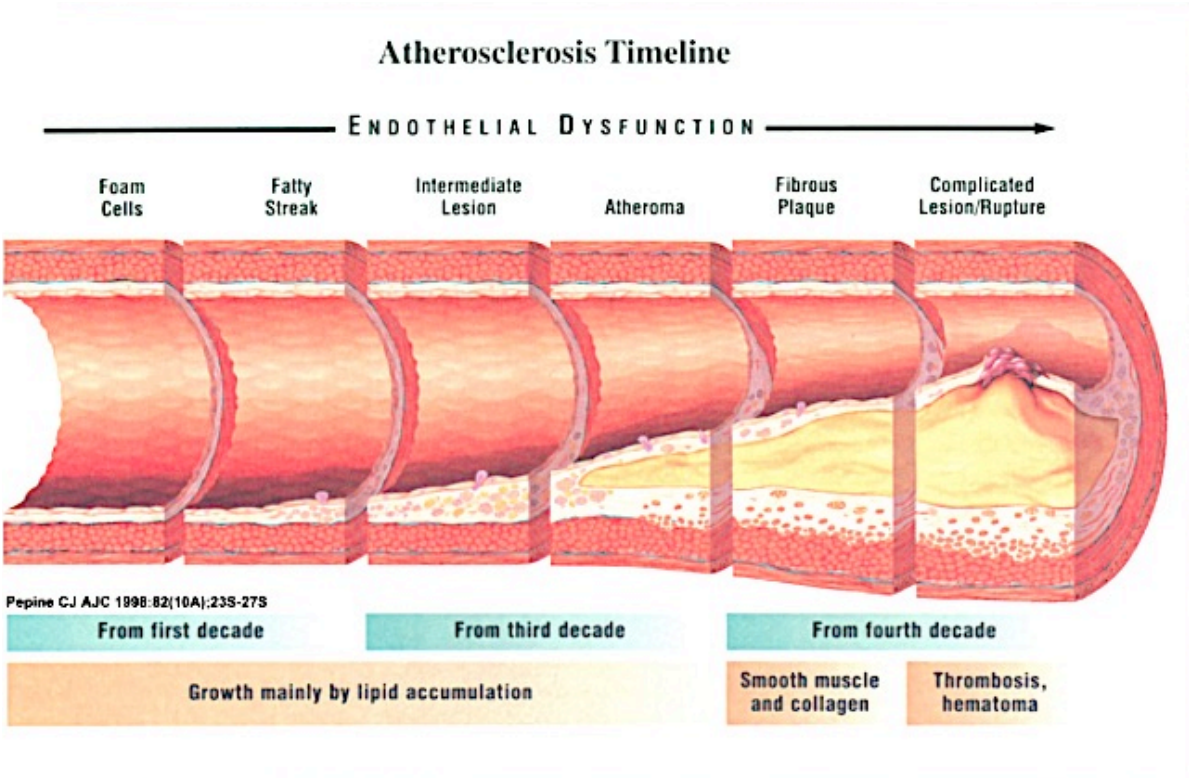


Figure 1: Atherosclerosis progression. Atherosclerosis is a gradual process of lumen narrowing, which can ultimately cause obstruction of blood flow, resulting in a cardiac event. Initial changes to vessel wall structure can occur within the first decade and progress onward with the development of atherosclerotic lesions to ultimately form an atheroma with a fibrous covering. Complicated lesions may lead to rupture, thrombus formation, and vessel occlusion. (Adapted from Pepine et al. (1998)).

Non-invasive Markers of Cardiovascular Disease. As a disease that remains clinically silent until a cardiac event, understanding childhood precursors of adult cardiovascular health was previously limited to pathologic studies, as described (Stary, 2000, Napoli et al., 1997). With advances in diagnostic imaging, non-invasive measures have developed to allow early detection of vascular changes associated with disease progression (Groner, Joshi, & Bauer, 2006). The intima, or endothelium, is the inner most layer of the vessel wall and is comprised of a monolayer of endothelial cells that mediates vascular tone. Damage to the endothelium can result in physiological changes (impaired shear-stress-induced vasorelaxation), mechanical changes (arterial stiffening), and anatomical changes (arterial thickening) that are associated with atherosclerosis (Groner et al., 2006). Ultrasonography imaging techniques can document these changes, via flow mediated-dilation, pulse-wave velocity, and intima-media thickness, respectively, and have been used extensively as markers of disease progression in the literature. The evidence supporting the use of cIMT as a marker of CVD is briefly summarized in the following sections.

Carotid Intima-Media Thickness. Carotid intima-media thickness (cIMT) is structural measure of the vascular wall that is used as a surrogate marker of subclinical atherosclerosis in both children and adults (Alpsoy et al., 2013; Simsek, Balta, Balta, & Dallar, 2010; Pacifico et al., 2009; Stein et al., 2008; Chambless et al., 1997). cIMT is a validated measure and predicts future risk of cardiac events (i.e. stroke, angiographic coronary artery disease) in adults (Chambless et al., 1997; Marini et al., 2012; Riley et al.,

1992, Stein et al., 2008). In the ARIC study, adults (age 45-70 years, n=12,841) with increased cIMT (≥1.0mm) had a 6.7-fold increased risk of a myocardial infarction and 2.9-fold increased risk of cardiovascular-related death (Chambless et al., 1997). A subsequent 2007 meta-analysis of 8 clinical trials reported that every 0.1mm increase in cIMT was associated with a 10-15% increased risk of myocardial infarction and 13-18% increased risk of stroke (Lorenz, Markus, Rosvall, & Sitzer, 2007).

In adults, cIMT has been adapted into clinical practice for CVD risk assessment and with patients at intermediate risk potentially benefiting most from subclinical disease measurement (Greenland et al., 2000; Roman, Naqvi, Gardin, Gerhard-Herman, Jaff, & Mohler, 2006; Stein et al., 2008). Systolic blood pressure (SBP) has been shown to be a significant determinant of cIMT in youth adults from the Bogalusa Heart study (mean age: 36y, n=1203) (r^2=12.3%, p<0.002), as was Total Cholesterol to HDL-C ratio (TC: HDL) (r^2=5.4%, p<0.002), and age (r^2=5.6 %, p<0.002) (Tzou et al, 2007). Similar to this, 36% of cIMT variance was explained by age, sex, BMI, pulse pressure, and LDL-cholesterol in a cohort of 750 healthy young adults (mean age: 28.4 years) (Oren et al., 2003). As a significant portion of cIMT variance can be explain by modifiable CVRFS, studies have used cIMT as endpoints for pharmacological efficacy trials in both children and adults (Wiegman et al., 2004a; Lonn et al., 2001).

Carotid Intima-Media Thickness in Children. Over the last 20 years, the evaluation of cIMT in the pediatric population has been increasingly reported (Groner et al., 2006;

Lamotte et al., 2011). Early studies were mainly focused on populations with increased risk of premature CVD, including children with familial hypercholesterolemia (FH) and type 1 diabetes (T1DM). In cross-sectional studies, children with heterozygous FH have greater thickening compared to healthy controls and unaffected siblings (Wiegman et al., 2004a; Wiegman et al, 2004b). Similar to findings in adult studies, age, male sex, and LDL-cholesterol were significant correlates of cIMT in these studies (Wiegman et al., 2004b) and others have reported a synergistic effect of high blood pressure in the youth with dyslipidemia (Krebs et al, 2009). Children with type 1 diabetes have also consistently demonstrated higher cIMT (Schwab et al., 2007; Gul et al., 2010; Jarvisalo et al, 2002) with similar determinants as LDL-cholesterol explained 12-16% ($p<0.001$) of variance amongst cIMT in children with type 1 diabetes (Urbina et al., 2013). Duration of risk factor exposure may also contribute to cIMT during childhood, as age and duration of diabetes have been reported as independent determinants in children with familial hypercholesterolemia (Wiegman et al., 2004b) and type 1 diabetes (Yamasaki et al., 1994, Schwab et al., 2007, Gul et al., 2010), respectively.

Carotid Intima-Media Thickness in Children and Youth with Obesity. In a recent review examining cIMT in different pediatric populations (Lamotte et al., 2011), 22 of 26 studies reported higher cIMT values in children with obesity compared children without obesity. Tounian and associates (2001) were among the first to study this population and found that cIMT was not increased in children with obesity (age 4-17 years) compared to normal weight controls. In a subsequent study of 147 children (100 with obesity and 47

age-matched controls, mean age: 10y), Iannuzzi and associates (2004) demonstrated that children with obesity had greater cIMT compared to controls, and others have reported similar findings when comparing lean and obese groups (Beauloye et al., 2007; Alpsoy et al., 2013; Elkiran et al., 2013; Simsek et al., 2010; Yilmazer et al., 2010). Reported differences between children with and without obesity (0.05-0.17mm) translate to an advanced vascular age of 2.5-10 years from their chronological age based on previously published progression rates of 0.017-0.020mm/year from the Bogalusa Heart Study (Urbina et al., 2013, Simsek et al., 2010; Pacifico et al., 2009; Iannuzzi et al., 2004, Stein et al., 2004). Given the high number of CVRFs in this population (Skinner et al., 2015), identifying determinants of cIMT in children and youth with obesity may assist clinicians in recognizing targets linked to early structural changes associated with atherosclerosis.

Determinants of cIMT in Children and Youth with Obesity. As the majority of studies in this cohort have shown signs of accelerated subclinical atherosclerosis progression (Lamotte et al., 2011; Park et al., 2015), perhaps the more important question is not whether obese children have higher cIMT but rather what are the determinants of cIMT in this population. As a progressive disease, age explains up to 17% of total cIMT variance in children with obesity (Urbina et al., 2009a; Ciccone et al., 2016; Shah et al., 2015). The relationship between BMI z-score (and other modifiable determinants) and cIMT has predominantly been studied in groups combining the entire study population (normal weight, overweight, and obese children) (Ryder et al., 2016; Mittelman et al., 2010; Vercoza et al., 2009) and few studies have exclusively looked at children with obesity.

Schiel and associates (2007) investigated potential correlates of cIMT specifically in children with obesity (n=81) and reported that BMI z-score was a prominent determinant of mean cIMT, explaining up to 26% cIMT variance (r^2= 0.263, p<0.001). This finding was slightly higher than those reported by Reinehr and colleagues (2006) who found that degree of overweight/obesity only explained 5% (p=0.013) in a group of 96 children with obesity, although Beauloye and colleagues (2007) reported a linear relationship between BMI z-score and cIMT (r=0.323, p=0.0036), even after adjustment for age and sex. These findings are reinforced by Shah and colleagues' (2015) recently finding that severe obesity is an independent determinant of cIMT in a group of 447 adolescents and young adults with obesity, thus underlining the impact of adiposity on vascular structure.

Interestingly, in a recent review of cIMT in children, no direct correlation between measures of adiposity (BMI z-score, WC, % body fat) and cIMT (mm) in pre-pubescent children (<12 y) were noted but in adolescence, there was a prominent positive relationship (Park et al., 2015). Throughout the pediatric literature, the variability of study populations, with regards to age, sample size, and inclusion of normal weight controls, create difficulties in elucidating how adiposity contributes to cIMT in children and youth. Gao and colleagues (2016) recently reported that adiposity indirectly impacts cIMT in children and young adults. In the 2016 study utilizing structural equation modelling, authors aimed to delineate the relationship between modifiable CVRFs and cIMT in a population sample of 784 children and young adults (mean age: 18y, range:10-24y). The effect of BMI z-score on cIMT was mediated through other modifiable CVRFs such as

blood pressure, insulin levels, and non-HDL cholesterol and was subject to the influence of unmodifiable CVRFs including age, race, and sex (Gao et al., 2015). Despite this indirect relationship between BMI z-score and cIMT, the magnitude of its effect on cIMT in children and young adults was significant (β: 0.2086, p<0.0001) and the 3 modifiable risk factors with the greatest overall impact (direct and indirect) on cIMT were: adiposity, BP, and blood glucose (Gao et al., 2016).

Similar to the adult population, SBP is consistently higher in children and youth with obesity (Elkiran et al., 2013, Yilmazer et al., 2010; Leite et al., 2012; Simsek et al., 2010; Beauloye et al., 2007; Iannuzzi et al., 2004; Jarvisalo et al., 2001) and explains up to 15% of cIMT variance in children and youth with obesity (Urbina et al., 2009a; Reinehr, Kiess, de Sousa, Stoffel-Wagner, & Wunsch, 2006). Elevated BP (either SBP or DBP) predicts a 5-fold increased prevalence of high cIMT (≥90th percentile of lean control) in children and youth with obesity in children from 6-16y (OR: 5.13, p=0.046) (Pacifico et al., 2014). Similarly, Reinehr and colleagues (2013) reported that every unit increase in SBP (mmHg) was associated with a 5% higher risk of increased IMT (≥0.7mm) in youth age 10-18y with obesity, illustrating a prominent effect of SBP on cIMT in this population.

The importance of glycemic control has been repeated implicated in arterial thickening in children with obesity. As an independent determinant of cIMT (r^2:0.05, p=0.028) (Reinehr et al., 2006), Pacifico and associates (2010), showed a 7-fold higher risk of increased cIMT (≥90th percentile of lean control) in children age 10-12y with obesity and

IFG (≥5.6mmol/L; OR:7.77, p=0.009). Additional studies by Reinehr and colleagues (2008, 2013) have shed light on the importance of glucose metabolism on cIMT. In a 2008 study of 264 children with overweight and obesity (median age: 11y, range: 7-16y), IGT was the best predictor of increased cIMT (≥0.7mm) and remained a significant correlate after adjusting for age, sex, and pubertal status in a following study of 461 children with overweight and obesity (Reinehr et al., 2013). Additionally, Chen and colleagues (2014) reported an independent relationship between elevated HbA1c (5.7-5.9%) and cIMT in a group of 524 Chinese children with obesity (mean age: 10-11years). Notably, every 1% increase in HbA1c was associated with a 2.7-fold (95%CI: 1.64-4.45) increased risk of abnormal cIMT, (≥75th for age and sex as per Bohm, Hartmann, Buck, & Oberhoffer, 2009), in boys after controlling for CVRFs (including age, blood pressure, waist circumference, triglycerides, atherogenic lipoproteins (ApoB, ApoA1), uric acid and liver enzymes (ALT, AST)) (Chen et al., 2014). To date, few studies have stratified children with obesity according to prediabetes status to investigate group differences in cIMT and those that have report conflicting results. Shah et al (2014) demonstrated higher cIMT in the internal carotid segment of the carotid artery in a group of youth with obesity (n=102, mean age: 18.3y, p=0.04), but not in the common carotid or carotid bulb segments, compared to normoglycemic controls with obesity (n=139, mean age: 18.0y) (See **Figure 2**). Similarly, Eklioglu reported no difference in cIMT in the common carotid segment between groups in a younger cohort (mean age: 11.8y). Reported associations in the internal carotid but not common or carotid bulb indicates a unique relationship between prediabetes and segment-specific cIMT, and highlights the difficulty of assessing

atherosclerotic risk when cIMT quantification methodologies differ. Given the heterogeneity of methodologies used to assess arterial thickness in the pediatric population, it is important to understand the nuances of the commonly used measures which are addressed in the following sections.

Carotid Intima-Media Thickness Methodology in Children. The American Heart Association published a statement relating to proper measurement of cIMT in children (Urbina et al., 2009b), which was followed by a similar statement from the Association for European Paediatric Cardiology in 2015 (Dalla Pozza et al., 2015). Highlights of both reviews identify the need for standardized, accurate, and reproducible methods of measurement in addition to longitudinal studies (Urbina et al., 2009b). The Mannheim Carotid Intima-Media Thickness and Plaque Consensus, a globally accepted guideline for standardized IMT in adults (2012) (Touboul et al., 2012), recommends a similar scanning and analyses protocol for children. Briefly, examinations should be performed on participants in the supine position with neck angled 45 degrees opposite of the examination probe. Scanning protocols should include a transverse scan to first identify the anatomical structures. Thereafter, a longitudinal scan should be performed by turning the transducer/examination probe to clockwise 90 degrees. Once near and far walls (with clear blood-intima (or lumen-intima) and media adventitia boundaries) of a 10mm segment can be visualized, video clips of at least 5 seconds should be recorded to allow appropriate selection of image frame during offline analysis. A minimum of 4 still frames should be used for analysis (from both left and right sides for each carotid segment

measured) (Stein et al., 2008). Although guidelines have been provided, inconsistent techniques and arterial segments (and walls) used to measure cIMT in studies to date create challenges in comparison of findings, as described below.

Quantification Methodology of cIMT: Semi-automated vs. Manual Edge Detection. Analysis of cIMT is primarily done offline. Pre-recorded video clips and images are used for measurement of cIMT with either a semi-automated or manual caliper imaging systems used for edge (or boundary) detection. It has been recommended that for small distances (i.e. limited thickness), automated (or semi-automated) boundary detected imaging systems are preferred. This newer technology system relies on the degree of image clarity and quality of scan to detect IMT boundaries and measure distance between interfaces based on pixilation of the image. The AEPC statement reports a typical number of 64 pixel measurements per 10mm segment, thus producing 64 individual measurements to calculate mean cIMT (Dalla Pozza et al., 2015). Preference to use semi-automated edge detection programs is centred around ease-of-use, as it is less resource-intensive, time-consuming, and is subject to less inter-rater variability (Peters and Bots, 2013). These programs allow for manual correction, however, excessive correction can diminish advantages of using automated technology (i.e. re-introduce reader drift or bias).

Alternatively, the more traditional method of cIMT measurement involves the placement of individual cross-hairs along defined IMT boundaries. This process is more challenging and laborious compared to semi-automated programs and require extensive training for

cross-hair placement. Although erroneous placement of individual cross-hairs may lead to over- or under-estimation, this protocol is valid and reproducible in the adult population (Lonn et al., 2001; Riley et al., 1992). Interestingly, similar reports of reproducibility and treatment effects have been shown in studies comparing both methodologies in adults (Bots & Sutton-Tyrrell, 2012; Polak, Pencina, Herrington, & O'Leary 2011) and preference to select one method over another is mostly personal preference, resources, and feasibility.

Selection of Carotid Arterial Segments. While there is debate as to which segments are best for cIMT quantification, the most commonly published cIMT protocols include either 2 or 12 segments (Urbina et al., 2009b). (2 segments: far wall of left and right common carotid artery; 12 segments: near and far wall of left and right common carotid artery (CCA), carotid bulb, and internal carotid artery (ICA) (see **Figure 2B**)). The 12 segments approach for cIMT assessment has consistently been used in large scale adult studies, including the Asymptomatic Carotid Artery Progression Study (ACAPS, n=919) (Furberg et al., 1994) and the Study to Evaluate Carotid Ultrasound changes in patients treated with Ramipril and vitamin E (SECURE, n=732) (Lonn et al., 2001; Riley et al., 1992) as endpoints to evaluate atherosclerotic risk and treatment effects. Although the acquisition of 12 segments is technically rigorous (Bots & Sutton-Tyrrell, 2012; Urbina et al., 2009b), both studies have validated their protocols and demonstrated high reliability when sonographers are uniformly trained (Espeland et al., 1996; Riley, Craven, Romont, & Furberg, 1996). Some studies have elected to restrict cIMT measurements to the CCA

segment primarily because it is highly reproducible and is consistently measured adequately (Bots & Sutton-Tyrell, 2012; Peters & Bots, 2013), as other segments are often more challenging to image. For example, in a study of 270 adults, Crouse and colleagues (1995) reported that 99% of common carotid sites and 94% percent of carotid bulb sites were evaluated adequately, whereas only 78% of internal carotid sites were acceptable for analyses. While some have shown that arterial thickness in the CCA is predictive of future cardiovascular events (Lorenz et al., 2007, Stein et al., 2008), atherosclerosis progression is not uniform throughout the carotid artery (Mackinnon et al., 2004) and may be missed when select carotid segments are overlooked. Inclusion of the carotid bulb and internal carotid artery is more informative of overall atherosclerotic burden in adults (Crouse et al., 1995, Peters & Bots, 2013) and is recommended for measurements of suspected focal thickening (or detection of plaque) regardless of age (Touboul et al., 2012).

With the limited degree of thickening present in children, the internal carotid and carotid bulb segments are often omitted when cIMT is measured in pediatric studies (Dalla Pozza et al., 2015; Urbina et al., 2009b). Many have focused on quantifying cIMT solely on the far wall of the common carotid arteries (2 segments approach) because it is less prone to inter-observer variability and is reliable in children (Urbina et al., 2009b; Bots & Sutton-Tyrell., 2012). For this reason, the normative data in children youth has been published in these segments (Doyon et al, 2013; Jourdan et al, 2005; Weberrub et al., 2015) and has contributed to the acceptance of this standard for children (Urbina et al., 2009a).

Inclusion of the near wall vs. far wall only. Clear detection and distinction of interface boundaries are pertinent for IMT measurement. cIMT is visualized by a double-line pattern produced by B-mode sonography, that is separated by the hypoechoic space. The distance between the double line pattern represents the intima-media layer and allows differentiation of the intima from the lumen (blood-intima interface) and media from adventitia (media-adventitia interface) (see **Figure 3**). This pattern is easily identifiable on the far wall of arterial segments; however, the media-adventitia interface is often more difficult to distinguish on the near wall (Wikstrand, 2007). This is primarily because the bright echoes from adjacent adventitia tissue often overlap and impede the visualization of the media-adventitia interface (Wikstrand, 2007) which create complications regarding reproducibility in poor-quality scans. For this reason, many pediatric studies have elected only to measure the far wall of specified segments (Doyon et al., 2013; Chambless et al., 1997), specifically the CCA. Although the use of the near wall is met with lower reliability, the ACAPS study has previously validated its protocol and reported high reproducibility (r=0.79), while emphasizing the importance of rigorous and standardized training of sonographers (Furberg et al., 1994; Wikstrand, 2007). The amalgamation of near wall measures with far wall measures can enhance precision without diminishing validity in adults (Furberg et al., 1994) and it is argued that the statistical power of averaging reduces random error and outweighs the possibility of increased variability (Bots & Sutton-Tyrrell, 2012; Furberg et al., 1994).

Normative cIMT values in children. As described previously, many studies have reported significant differences when comparing between groups. However, the presentation of statistically different mean cIMT values is less meaningful when not accounting for the physiological progression associated with growth (Dalla Pozza et al., 2015; Urbina et al., 2009b). Nevertheless, the literature is lacking in longitudinal normative values for children. Several published reports have attempted to provide cross-sectional normative scores for the pediatric population, however, there remain challenges with children <8 years (Bohm et al., 2009, Weberrub et al., 2015), lack of standardized methodology (Sass et al., 1998), and small sample size (Dalla Pozza et al., 2015; Jourdan et al., 2005; Bohm et al., 2009). To date, two sizeable reference datasets have been published in the pediatric age group (Doyon et al., 2013; Weberrub et al., 2015). It is important to note, however, that normative values have only been available for the far wall of the common carotid artery (CCA) segments, although recommendations by the American Heart Association suggest inclusion of the ICA and bulb (Urbina et al., 2009). Doyon and colleagues (2013) published normative mean cIMT values in a group of 1055 healthy children and youth (6-17y). This international study is the largest reference dataset of children and youth free of chronic conditions. cIMT was measured using either manual caliper or semi-automated measures in the CCA only (Doyon et al., 2013) and despite the use of different methods (semi-automated vs. manual caliper), the intra-class correlation was high (r=0.83), with a small coefficient of variation (9.9%) and limited inter-observer variability (7.3%). A more recent dataset was published in 2015 by Weberrub and colleagues in a group of 690 healthy children (7-17y) using semi-

automated measure of the far wall CCA and also demonstrated limited variability (4.79%). However, inadequate reports on children <8y (n=6) and inclusion of children with hypertension were drawbacks to using this dataset as reference population. Interestingly, Weberrub and colleagues (2015) reported similar findings to Doyon and colleagues' (2013) emphasizing the impact of SBP and adiposity (BMI SDS or z-score) on cIMT in children and youth. Although Weberrub et al. (2015) did not find any effect between age or sex on cIMT, Doyon et al. reported that boys had a higher cIMT compared to girls starting at the age of 15y, which is consistent with others that have noted sex as an important determinant of cIMT in children and youth (Morrison et al., 2010; Mittelman et al., 2010; Wiegman et al., 2004b). Both datasets have provided valuable information on cIMT throughout childhood, however the lack of longitudinal measures and those associated with long term outcomes in the literature remains problematic. Furthermore, although both studies are large, whether these data are appropriate and generalizable to all children remains to be confirmed, noting that the majority of both studies were Caucasian in race.

Summary. Given the increased risk of adult cardiovascular disease and diabetes associated with obesity and dysglycemia in childhood, it is important to initiate early treatment strategies in children to monitor risk by using inexpensive, non-invasive measures. Traditional CVRFs have been reviewed in multiple studies and age, SBP, indices of glucose regulation, and measures of adiposity have come forth as relatively consistent correlates. However, the studies cited in this review of the literature vary

widely in the methodology with regards to selection of technology (manual or semi-automated measurement), carotid segment (CCA vs. CCA + ICA + carotid bulb), and carotid wall (near or far) used to assess cIMT. In fact, inconsistent and poorly described cIMT protocols to have led to variable results between pediatric studies. With the breadth of literature dedicated to understanding determinants of cIMT in the pediatric population, there remains a gap in knowledge regarding standardized cIMT methodology and lack of conclusive longitudinal studies that relate cIMT in children to CVD outcomes in adulthood and limit our ability to determine best practices in children. Additionally, data in the obese cohort remain all the more unclear in studies that pool results from lean controls. Taken together, the inability to directly compare between studies due to methodological considerations and inconsistent study populations hinder the capacity to critically appraise published results to date in children with obesity. For this reason, the purpose of this project is to objectively characterize correlates of cIMT in children and youth with obesity and assess the comparability of the common cIMT quantification methods.

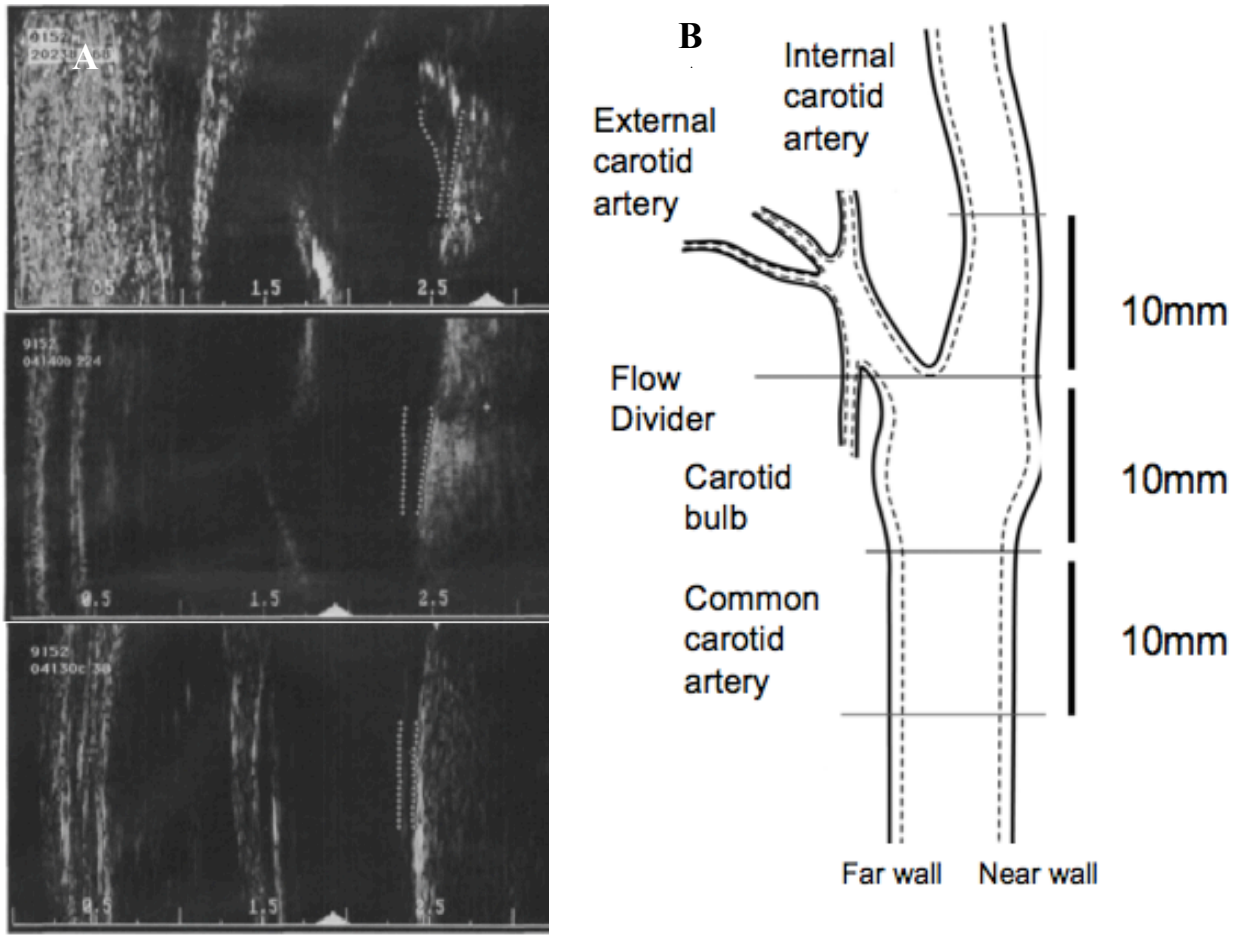


Figure 2. Measurement of Intima-media thickness using B-mode ultrasonography. **(A)** Individual cross-hairs were placed along each 10mm carotid arterial segment at every 0.05mm interval. The left line of cross hairs (seen in each panel; top-bottom) demonstrates the blood-intima interface. The right series of cross-hairs define the media-adventitia boundary. A minimum of 3 images per segment, per side were measured and used to calculate average mean cIMT. *Top panel:* Internal carotid artery was measured 0-10mm distal to the carotid flow divider. *Middle panel:* Carotid bulb was measured 0-10mm proximal to the carotid flow divider. *Bottom Panel*: Common carotid artery was measured 10-20mm proximal to the carotid flow divider. (Adapted from Riley et al., 1992). **(B)** Simplified diagram of carotid artery identifying arterial segments commonly measured. (Adapted from Polak et al., 2010).

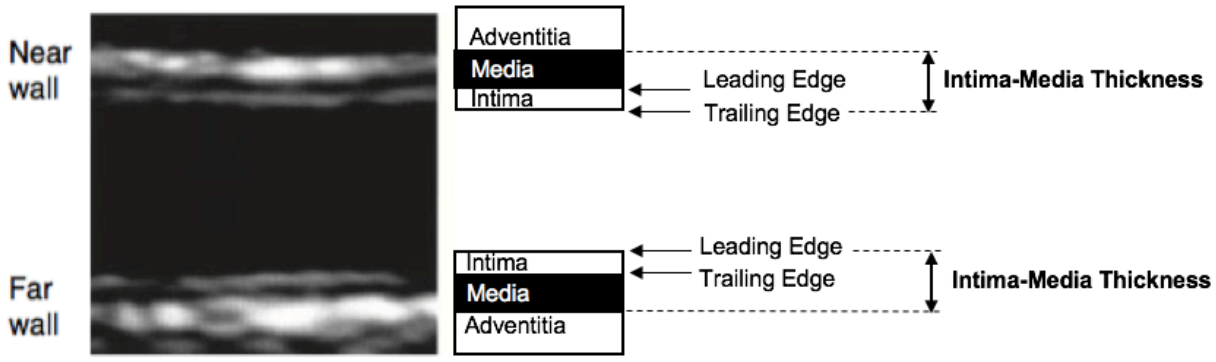


Figure 3. Echographic images of arterial intima-media thickness. The double line pattern reflects the intima and adventitia layer, which is separated by the hypoechoic space, representing the media layer. To evaluate the far wall, the leading edge of the intima layer and the media-adventitia interface are used to define boundaries for intima-media thickness (IMT) measurement. To assess the near wall, the trailing edge of the intima layer and the media-adventitia interface is used to define the margins for IMT measurement. As previously described, the media-adventitia interface is often poorly defined on the near wall, due to the hyperechoic nature of the adjacent adventitia layer. The dotted lines represent respective defining edges for IMT assessments on the near and far wall.

CHAPTER 2: STUDY DESIGN AND METHODS

Study Objectives, Rationale, and Hypotheses

Study Objectives. With a high prevalence of abnormal CVRFs in children with obesity and dysglycemia (Skinner et al., 2015), it is critical to understand underlying drivers of accelerated atherosclerosis progression. Identification and characterization of cIMT correlates would provide targets for clinical management of CV risk in this population. Additionally, evaluation of commonly used cIMT methodologies would elucidate any bias between methods and allow better interpretation of results to date.

Objective 1: The main objective of the project was to evaluate the determinants of carotid intima-media thickness (cIMT), with a specific focus on measures of glycemia, in children (age 5-17y) with obesity. As previously reported in adult studies, individuals with prediabetes have increased risk of CVD (DeFronzo & Abdul-Ghani., 2011; Levitan et al., 2004). The contributing role of dysglycemia on vascular structure warrants investigation in high-risk pediatric populations such as children and youth with obesity. As a non-invasive marker of early structural changes associated with atherosclerosis progression, carotid intima-media thickness (cIMT) was used in this study as a surrogate marker of subclinical disease and was the outcome of interest. To thoroughly assess how glycemia may contribute to subclinical thickening of cIMT in this population, measures of glycemic status were examined as exposures of interest and the following research questions have been examined.

Research questions:

1. What are the potential determinants of cIMT in children with obesity (5-17y)?
2. Is any measure of glycemia a correlate of mean cIMT in children with obesity (5-17y)?
    a) If so, is any measure of glycemia an independent correlate of mean cIMT?
    b) Do children with obesity and prediabetes have higher measures of mean cIMT compared to children with obesity and normal glycemia?

Objective 2: Given the heterogeneity of methods used to quantify cIMT in the pediatric population, the second objective of this project was aimed at examining and comparing published methodologies. To do so, the following research questions have been developed.

Research questions:

3. How similar are mean cIMT (mm) calculated using only the far wall of the CCA (2 segments approach) compared to the far and near wall of the CCA, ICA, and carotid bulb (12 segments approach)?
4. Do correlates of mean cIMT (mm) when calculated using the 12 segments approach differ from those correlates of cIMT z-scores when controlling for age, height, and sex?

Study Rationale. The foundation of the first study objective was to describe the relationship of cardiovascular disease risk factors and cIMT in children and youth with

obesity. As described previously, the majority of studies compare absolute cIMT (mm) to normal-weight controls and few report on determinants of cIMT exclusively in the obese cohort. Additionally, the role of prediabetes on the cIMT in obese children without type 2 diabetes remains unclear in children with obesity. Given that the up to 25% of obese children presenting at weight management programs have glucose dysregulation (Morrison et al., 2012, Weiss et al., 2005), it is imperative to understand the impact of dysglycemia on cIMT and potential correlates of this measure to prevent further progression and risk of accelerated cardiovascular disease.

In order to speak to the relationship between various CVRFs and cIMT in this population, it is essential to have a consistent methodological approach to assess cIMT in children. However, with varying protocols published in the pediatric literature (Dalla Pozza et al., 2015), the consensus of which carotid segments provide the best information on adult CVD risk is a current gap in the literature (Urbina et al., 2009, Bots et al., 2012). For this reason, the second objective of this project is to assess the comparability of the two most published methods used to assess cIMT in children. As described, differences in methodologies used in pediatric studies relate to the selection of carotid segments and walls used to calculate mean cIMT. The two most common methods are: the far wall of the common carotid artery (CCA) alone (a total of 2 segments) and the near and far wall of the internal carotid artery (ICA), carotid bulb, and CCA (12-segments) of the left and right arteries. By assessing comparability, this objective was aimed at identifying

methodological biases between the two most common approaches of assessing mean cIMT in children.

Study Hypotheses. With limited and inconsistent literature in children with obesity and prediabetes, our hypothesis that children with obesity and prediabetes will have a marginally higher cIMT compared to children with obesity and normal glycemia is based on similar reports in pediatric diabetic studies (Kotb et al., 2012; Urbina et al., 2009a, Urbina et al., 2013). It was also hypothesized that measures of glycemia will be positively correlated to measures of cIMT.

It was also hypothesized that the 12-segments approach will be consistently greater thickness compared to measures of 2-segments mean cIMT. This is partially due to the inclusion of bulb, which is prone to thickening compared to the CCA in adults (Crouse et al, 1995). Indeed, although measures of IMT in the CCA is reasonably is related to extent of coronary atherosclerosis, inclusion of all 12 sites has been shown to have better predictive power with coronary artery disease (Crouse et al., 1995; Bots & Sutton-Tyrell, 2012). For this reason, it is also hypothesized that the CVRFs will have a stronger correlation to the 12-segment measure of mean cIMT (mm) compared to the 2-segment measure of mean cIMT (mm).

Study Methodology

Canadian Pediatric Weight Management Registry (CANPWR). The Canadian Pediatric Weight Management Registry (CANPWR) is a prospective cohort study designed to evaluate and assess determinants of health outcomes of children and youth enrolled in weight management programs across Canada. Characterization of individual-, family- and program-level determinants of change in anthropometrics, obesity-related co-morbidities, and program attrition will provide key knowledge on the management of pediatric obesity in Canada (Morrison et al., 2014). Since the pilot study of 5 centres, the CANPWR study has expanded to 10 centres across the country. As part of the CANPWR Main study, a sub-study investigating early markers of cardiovascular disease risk factors was initiated at McMaster Children's Hospital in the fall of 2014. The CANPWR Main Study and Sub-study are 3 years in length, with follow-ups at 6 months, 12 months, 24 months and 36 months from baseline (see **Appendix A2**). The primary outcome measurement of this sub-study is carotid intima-media thickness, which is also the primary outcome measure in this project.

Study Design and Population. This study was a cross-sectional study of children and youth with obesity, age 5-17 years, presenting at the Growing Healthy Weight Management Program in the Children's Exercise and Nutrition Clinic (CENC) at McMaster Children's Hospital. The Growing Healthy Weight Management Program is a physician-referred multi-disciplinary outpatient weight management program, which

offers family-based behaviour modification centred on sustainable lifestyle changes. Program structure is based on current Canadian clinical practice guidelines (Lau et al., 2007) and evidence of modest reduction in weight from family-centred pediatric weight management interventions (Peirson et al., 2015). Study participants were consented as per the CANPWR Main and cIMT Sub-study at the time of entry into the weight management program. Potential participants were initially asked if they were interested in learning more about the CANPWR study and cIMT sub-study through a Consent-to-Contact process by an impartial health professional. If interested, parents or legal guardians provided informed consent with research personnel and the child (participant) provided informed assent, if capable. Upon consent, each participant was assigned with a non-identifiable participant code to ensure anonymous confidential participation. All potentially identifiable information was stored in a locked cupboard and separate from study data. Eligibility for the study included children and youth (5-17y) enrolled in tertiary weight management program. Exclusion criteria included children with clinically diagnosed diabetes or familial hypercholesterolemia at entry into the weight management program and children <5y. Data for this project was taken from the baseline questionnaires (and chart review) of the CANPWR Main and cIMT Sub-study at the McMaster Children's Hospital site (see **Appendix A1).** Ethics approval was received in September 2014 for the CANPWR cIMT Sub-study and has since been renewed annually, as per Hamilton Integrated Research Ethics Board guidelines.

Study Measures. Potential determinants and covariates included in this study were based on previously reported determinants and correlates of cIMT in the literature (See **Determinants of cIMT in children with obesity**). Study data was collected prospectively from review of participant clinic charts and evaluated at time of entry to pediatric weight management. These data were limited to those collected as part of clinical practice (Morrison et al., 2014). Measures of cIMT were collected within 4 weeks of CANPWR (and cIMT Sub-study) consent and enrolment.

Primary Outcome Measures. The primary outcome measure of the project is mean carotid intima-media thickness (cIMT). To address both study objectives, cIMT was evaluated using 4 measures:

1. 2 segments mean cIMT (mm): as calculated with the far wall of the CCA of the left and right carotid arteries
2. 12 segments mean cIMT (mm): as calculated with the near and far wall of the ICA, CCA, and carotid bulb of the left and right carotid arteries
3. cIMT-for-age z-score: based on published pediatric normative values for the far wall CCA of the left and right carotid arteries (2-segments) (Doyon et al., 2013)
4. cIMT-for-height z-score: based on published pediatric normative values for far wall CCA of the left and right carotid arteries (2-segments) (Doyon et al., 2013)

Carotid IMT Scanning and Measurement Protocol. Quantitative ultrasonography was performed on both right and left carotid arteries with 7.5-13 MHz linear array transducers

(GE VIVID 7 imaging system) with Doppler capabilities by a trained senior sonographer. Scans began with a transverse examination of the vessel, identifying structural landmarks (e.g. carotid flow divider), followed by a circumferential longitudinal scan to identify 3 pre-defined arterial segments for image acquisition (Morrison et al., 2010; Lonn et al., 2001; Polak et al., 2010). These segments included the: carotid bulb, internal carotid artery (ICA) and common carotid artery (CCA). Carotid arterial segments were defined relative to the carotid flow divider, which is a replicable anatomical marker (ICA: proximal 1cm from the flow divider, Bulb: tip of flow divider extending distally 1 cm, CCA: distal 1cm from flow divider extending distally 1cm) (see **Figure 2B**) (Morrison et al., 2010; Lonn et al., 2001).

A minimum of 3 video clips of at least 5 seconds (or cardiac cycles) were captured. 3 separate frames per arterial segment with clear cIMT boundaries were used for offline analysis. A minimum of 3 measurements were taken along each 10 mm segment and used to calculate the average mean cIMT. Individuals with a minimum of 4 segments with clear blood-intima and media-adventitia interfaces were included in analyses (Riley et al., 1992). cIMT scans were performed with electrocardiographic gating; images were digitized at the tip of the R-wave of the electrocardiogram (ECG) (end diastole). Measurements were taken via manual caliper to the nearest 0.05mm. Individual cross hairs were placed on the boundaries of respective interfaces (i.e. blood intima or media-adventitia) at 0.5mm intervals along each 10mm carotid arterial segment (see **Figure 2A**).

Carotid IMT z-score Calculation. Absolute cIMT measures (as described above) were converted to sex-specific z-scores for-age and for-height based on recently published normative values (Doyon et al., 2013). Reference values were reported in 1051 normotensive children and youth (6-18 years). To compute cIMT z- or SD-scores (SDS), the LMS method for developing normalized cIMT centile standards was utilized to generate a publicly available SAS macro code (or Syntax) by Quazi Ibrahim. The LMS method describes the distribution of cIMT measurements through mean (M), coefficient of variation (S) and skewness (L) (Doyon et al., 2013).

Potential determinants and covariates. Adiposity was evaluated based on sex-specific BMI percentile for age (BMI z-score), waist circumference (cm), and body fat percentage (%). BMI z-score used ***height (cm)*** and ***weight (kg)*** measured using Harpenden Stadiometre (London, UK) and an Inbody Body Composition Analyzer scale (California, USA), respectively, and were converted to ***BMI z-scores*** according to World Health Organization (2006) definitions (de Onis et al., 2007). ***Body fat %*** was also assessed (via 4-point bioelectric impedance analysis) using a Inbody Body Composition Analyzer scale (California, USA). ***Waist circumference*** was measured half-way between iliac crest and lower rib (Fernandez et al., 2004). Guardians of study participants reported ***family history of cardiovascular disease (CVD)*** – angina, myocardial infarction, coronary artery bypass surgery – in first and second degree relatives. ***Parental history of premature CVD*** was reported in biological mother and father by guardians of study participants (<55y for men, <65 for women). Family history was not verified.

Blood pressure (mmHg) was evaluated as systolic and diastolic measures via 6 serial measurements using the automated oscillometric BPTru device (Bristish Columbia, Canada) on the day of cIMT ultrasound after sitting for approximately 10 min at rest. Blood pressure was considered abnormal if SBP or DBP ≥95th percentile for age, height, and sex (Falker et al., 2004). Fasting laboratory measures were performed after a minimum of 8-hr overnight fast. Traditional lipid profiles (***low-density lipoprotein – LDL-C, high-density lipoprotein – HDL-C, triglycerides – TG, total cholesterol – TC***) were measured in a certified lab within the Greater Hamilton Area, as provided in the clinical chart during baseline medical assessment. ***Non-HDL cholesterol*** and ***TC/HDL ratio*** were also calculated and provide a more holistic measure of all of atherogenic particles (including vLDL, IDL, and Lp(a)). Abnormal lipid values were defined according to the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in children and adolescents (2011).

Glycemic measures were assessed using ***fasting plasma glucose (FPG), 2hr plasma glucose (2hrPG),*** and ***glycated hemoglobin (HbA1c)***. 2hrPG was measured using 1.75g/kg (up to 75g) oral glucose tolerance test. FPG, 2hrPG, TC, TG, and HDL cholesterols were measured using Roche analyzer and LDL was calculated using the Friedewald formula (Morrison et al., 2010). Abnormal glycemic measures were defined according to ADA 2010 Guidelines (see **Table 1**).

Confounding variables. As a growing population, ***age*** was measured to the closest month and adjusted for potential confounding effects in multivariable analyses, as was height. As a consistently reported determinant of cIMT in children with obesity (Mittelman et al.2010; Beauloye et al., 2007), ***sex*** was also accounted for in multivariable analyses.

Prediabetes Definition. As one of the exposures of interest, prediabetes was evaluated based on 3 glycemic tests: fasting plasma glucose (FPG), 2-hour plasma glucose (2hrPG) and glycated hemoglobin (HbA1c). Given that HbA1c is a measure of glycemia over a 3-month period and its current use in the pediatric clinical setting, it was included as one of the 3 glycemic tests used for prediabetes classification. As the risk for CVD and diabetes progression exists on a continuum (Shaw et al., 2000; Tirosh et al., 2011), prediabetes was defined using ADA 2010 guidelines to capture a greater number of participants at risk of developing diabetes (see **Table 1**).

Data Management and Statistical Analyses. As part of the CANPWR Main Study, all collected data was entered into the iDataFax web-based application, which is stored at the Population Health Research Institute (Hamilton, Ontario). Continuous variables are presented as mean±standard deviations. Categorical variables are presented as percentages. Baseline descriptive statistics were calculated to compare differences of children with obesity with or without prediabetes. Subsequent analysis of anthropometric characteristics, cardiovascular risk factors and cIMT values were conducted via Independent Samples t-tests. To examine the contribution of glycemia to cIMT in

children with obesity (with or without prediabetes), univariable linear regression analyses were performed. Clinically significant variables ($p<0.1$) from the univariable analysis were evaluated with multivariate linear regression analyses. This model included significant test variables (exposures) in addition to confounders to determine independent correlates of cIMT ($p<0.05$). Exposures of interest included the following modifiable CVRFs: measures of adiposity (WC, BMI z-score), blood pressure (systolic and diastolic), glycemia (HbA1c, FPG, 2hrPG), lipid profile (LDL, HDL, TG, TC, non-HDL-C, and/or TC/HDL ratio). Covariates were: family history, age, height, and gender. Collinearity was evaluated based on variance inflation factor (VIF). Any variable with VIF>10 was removed from multivariate regression model. All analyses were performed used Statistical Analysis System (SAS) version 9.2 (Cary, NC, USA) with $p=0.05$ to used as a cutoff for significance.

To examine the comparability of methods used to quantify cIMT, a series of statistical tests were performed. First, an intra-class correlation (ICC) was completed to assess the similarity between 2-segments and 12-segments mean cIMT (mm). A paired t-test was also performed to assess any mean differences between both measures and a one-sample t-test was used to evaluate if difference between measures were statistically different from zero. A Bland-Altman plot was created to illustrate presence of any bias between cIMT methodologies. Regressions analyses were subsequently performed on differences between 2-segments and 12-segments mean cIMT (mm) to assess presence and direction of other potential biases. Lastly, a comparison of independent correlates of primary

outcome variables (outlined above) was performed further understand similarities or differences of mean cIMT quantification methods.

Sample Size Calculation. The sample size was based on the primary research question aimed to define determinants of cIMT in obese children (see **Study objectives: Research Question 1**). A multi-variable model consisting of 10 variables (exposure and covariate) was used to calculate the appropriate recruitment goal for this project. The planned variables included (but were not limited to): measures of glycemia (HbA1c, FPG, 2hrPG), BMI z-score, age, height, family history of CVD, systolic BP, HDL-, and either non-HDL- or LDL-cholesterol, acknowledging that these would be dependent on univariable analyses. With this, a sample size of 170 participants was determined, by Quazi Ibrahim (CANPWR statistical analyst), to be sufficient to explain 20% of total cIMT variation. This calculation is based on a multi-variable model with 80% power (β=0.8) at 95% confidence (α=0.05) while accounting for 10% missing values (See **Appendix B1**).

To answer Research Question 2 (see **Study objectives: Research Question 2**), a clinically significant difference in mean cIMT between children with obesity and prediabetes and children with obesity without prediabetes is needed to calculate an appropriate sample size. Given the limited studies of cIMT and prediabetes in pediatric cohorts, a relative difference of 6.6% of mean cIMT (mm) is based on previously published differences in an overweight/obese prediabetic adult cohort comparing mean

cIMT (far wall CCA) (n=145, 0.76 ±0.18 vs. 0.71±0.18) (Marini et al., 2012). Using this relative difference between individuals with prediabetes (HbA1c: 5.7-6.4%) and without prediabetes (HbA1c: <5.7%), a minimum of 30 participants per group would be needed (see **Appendix B2**).

CHAPTER 3: RESULTS

Recruitment and Study flow. 346 participants were consented into the CANPWR Main study at time of entry into the Growing Healthy Weight Management Program since Spring 2013. Of the 251 participants consented in to the CANPWR Main Study since the commencement of the cIMT sub-study (Fall 2014), 165 participants consented to the sub-study, 12 individuals were not approached based on exclusion criteria (<5y), and 74 refused cIMT sub-study participant based on lack of time or inconvenience of travel. A total of 165 CANPWR participants were consented into the cIMT sub-study, yielding a 69.0% enrollment rate (See **Figure 4**).

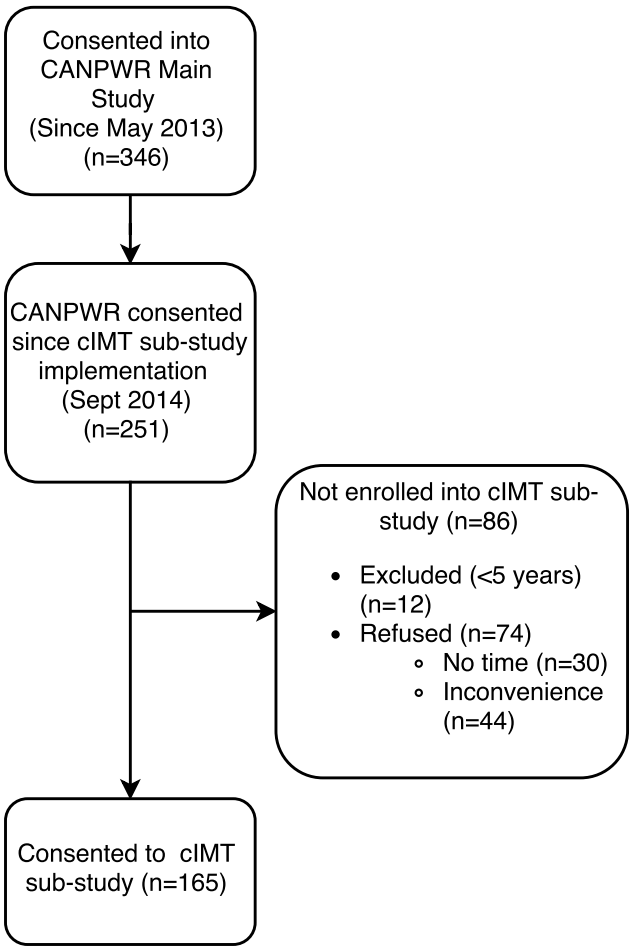


Figure 4: Study recruitment and flow. Since implementation of the cIMT sub-study in September 2014, 251 CANPWR participants were enrolled and 239 CANPWR participants were eligible for cIMT study participation. Of these, 165 have been recruited as of January 20th, 2017, yielding a 69.0% recruitment rate.

SECTION A - PARTICIPANT CHARACTERISTICS

Participant Demographics. Data from 162 participants were included for this analysis. Although 165 participants were consented, 3 participants were excluded retrospectively from analyses based on: <5 years of age (n=1) and HbA1c>6.5% (n=2). The participant excluded based on age was enrolled as a sibling to another CANPWR cIMT sub-study participant and the 2 participants excluded based on HbA1c levels met diabetes criteria. These laboratory data were collected from patient charts from participants enrolled in CANPWR study after study and sub-study consent. Participant characteristics are reported in **Table 2: Participant Characteristics**. Average age was 12.8 ±2.95y and there was a relatively equal distribution between sexes (73 male participants, 45.1%) amongst study participants. 75.3% (n=122) of study participants were Caucasian. With regards to socioeconomic status, 25.3% of study participants came from households with a total annual income of <$50,000/year (n=41). 77.1% (n=125) and 67.9% (n=110) of primary female and male caregivers attended university or college, respectively. Average BMI z-score was 3.4 ±1.03. According to WHO 2006 criteria (de Onis et al., 2007), 5.6% (n=9) of participants met overweight classification, 34.0% (n=55) of participants met obesity classification, and 59.9% (n=97) of participants met severe obesity classification for their age and sex (de Onis et al., 2007). Average waist circumference was 96.58±14.66 cm and mean body fat % was 43.71 ±7.04%.

Participant Cardiovascular Risk Factors. Family history of cardiovascular disease was reported in 44.4% (n=72) of study participants. (Family history was defined as presence

of CVD in first and second degree relatives – parents, siblings, aunts/uncles, and grandparents.) Parents of 13 participants (8.0%) reported premature CVD (angina, myocardial infarction, or stroke; <55y for males, <65y for females). Mean systolic and diastolic blood pressures were 110.3 ±11.38 mmHg and 69.6 mmHg ± 9.11, respectively. Mean total cholesterol was 4.28 ± 0.68 mmol/L, mean LDL-cholesterol was 2.54 ± 0.62 mmol/L, and mean HDL-cholesterol was 1.15 ± 0.21 mmol/L. Non-HDL cholesterol and total cholesterol-to-HDL ratio were 3.12 ± 0.69 mmol/L and 3.83 ± 0.90 mmol/L, respectively. Average fasting plasma glucose was 4.74 ± 0.39 mmol/L and average HbA1c was 5.26 ±0.31 %. Average blood glucose value after 2hr oral glucose tolerance tests was 5.92 ± 1.22 mmol/L.

Prevalence of CVRFs. Abnormal laboratory measures were reported in over one-third of participants. 34.3% (n=50) of study participants assessed had abnormal triglycerides (≥1.5 mmol/L) and 24.7% (n=36) of study participants assessed had abnormal HDL cholesterol levels (<1.0 mmol/L). Elevated total cholesterol and LDL cholesterol were less prevalent in the study population, with 9.7% (n=14) and 9.0% (n=13) participants meeting abnormal criteria, respectively. 18.6% (n=27) of participants assessed had abnormally high levels of non-HDL cholesterol. 2.1% (n=3) of participants had impaired fasting glucose and 5.1% (n=4) of participants assessed had impaired glucose tolerance. 9.7% (n=13) of participants met prediabetes criteria based on elevated HbA1c levels. Abnormal SBP and DBP were present in 6.9% (n=11) and 8.2% (n=13) of participants, respectively.

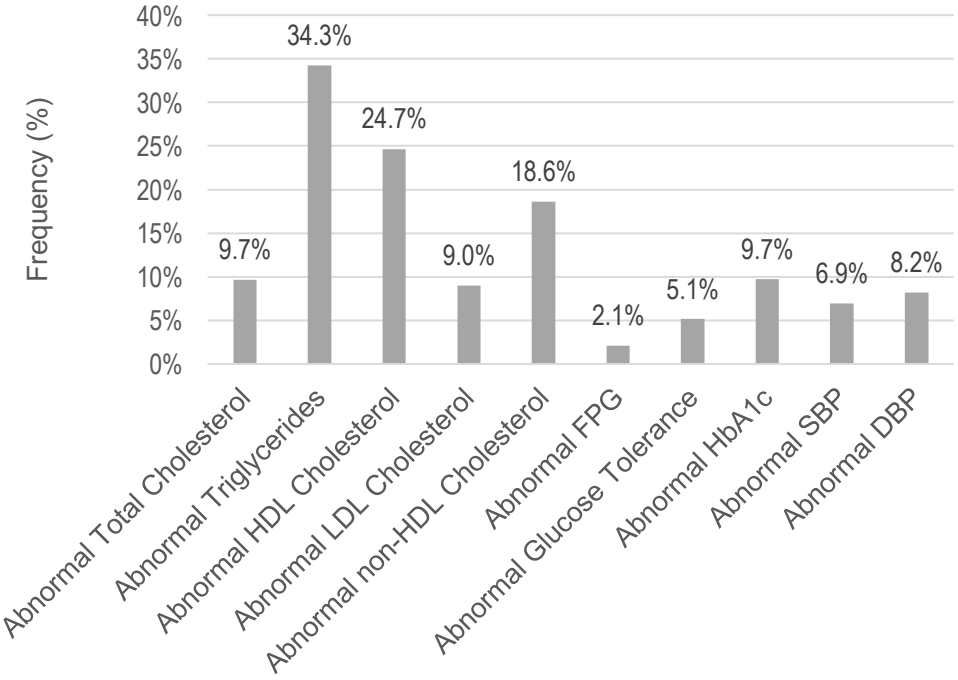


Figure 5. Prevalence of CVRFs. Cardiovascular risk factors were assessed based on current pediatric guidelines. (Falker et al., 2004; Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, 2011; American Diabetes Association, 2010). Abnormal values included: TC ≥5.2 mmol/L; TG ≥1.5 mmol/L; HDL <1.0 mmol/L; LDL≥3.4 mmol/L; Non-HDL ≥3.8 mmol/L; FPG ≥5.6 mmol/L; 2hrPG ≥7.8mmol/L; HbA1 ≥5.7%; SBP and DBP ≥95th percentile for age, height and sex.

Table 2: Participant Characteristics

Variable	All*	Normal Glycemia	Prediabetes	p-value
N	162	129	17	
Participant Demographics				
Age(y), n	12.77±2.95,162	12.57±3.00,129	13.65±2.62,17	0.163
Sex: Male (%), n	73(45.06),162	55(42.64),129	8(47.06),17	0.729
Race: Caucasian (%), n	122(75.31),162	101(78.29),129	8(47.06),17	**0.014**
Annual Household Income: <$50,000/year (%), n	41(25.31),162	33(25.58),129	5(29.41),17	0.771
Female Caregiver Education: attended university/college (%), n	125(77.16),162	102(79.07),129	11(64.71),17	0.218
Male Caregiver Education: attended university/college (%), n	110(67.90),162	89(68.99),129	11(64.71),17	0.721
Weight (kg), n	83.69±25.71,161	80.87±24.50,128	93.41±29.90,17	0.055
Height(m), n	1.57±0.14,161	1.56±0.14,128	1.62±0.13,17	0.091
BMI (kg/m^2), n	33.03±6.20,161	32.47±5.89,128	34.92±8.42,17	0.260
BMI z-score, n	3.37±1.01,161	3.33±1.01,128	3.41±1.27,17	0.756
Waist Circumference (cm), n	96.56±14.66,107	95.30±14.71,88	100.05±13.46,10	0.332
Body fat %, n	43.71±7.04,140	43.48±7.16,111	44.51±7.48,16	0.591
Participant Cardiovascular Risk Factors				
Family Hx of CVD (%), n	72(44.44),162	53(41.09),129	8(47.06),17	0.639
Parental Hx of premature CVD (%), n	13(8.02),162	8(6.20),129	1(5.88),17	1.000
Systolic BP (mmHg), n	110.27±11.38,160	109.70±11.05,128	114.67±12.87,17	0.090
Diastolic BP (mmHg), n	69.57±9.11,160	69.06±9.12,128	73.19±8.98,17	0.081
Total Cholesterol (mmol/L), n	4.28±0.68,145	4.26±0.69,128	4.40±0.58,15	0.450
Triglycerides (mmol/L), n	1.36±0.67,146	1.31±0.65,129	1.80±0.70,15	**0.007**
HDL Cholesterol (mmol/L), n	1.15±0.21,146	1.16±0.21,129	1.07±0.26,15	0.131
LDL Cholesterol (mmol/L), n	2.54±0.62,145	2.53±0.63,128	2.58±0.49,15	0.809

Variable	All*	Normal Glycemia	Prediabetes	p-value
Non-HDL Cholesterol (mmol/L), n	3.12±0.69,145	3.10±0.70,128	3.33±0.55,15	0.225
TC/HDL ratio, n	3.83±0.90,145	3.77±0.85,128	4.32±1.14,15	**0.023**
Fasting Plasma Glucose (mmol/L), n	4.74±0.39,145	4.71±0.37,128	4.96±0.50,17	**0.013**
2-hr Plasma Glucose (mmol/L), n	5.92±1.22,78	5.75±0.97,65	6.77±1.91,13	0.083
HbA1c (%),n	5.26±0.31,134	5.19±0.25,118	5.79±0.22,16	**<0.001**
Participant cIMT measurements				
Average Mean cIMT, 12-segments (mm), n	0.40±0.03,162	0.40±0.03,129	0.40±0.03,17	0.754
Average Mean cIMT, 2-segments (mm),n	0.39±0.03,162	0.39±0.03,129	0.39±0.04,17	0.940
Mean cIMT-for-age z-score, n	0.10±0.72,161	0.13±0.72,128	0.05±0.82,17	0.656
Mean cIMT-for-height z-score, , n	0.07±0.72,160	0.10±0.71,127	0.04±0.90,17	0.753

All measures are reported as mean±standard deviation unless otherwise stated.

***n=16 participants did not have any measure of glycemia at time of entry into weight management.**

Prevalence of Prediabetes in Children with Obesity. Prediabetes was defined using three measures: fasting plasma glucose, 2hr plasma glucose (2hr PG), and glycated hemoglobin (HbA1c). Current guidelines of screening for dysglycemia in children suggest the use of oral glucose tolerance tests be exclusive to children ≥10y with the exception of children <10y who are very obese (BMI ≥99th percentile for age and gender) or present with multiple risk factors (high-risk ethnic group, family history of T2DM, signs of insulin resistance) (Canadian Diabetes Association, 2013). For this reason, the number of tests used to screen for dysglycemia is presented based on age (participants <10y: n=33, participants ≥10y: n=129) in **Table 3**. FPG values were missing in 6% of children under the age of 10 (n=2) and 11.6% of those ≥10y (n=15). 45.3% of children ≥10y did not have an OGTT done (n=58). HbA1c was not assessed in 18.2% of children <10y (n=6) and 17% of children ≥10y (n=22).

46.9% (n=76) of study participants had all 3 glycemic measures (FPG, 2hrPG, and HbA1c). An additional 35.2% (n=57) of the population only had FPG and HbA1c assessed (See **Figure 6**). A total of 145 fasting plasma glucose measures (or 89.5% of the total population) were available for analyses, of which 31 were done in children <10y. Impaired fasting glucose was found in 3 participants (2.2% of those who had FPG tested), n=1 <10y and n=2 ≥10y (See **Table 3**). 78 OGTTs were available for analyses and 89.7% of tests were conducted in children ≥10y (n=70). IGT was detected in 5.1% of children who had an OGTT performed and all were in participants ≥10y (n=4). HbA1c was measured in 134 participants and 9.7% (n=13) of those participants met ADA 2010

prediabetes criteria (HbA1c ≥5.7%). Overall, n=17 participants had at least one glycemic measure within the prediabetic range and these individuals were grouped together to compare with children and youth with normal glycemia. N= 1 participant had IFG only, n=2 had IGT only, n=1 had IGT + IFG, n=1 had IFG + elevated HbA1c, n=1 had IGT + elevated HbA1c, and n=11 had only elevated HbA1c (as seen in **Table 3**). No participant presented with IFG + IGT + elevated HbA1c at time of entry into weight management. With 9.9% of study participants (n=16) without any glycemic tests, there is a possibility that prediabetes was missed in participants without any measure of glycemic status.

Table 3. Number of tests and Prevalence of Prediabetes based on FPG, 2hrPG, and HbA1c tests

Glycemic Test	Normal Glycemia Range		Prediabetes Range	
	<10y	≥10y	<10y	≥10y
Fasting Plasma Glucose (n=145)	30	112	1	2
Oral Glucose Tolerance Test (2hr PG) (n=78)	8	66	0	4
HbA1c (n=134)	27	94	0	13

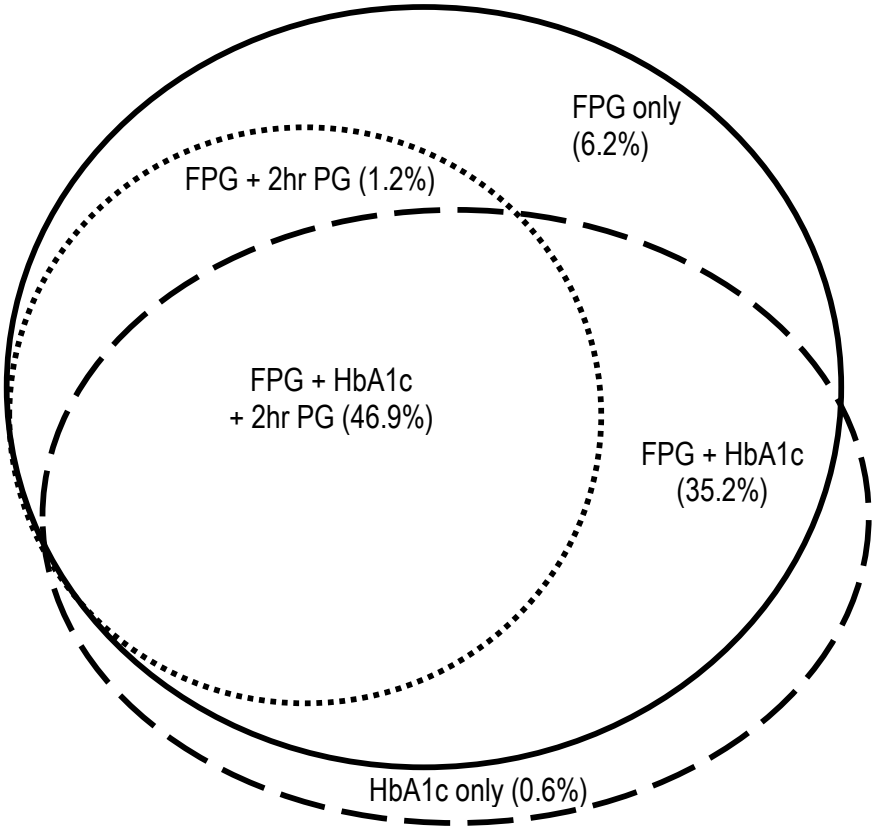


Figure 6. Proportion of glycemic tests performed. At least one measure of glycemia was available to screen for glycemic status in 146 participants (90.1% of total study population). 46.9% of study participants had all 3 tests performed (n=76). 6.2% of study participants had glycemia assessed solely with FPG (n=10), 1.2% with FPG and 2hr PG alone (n=2), 35.2% with FPG and HbA1c alone (n=57), and 0.6% (n=1) with HbA1c alone. Solid line = FPG (n=145); Dotted line = 2hr PG (n=78); Dashed line = HbA1c (n=134).

Participant Characteristics between Children with Normal Glycemia and Prediabetes. Differences between groups are reported in **Table 2**. Participants did not differ in age or sex (p=0.163 and p=0.729, respectively). A smaller proportion of individuals with prediabetes were Caucasian (47.1% vs. 78.3%, p=0.014) and the 9 participants that were not Caucasian were of South East Asian (n=4), African Canadian (n=1), Arab (n=1), Latin American (n=2) and Caribbean (n=1) descent. Chi-square tests revealed no proportional differences in reported socioeconomic factors such as annual household income (p=0.771) or parental education (p=0.218 for female caregiver, p=0.721 for male caregiver). With regards to anthropometry, children with prediabetes had similar BMI (32.47 ±5.89 vs. 34.92±8.42, p=0.260), and BMI z-score (3.33 ±1.01 vs. 3.41 ±1.27, p=0.756) as children with normal glycemia. Participants with prediabetes did not differ in body fat % (p=0.591) or waist circumference (p=0.332). There was no difference in systolic or diastolic blood pressure between children with or without prediabetes (109.70±11.05 vs. 114.67±12.87, p=0.090; 69.06±9.12 vs. 73.19±8.98, p=0.081, respectively). Children with prediabetes had higher triglyceride values (1.31±0.65 vs. 1.80±0.70, p=0.007) and TC/HDL ratio (4.32±1.14 vs. 3.77±0.85, p=0.023), however no other lipid measure was significantly different between groups.

Not surprisingly, measures of glycemia were higher in children with prediabetes, although the difference between 2hr PG was not significant (p=0.083). HbA1c was higher in children with prediabetes compared to children with normal glycemia (5.79±0.22 mmol/ L vs. 5.19±0.25 mmol/L, p<0.001) as was mean FPG (4.96±0.50 mmol/L vs.

4.71±0.37 mmol/L, p=0.013). When prediabetes was classified using solely IFG and IGT, only n=6 participants had positive glycemic values. Using this classification, participant demographics and CVRFs were similar in range and no statistical differences were found between groups, aside from all glycemic measures (See **Appendix C1**).

SECTION B - GLYCEMIA AND cIMT

Mean cIMT between Children Normal Glycemia and Prediabetes. There was no significant difference between measures of cIMT in children with or without prediabetes (12-segment mean cIMT (mm): 0.40±0.03 vs. 0.40±0.03, p=0.754; 2-segment mean cIMT (mm): 0.39±0.03 vs. 0.39±0.04, p=0.940; cIMT-for-age z-score: 0.13±0.72 vs. 0.05±0.82; cIMT-for-height z-score: 0.10±0.71 vs. 0.04±0.90, p=0.753)

Measures of Glycemia and cIMT. Measures of glycemia were assessed for their relation to measures of cIMT using Pearson correlations. Although there was no statistically significant relationship between FPG and cIMT when measured using the far wall of the CCA (2 segments) (r=0.126, p=0.131 for mean cIMT (mm, 2 segment); r=0.070, p=0.403 for FPG and cIMT-for-age z-score; r=0.072, p=0.390 cIMT-for-height z-score), there was a weak correlation present between FPG and mean cIMT when measured by the 12-segment approach (r=0.166 p=0.047). The distributions of these relationships is reported in **Figure 7**.

In those children that had an OGTT (n=78), 2hr plasma glucose values were not associated with any measure of cIMT (r=0.044, p=0.703 for 12-segment mean cIMT; r= -0.116, p=0.314 for 2-segment mean cIMT; r= -0.088, p=0.443 for cIMT-for-age z-score; r= -0.105, p=0.361 for cIMT-for-height z-score) (see **Figure 8)**. Similar to 2hr PG, using Pearson correlations, no statistical association between HbA1c values and measures of cIMT (r=0.054, p=0.537 for 12-segment mean cIMT; r= -0.070, p=0.419 for 2-segment mean cIMT; r= -0.099, p=0.259 for cIMT-for-age z-score; r= -0.097, p=0.268 for cIMT-for-height z-score). Distribution of mean cIMT measures across HbA1c values are illustrated in **Figure 9**.

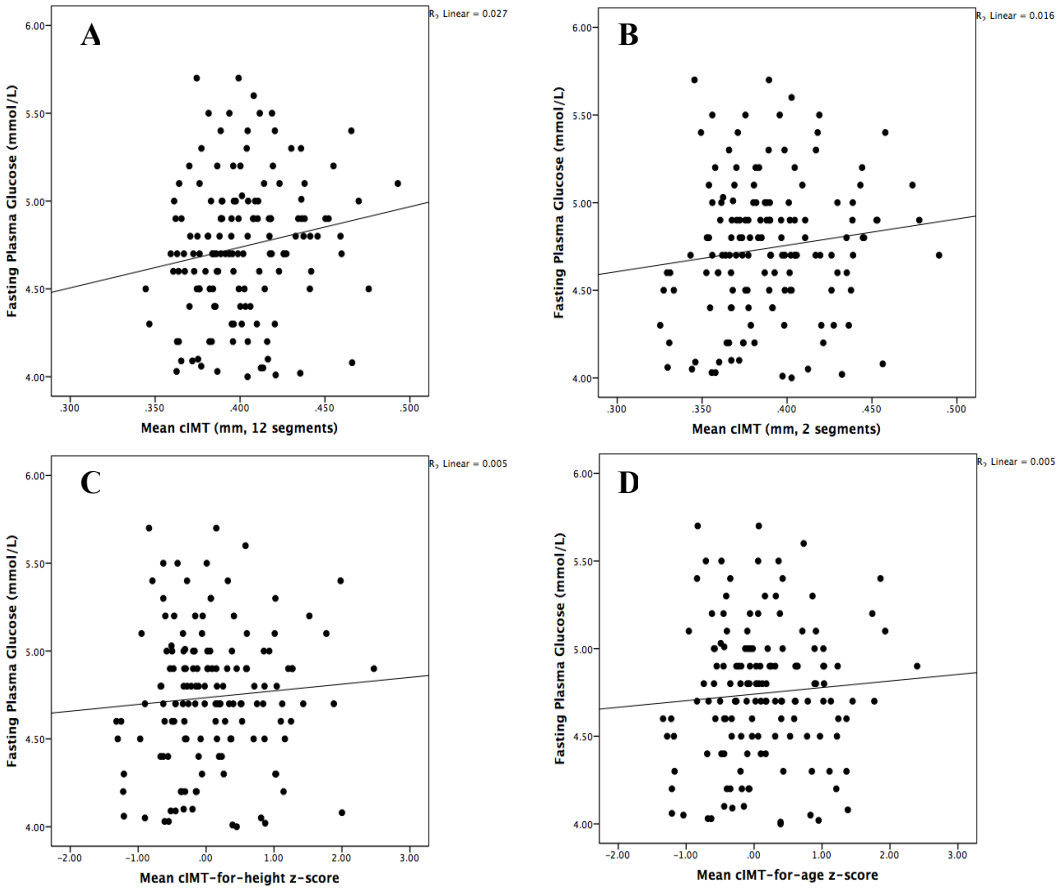


Figure 7. Distribution of FPG values and measures of cIMT. **(A)** FPG and mean cIMT (as measured by far and near wall of the CCA, ICA, bifurcation) (mm): mean cIMT as calculated with 12 carotid segments was associated with baseline FPG (r=0.166, p=0.047). **(B)** FPG and mean cIMT (as measured using the far wall of the CCA segment) (mm): far wall CCA cIMT was not associated with FPG (r=0.126, p=0.131). **(C)** FPG and cIMT-for-age z-score: similar to 2-segment mean cIMT, when these values were converted to z-scores that accounted for age and sex, the two variables were not statistically related (r=0.070, p=0.403). **(D)** FPG and cIMT-for-height: FPG was not associated with cIMT-for-height z-score (r=0.072, p=0.390).

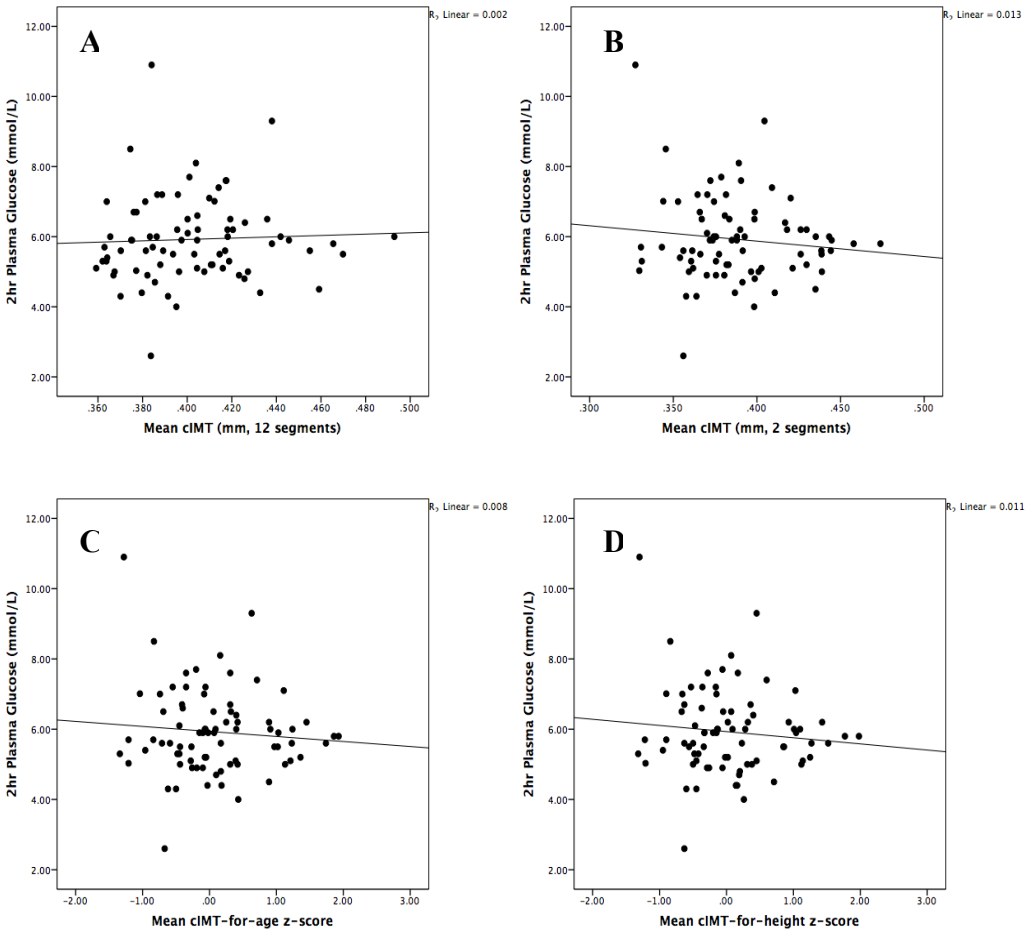


Figure 8. Distribution of 2hr PG values and measures of cIMT. (A) 2hr PG and mean cIMT (as measured by far and near wall of the CCA, ICA, bifurcation) (mm): mean cIMT, as calculated with 12 carotid segments, was not associated with baseline 2hr PG (r=0.044, p=0.703). **(B)** 2hr PG and mean cIMT (mm): far wall CCA cIMT was not associated with FPG (r= -0.116, p=0.314). **(C)** 2h PG and cIMT-for-age z-score: were not significantly associated (r= -0.088, p=0.443). **(D)** 2hr PG and cIMT-for-height: 2hrPG was not correlated with cIMT-for-height z-score (r= -0.105, p=0.361).

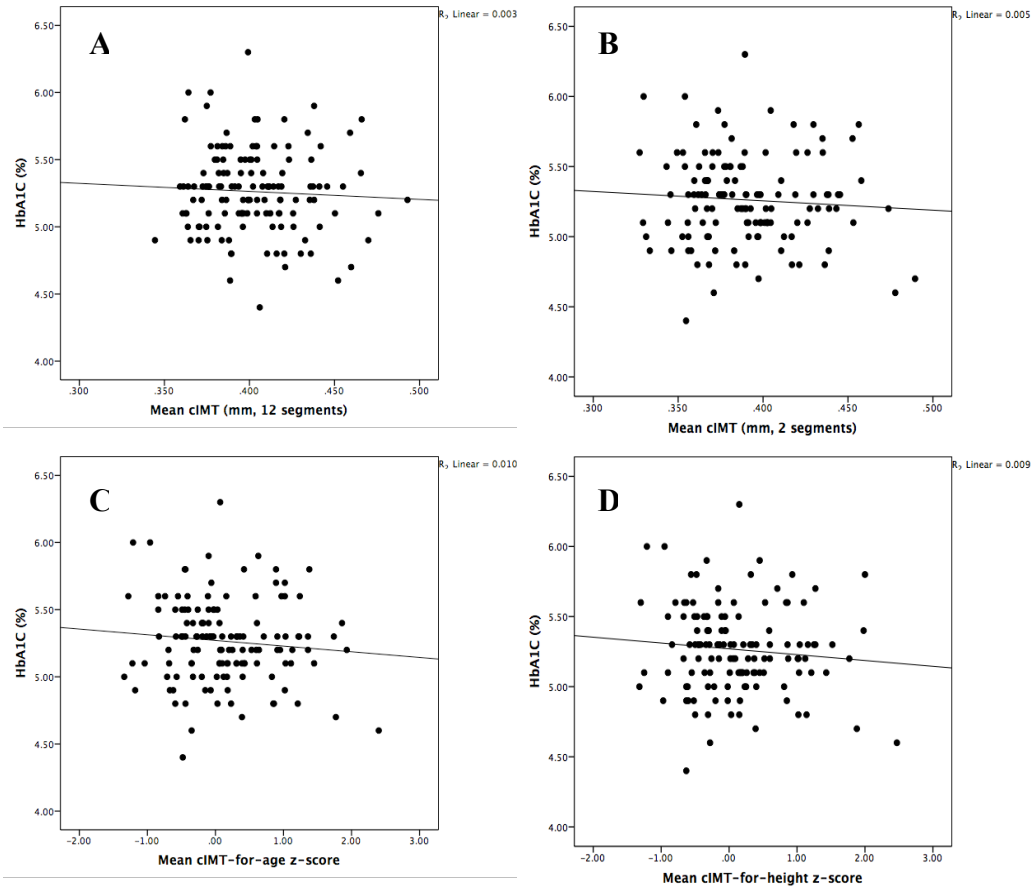


Figure 9. Distribution HbA1c and measures of cIMT. (A) HbA1c and mean cIMT (as measured by the far and near wall of the CCA, ICA, bifurcation) (mm): mean cIMT (mm), as calculated with 12 carotid segments, was not associated with HbA1c (r= -0.054, p=0.537). **(B)** HbA1c and mean cIMT (mm): mean cIMT (mm) from the far wall of the CCA was not related with HbA1c (r= -0.070, p=0.419). **(C)** HbA1c and cIMT-for-age z-score: did not exhibit a significant correlation (r= -0.099, p=0.259). **(D)** HbA1c and cIMT-for-height was not associated with cIMT-for-age z-score (r= -0.097, p=0.268).

SECTION C - CAROTID INTIMA-MEDIA THICKNESS MEASUREMENTS

Mean cIMT across cIMT Measures and Carotid Segments. Carotid intima media thickness (cIMT) was measured in all 162 participants. Common carotid artery (CCA) and carotid bulb IMT was available for all 162 participants and internal carotid artery (ICA) thickness was only available for 145 participants (see **Table 4**). cIMT-for-age and for-height z-scores were calculated for 161 and 160 participants, respectively, based on available reference values (Doyon et al., 2013). cIMT-for-age z-scores were not calculated for 1 participant because reference values were only available for 6-18y (participant was 5y) and cIMT-for-height z-scores were not calculated for 2 participants as reference values were only available for individuals 120cm or greater. Average Mean cIMT (mm) was calculated using the 2-segment and 12-segment approach (n=162 for each). The inclusion of near and far wall of the carotid bulb and internal carotid segments (12 segment approach) yielded greater thickness (0.40 ±0.03mm vs. 0.39±0.03mm, $p<0.001$) (see **Table 2**). Compared to only including the far wall, inclusion of the near wall resulted in increased IMT in the carotid bulb (0.43±0.04 vs. 0.44±0.04mm, $p<0.001$) and CCA segment (0.39±0.03 vs. 0.40±0.03mm, $p<0.001$) (see **Table 4**). When carotid segments were compared, the carotid bulb was consistently higher than CCA ($p<0.001$) and ICA ($p<0.001$) segments, irrespective of including the near wall (see **Table 4**).

Table 4. Mean cIMT by arterial segment

	Far wall only	Far and Near walls	p-value
CCA IMT (mm) (n=162)	0.39±0.03*	0.40±0.03*	**p<0.001**
Carotid Bulb IMT (mm) (n=162)	0.43±0.04	0.44±0.04	**p<0.001**
ICA IMT (mm) (n=145)	0.36±0.03*	0.36±0.03*	0.282

*All measures are presented as mean±SD unless otherwise stated. *significantly different than carotid bulb IMT (p<0.001).*

Comparability of Average Mean cIMT: 2- vs. 12-segment. An intra-class correlation revealed a moderate correlation between methodologies (ICC: 0.65, 95%CI: 0.55-0.73, see **Figure 11**). The Bland-Altman plot revealed an average variability of 0.012mm between measures (mean difference: -0.012mm, 95%CI: -0.016 to -0.009) (see **Figure 12**) and suggests that inclusion of all arterial segments yields an average mean cIMT value 0.012mm greater than measures limited to the far wall CCA segment. Although the recommended 95% of measures were within the limits of agreement (±1.96SD of mean difference), a one-sample t-test revealed that the mean difference of 0.012mm was significant. Further regression analysis revealed a proportional bias between differences of the two cIMT quantification methods. This was assessed by the following regression equation: (difference between cIMT quantification methods) = intercept + slope (average between cIMT quantification methods), where there was a significant slope, or bias, of 0.19 (95%CI: 0.06 to 0.31, p=0.003). This positive proportional bias implies that the difference between measures increases as the magnitude of mean cIMT increases.

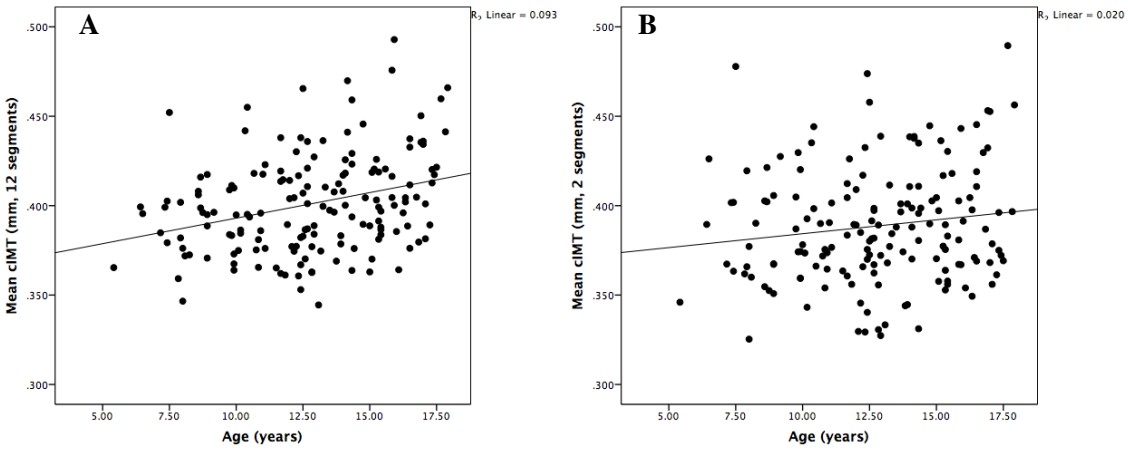


Figure 10. Mean cIMT dataset over age using different cIMT quantification approaches. The distribution of mean cIMT over age is shown between the two common methodologies used to assess cIMT in children: **(A)** near and far wall of the CCA, ICA, and carotid bulb (12-segments), and (**B**) Far wall of the CCA (2-segments).

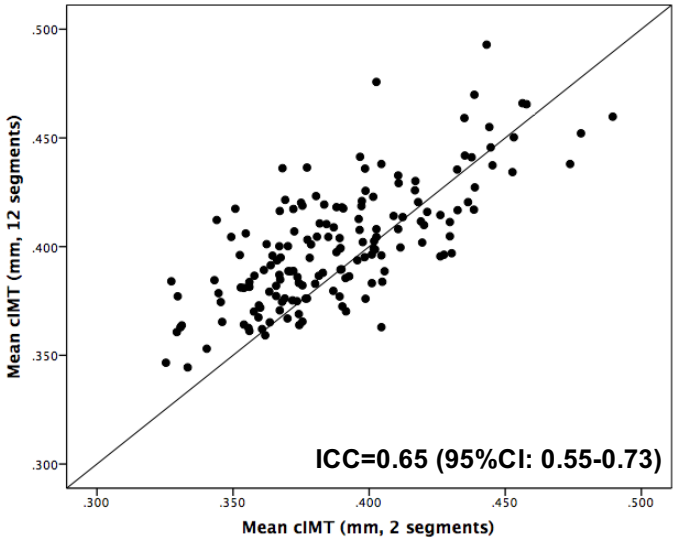


Figure 11. Intra-class correlation coefficient between cIMT methodologies. An Intra-class correlation was performed to assess similarity of cIMT methodologies commonly used in pediatric literature. Between the 2-segment and 12 segment approach, ICC=0.65 (95%CI: 0.55-0.73), suggesting a moderate resemblance between the two measures.

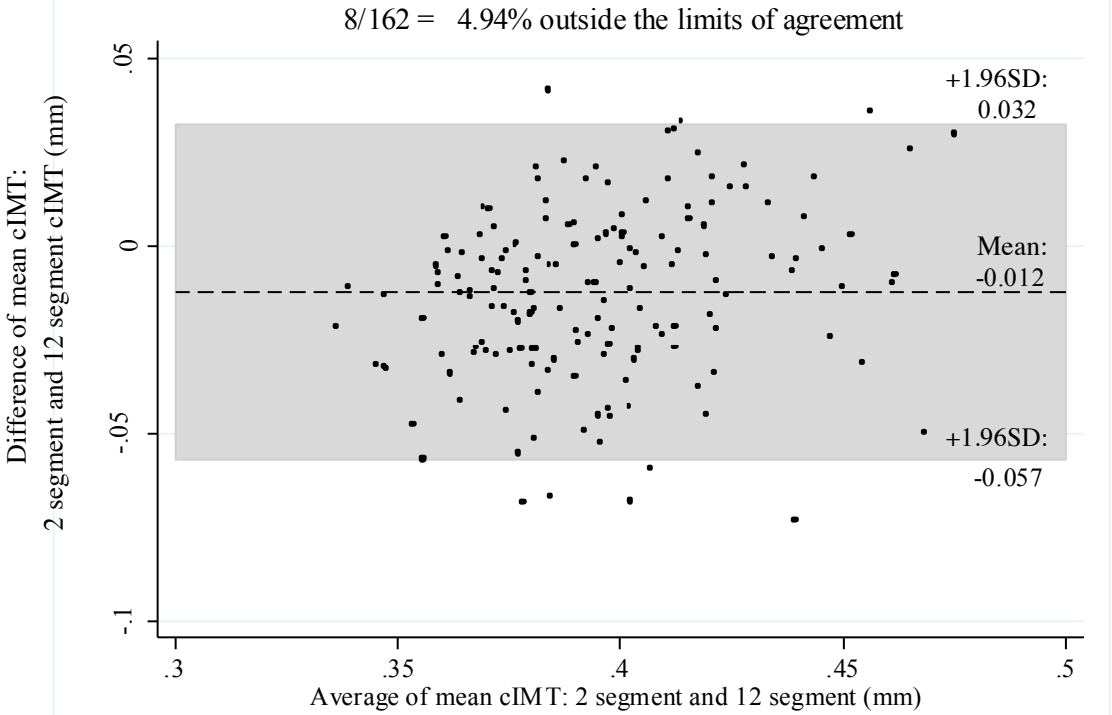


Figure 12. Bland Altman Plot for cIMT method comparisons Absolute difference between mean cIMT (mm) as calculated by 2- and 12-segment approaches are displayed on the y-axis. Average of both measures of mean cIMT is shown on the x-axis. Regression analyses revealed a slightly positive proportional bias between measures of mean cIMT (slope= 0.19, 95%CI: 0.06 to 0.31, p=0.003), indicating that variability between measures increases significantly with greater arterial thickness. Shaded area indicates agreement interval (defined by ±1.96SD of mean difference between measures). This area represents 95% of the differences between 2-segment and 12-segment mean cIMT.

Distribution of cIMT z-scores. Distribution of cIMT-for-age and for-height z-scores are presented in **Figure 13**. The distribution of z-scores in both populations were normal and slightly shifted to the right (mean cIMT-for-age z-score: 0.10 ± 0.72 and mean cIMT-for-height z-score: 0.07 ± 0.72). 13.0-13.2% of study participants had a cIMT-for-age and -height z-score ≥1 whereas only 4.4-5.6% of study participants presented with cIMT z-scores ≤1 (See **Figure 13**). Although a mean cIMT z-score greater than 0 would suggest that our current study population had slightly higher mean cIMT z-scores as compared to reference values matched for age or height (Doyon et al, 2013), a one-sample t-test revealed that mean cIMT z-scores were not statistically different from 0 (p=0.07 for cIMT-for-age z-score; p=0.23 for cIMT-for-height z-score). Skewness coefficients for mean cIMT-for-age and cIMT-for-height were 0.451± 0.191 and 0.511 ± 0.192, respectively, which are within limits for normality (Tabachnik & Fidell, 2001) (see **Appendix C3**) and Kolmogorov-Smirmov test for normality supported these findings (p=0.07 for cIMT-for-age z-score and p=0.23 for cIMT-for-height z-score).

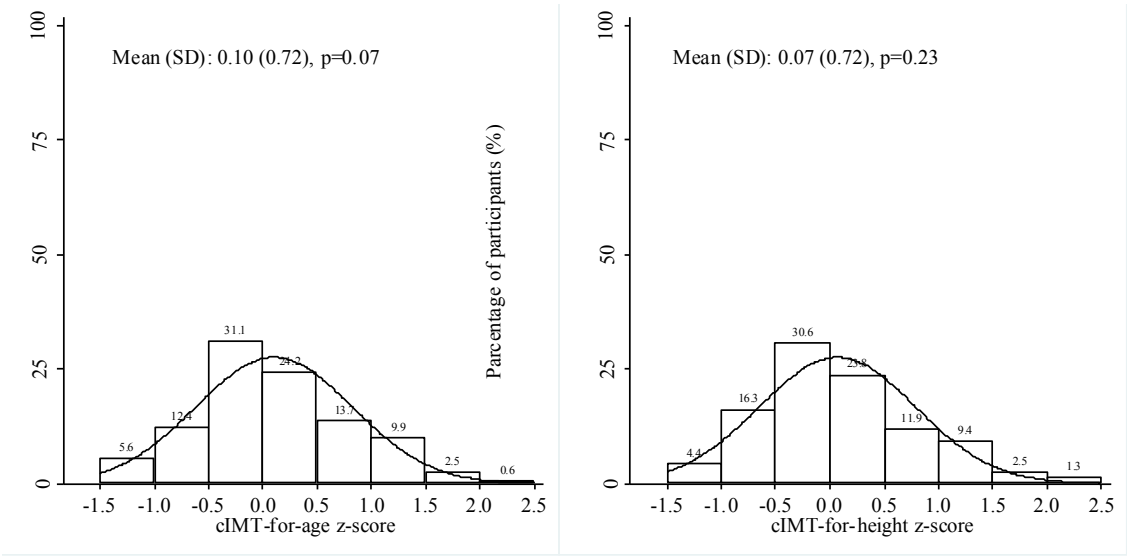


Figure 13. Distribution of cIMT-for-age and for-height z-scores. cIMT z-scores were normally distributed according to and Kolmogorov-Smirmov test for normality (p=0.07 for cIMT-for-age z-score and p=0.23 for cIMT-for-height z-score). cIMT z-scores were calculated using the 2-segment (far wall CCA) approach according to reference values published by Doyon et al (2013).

SECTION D – DETERMINANTS OF cIMT

Correlates of Mean cIMT Measures. Univariable regressions were conducted to identify potential correlates of mean cIMT measures. As expected based on previous literature, sex, age, and height were prominently associated with measures of mean cIMT (as calculated with 2 and 12 segments, see **Table 5**). Interestingly, age was only related to mean cIMT (mm) when calculated using all 12 carotid segments and explained 9.3% of variance ($\beta = 0.003 \pm 0.0007$, $r^2 = 9.3\%$, $p<0.001$). This finding was attributable to the inclusion other arterial segments (and both near and far walls were included), as age was related to carotid bulb IMT ($\beta = 0.004 \pm 0.0009$, $r^2 = 11.9\%$, $p<0.001$) and internal carotid IMT ($\beta = 0.003 \pm 0.0007$, $r^2 = 8.8\%$, $p<0.001$) in a subsequent segment-specific analyses (**Appendix C5**). (Age was also significantly related to thickness in the carotid bulb ($p<0.001$) and ICA segments ($p=0.004$) when limited to far wall only). Age was not significantly related to 2-segment mean cIMT (mm) ($p=0.07$) or cIMT-for height z-score ($p=0.77$). It is possible that most of the age effect is corrected for when height is standardized, indicating that its impact may be secondary to the influence of height. Indeed, height was related to both absolute measures of mean cIMT (mm) and explained 3.2% and 10.3% variance when calculated using the 2-segments and 12-segments approaches, respectively ($\beta = 0.0004 \pm 0.0002$, $r^2 = 3.2\%$, $p=0.02$; $\beta = 0.001 \pm 0.0001$, $r^2 =$

10.3%, p<0.01, respectively). Height was associated with all segments of the carotid artery (**Appendix C5**), but not when age and sex were adjusted for in cIMT-for-age z-scores (p=0.46). Taken together, these findings suggest a robust relationship between arterial thickness and height, which may be mediated by age.

Male sex was also associated with 12-segment mean cIMT (mm) (β = 0.011 ± 0.0043, r^2= 3.7%, p=0.015) but not when cIMT was calculated with the far wall of the CCA segments alone (2-segments) (p=0.07). When analyzed based on individual segments, male sex was only related to the carotid bulb IMT (near + far wall: β = 0.017 ± 0.0009, r^2= 5.65%, p=0.002; far wall only: β = 0.015 ± 0.0057, r^2= 4.15%, p=0.009), which may drive the relationship between sex and 12-segment mean cIMT (mm). History of premature CVD reported in biological parents was significantly related to cIMT z-scores (β = -0.485 ± 0.2059, r^2= 3.4%, p=0.02 for cIMT-for-age z-score; β = -0.415 ± 0.2070, r^2= 2.5%, p=0.047 for cIMT-for-height z-score) but no measures of absolute mean cIMT (mm) (2-segments: p=0.082; 12-segments: p=0.302). Similarly, presence of CVD in first- and second-degree family members (regardless of age) was also associated with cIMT z-scores (β = -0.291 ± 0.1124, r^2= 4.0%, p=0.01 for cIMT-for-age z-score; β = -0.261 ± 0.1132, r^2= 3.26%, p=0.02) but not absolute measures of cIMT (mm) (p=0.054 for 2

segment mean cIMT, p=0.136 for 12 segment mean cIMT). No measure of socio-economic status (annual household income, female, or male caregiver education) was related to any measure of cIMT (p>0.05).

Adiposity was assessed using BMI z-score, waist circumference, and body fat %. BMI z-score was not related to any measure of cIMT (or carotid segment). Greater waist circumference (cm) was associated with greater mean cIMT (mm) when all carotid segments were included ($\beta = 0.001 \pm 0.0002$, $r^2 = 7.3\%$, p=0.005) but not when cIMT was limited to measures using only the far wall of the CCA (p=0.27 for 2 segment mean cIMT (mm), p=0.0.55 for cIMT-for-age; p=0.67 for cIMT-for-height). Similar to male sex, the role of waist circumference on arterial thickness was limited to the carotid bulb (near + far wall: $\beta = 0.001 \pm 0.0002$, $r^2 = 4.7\%$, p=0.024; far wall only: $\beta = 0.001 \pm 0.0002$, $r^2 = 4.02\%$, p=0.038) as no relationship was found with the CCA (near + far wall: p=0.113, far wall only: p=0.265) or ICA segments (near + far wall: p=0.254; far wall only: p=0.635) (**Appendix C5**). Body fat % was only related to mean cIMT (mm) when assessed with the far wall CCA segments ($\beta = -0.001 \pm 0.0004$, $r^2 = 3.97\%$, p=0.02) but not when sex and age or height were adjusted for in cIMT z-scores (p=0.08 for cIMT-for-age z-score; p=0.11 for cIMT-for-height z-score). Body fat % was not associated to mean

cIMT when all 12 segments were included (p=0.09), despite being significantly correlated with each individual carotid segment (β = -0.001 ± 0.0004, r^2= 4.75%, p=0.01 for near + far wall carotid bulb; β = -0.001 ± 0.0003, r^2= 3.52%, p=0.033 for near + far wall ICA).

Systolic blood pressure was only associated with mean cIMT (mm) when all segments were included (12-segments) (β = 0.001 ± 0.0002, r^2= 4.3%, p=0.008). This relationship was again driven by the carotid bulb, as it was the only arterial segment that was significantly related to SBP (near + far wall: β = 0.001 ± 0.0002, r^2= 4.9%, p=0.005; far wall only: β = 0.001 ± 0.0003, r^2= 2.91%, p=0.031). Diastolic blood pressure was not significantly related to any measure of cIMT (p=0.94 for mean cIMT (mm) when using 2 segments; p=0.08 for mean cIMT when using 12 segments; p=0.45 for cIMT-for-age z-score; p=0.52 for cIMT-for-height z-score).

HDL cholesterol was significantly associated with mean far wall CCA IMT (2-segment) (β= -0.029 ± 0.0126, r^2= 3.6%, p=0.005 p=0.03), but not when calculated using the 12-segment approach (near and far wall of the CCA, ICA, and carotid bulb) (p=0.080). Indeed, HDL was not related when other arterial segments (p=0.068 for carotid bulb (near and far wall); p=0.306 for ICA (near and far wall)) or cIMT z-scores were the outcome

variable (p=0.08 for cIMT-for-age z-score; p=0.08 for cIMT-for-height z-score). Interestingly, neither non-HDL cholesterol, a measure of atherogenic particles, or TC/HDL ratio were related to any measure of cIMT. LDL was not related to any of the primary outcome measures of cIMT but was related to ICA IMT when including the near wall (p=0.038). Despite high prevalence of abnormal triglycerides (34% of study population), triglycerides levels were not related to any measure (primary or arterial segment) of cIMT.

As seen in the **Section B**, FPG was a significant correlate of 12-segments mean cIMT ($\beta = 0.012 \pm 0.0059$, p=0.03) but not when using only the far wall CCA segments to quantify cIMT (p=0.13 for 2-segment mean cIMT (mm); p=0.40 for cIMT-for-age; p=0.39 for cIMT-for height). FPG was related to CCA ($\beta = 0.016 \pm 0.0068$, $r^2 = 3.75\%$, p=0.02) and ICA IMT ($\beta = 0.013 \pm 0.0059$, $r^2 = 3.67\%$, pp=0.028) but not carotid bulb (p=0.19) when the near and far wall were included. FPG was not related to any segment of cIMT when only the far wall was used for analyses. No other measure of glycemia was related to cIMT. Additional analyses dichotomizing CVRFs into binary variables (normal vs. abnormal values) based on current pediatric guidelines, revealed that the presence of individual CVRFs in the abnormal range was not significantly related to any measure of

cIMT (**Appendix C7**). This may be due relatively low prevalence of abnormal and/or extreme values within the study population.

Table 5. Univariable Regression Analyses of mean cIMT measures

	Mean cIMT 2-segment, mm			Mean cIMT 12-segment, mm			Mean cIMT-for-age z-score			Mean cIMT-for-height z-score		
	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	P-value
Age (year)	0.0016 (0.0009)	2.00	0.072	0.0029 (0.0007)	9.34	**<0.001**	-	-	-	-0.0056 (0.0195)	0.05	0.772
Sex (Male)	0.0094 (0.0051)	2.10	0.066	0.0106 (0.0043)	3.67	**0.015**	-	-	-	-	-	-
Race: Caucasian	-0.0003 (0.0059)	0.00	0.955	-0.0004 (0.0050)	0.00	0.940	0.0074 (0.1321)	0.00	0.96	-0.0036 (0.1334)	0.00	0.978
Height(cm)	0.0004 (0.0002)	3.20	**0.023**	0.0006 (0.0001)	10.32	**<0.001**	-0.0030 (0.0041)	0.34	0.46	-	-	-
Parental Hx of Premature CVD	-0.0163 (0.0093)	1.87	0.082	-0.0083 (0.0080)	0.66	0.302	-0.4853 (0.2059)	3.38	**0.02**	-0.4151 (0.2070)	2.48	**0.047**
Family Hx of CVD	-0.0099 (0.0051)	2.29	0.054	-0.0065 (0.0043)	1.39	0.136	-0.2905 (0.1124)	4.03	**0.01**	-0.2612 (0.1132)	3.26	**0.022**
Annual Household Income (<$50,000/year)	0.0026 (0.0059)	0.12	0.664	0.0043 (0.0050)	0.46	0.390	0.0971 (0.1318)	0.34	0.46	0.0849 (0.1310)	0.27	0.518
Female Caregiver Education: attended university/ college	0.0060 (0.0061)	0.61	0.322	0.0009 (0.0052)	0.02	0.855	0.1745 (0.1363)	1.02	0.20	0.1373 (0.1354)	0.65	0.312
Male Caregiver Education: attended university/ college	-0.0052 (0.0055)	0.57	0.341	-0.0039 (0.0047)	0.43	0.409	-0.1058 (0.1217)	0.47	0.39	-0.1025 (0.1220)	0.45	0.402

BMI z-score	-0.0005 (0.0025)	0.02	0.844	0.0000 (0.0022)	0.00	0.993	0.0645 (0.0629)	0.66	0.31	0.0123(0.0567)	0.03	0.829
Waist Circumference (cm)	0.0002 (0.0002)	1.18	0.265	0.0005 (0.0002)	7.30	**0.005**	-0.0029 (0.0049)	0.34	0.55	-0.0022 (0.0051)	0.18	0.668
Body Fat %	-0.0009 (0.0004)	3.97	**0.018**	-0.0006 (0.0003)	2.08	0.089	-0.0155 (0.0087)	2.24	0.08	-0.0140 (0.0087)	1.83	0.112
Systolic BP (mmHg)	0.0002 (0.0002)	0.47	0.390	0.0005 (0.0002)	4.33	**0.008**	-0.0029 (0.0051)	0.22	0.56	-0.0028 (0.0051)	0.19	0.584
Diastolic BP (mmHg)	-0.0000 (0.0003)	0.00	0.939	0.0004 (0.0002)	2.03	0.072	-0.0048 (0.0063)	0.37	0.45	-0.0041 (0.0063)	0.27	0.519
Total Cholesterol (mmol/L)	-0.0003 (0.0041)	0.00	0.943	0.0008 (0.0035)	0.04	0.811	-0.0090 (0.0899)	0.01	0.92	0.0058 (0.0901)	0.00	0.949
HDL Cholesterol (mmol/L)	-0.0292 (0.0126)	3.57	**0.022**	-0.0192 (0.0109)	2.12	0.080	-0.4997 (0.2861)	2.09	0.08	-0.4962 (0.2838)	2.11	0.083
LDL Cholesterol (mmol/L)	0.0043 (0.0044)	0.64	0.338	0.0044(0.0038)	0.94	0.245	0.0670 (0.0982)	0.33	0.50	0.0770 (0.0982)	0.43	0.434
Non-HDL Cholesterol (mmol/L)	0.0025 (0.0040)	0.27	0.535	0.0027 (0.0034)	0.42	0.436	0.0364 (0.0879)	0.12	0.68	0.0508 (0.0879)	0.24	0.564
TC/HDL ratio	0.0046 (0.0030)	1.55	0.136	0.0037 (0.0026)	1.39	0.158	0.0642 (0.0677)	0.63	0.35	0.0691 (0.0675)	0.74	0.308
Triglycerides (mmol/L)	0.0004 (0.0041)	0.01	0.924	0.0018 (0.0035)	0.18	0.609	-0.0578 (0.0923)	0.27	0.53	-0.0349 (0.0914)	0.10	0.703
Fasting Plasma Glucose (mmol/L)	0.0106 (0.0070)	1.59	0.131	0.0119 (0.0059)	2.74	**0.047**	0.1318 (0.1570)	0.49	0.40	0.1361 (0.1579)	0.52	0.390
2-hr OGTT Glucose (mmol/L)	-0.0030 (0.0030)	1.33	0.314	0.0010 (0.0026)	0.19	0.703	-0.0542 (0.0704)	0.77	0.44	-0.0627 (0.0683)	1.10	0.361

HbA1c (%)	-0.0074 (0.0092)	0.49	0.419	-0.0048 (0.0078)	0.29	0.537	-0.2297 (0.2026)	0.97	0.26	-0.2265 (0.2038)	0.94	0.268

Independent Determinants of Mean cIMT Measures. Multivariable linear regression models were constructed to identify any potential independent relationships between CVRFs and cIMT in children with obesity (**Table 6 and 7** for mean cIMT (mm) and cIMT z-scores, respectively). Any variable that was clinically significant ($p<0.1$) for at least one measure of cIMT (mm or z-scores) was included in all models to allow a direct comparison of correlates. These included: age, height, family history of CVD, waist circumference, body fat %, SBP, DBP, HDL, FPG. When cIMT-for-age z-score was the outcome, age and sex were omitted in the model, as was height and sex for when cIMT-for-height was the outcome. Collinearity was not an issue in these models (VIF<10). These analyses explained a total variance of 20.08% for 2-segment mean cIMT (mm), 26.52% for 12-segment mean cIMT (mm), 15.19% for cIMT-for-age z-score, and 8.95% for cIMT-for-height z-score. Multivariable regression analyses revealed that height was independently associated with cIMT-for-age z-score (β = -0.0203 ± 0.0091, p=0.029) but not for 2-segment mean cIMT (mm) (p=0.069). The correlation between height and 12-segment mean cIMT (mm) in univariate analyses ($p<0.001$) was attenuated in the multivariate model (**Table 7**), which suggests that its relationship may be mediated through other CVRFs. Other non-modifiable CVRFs included in the models, such as age, sex, family history, were not independently associated with measures of cIMT ($p>0.05$).

Body fat % was related to cIMT-for-age z-score (β = -0.0312 ± 0.0151, p=0.042) and 2-segment mean cIMT (mm) (β = -0.0014 ± 0.0007, p=0.050). Its relation to 12-segment mean cIMT (mm) did not reach significance (p=0.052) and the lack of association of

body fat % with cIMT-for-height z-score (p=0.456) was consistent with previous univariable analyses (p=0.112). Similarly, although HDL was related to 2-segment mean cIMT in univariable analyses (p=0.047), it did not have an independent relationship in multivariate analyses (p=0.052). Waist circumference, SBP, DBP, and FPG were not independently related to any measure of cIMT (p>0.05). No one variable demonstrated a consistent and independent relationship with all 4 primary measures of cIMT. These findings support our previous results in **Section C** which highlighted differences and potential bias between mean cIMT measures.

Table 6. Determinants of mean cIMT (mm)

Variable	Reg Coeff (SE)	P-value	R-Square (%)
Mean cIMT, 2 segment (n=92)			20.08
Intercept	0.4726(0.0715)	<0.001	
Sex: Male	0.0063(0.0078)	0.424	
Age (y)	0.0032(0.0019)	0.106	
Height (cm)	-0.0009(0.0005)	0.069	
Family Hx of CVD	-0.0040(0.0075)	0.596	
Waist circumference (cm)	0.0003(0.0004)	0.552	
Body fat %	-0.0014(0.0007)	**0.050**	
Systolic BP (mmHg)	0.0007(0.0005)	0.145	
Diastolic BP (mmHg)	-0.0008(0.0005)	0.112	
HDL Cholesterol (mmol/L)	-0.0355(0.0180)	0.052	
Fasting plasma glucose (mmol/L)	0.0154(0.0094)	0.105	
Mean cIMT, 12 segment (n=92)			26.52
Intercept	0.4132(0.0589)	<0.001	
Sex: Male	0.0065(0.0064)	0.314	
Age (y)	0.0031(0.0016)	0.054	
Height (cm)	-0.0005(0.0004)	0.189	
Family Hx of CVD	-0.0040(0.0061)	0.516	
Waist circumference (cm)	0.0004(0.0003)	0.207	
Body fat %	-0.0012(0.0006)	0.052	
Systolic BP (mmHg)	0.0003(0.0004)	0.452	
Diastolic BP (mmHg)	-0.0003(0.0004)	0.438	
HDL Cholesterol (mmol/L)	-0.0258(0.0148)	0.086	
Fasting plasma glucose (mmol/L)	0.0122(0.0077)	0.118	

Table 7. Determinants of cIMT z-scores.

Variable	Reg Coeff (SE)	P-value	R-Square (%)
Mean cIMT-for-age z-score (n=91)			15.19
Intercept	2.7352(1.5479)	0.081	
Height (cm)	-0.0203(0.0091)	**0.029**	
Family Hx of CVD	-0.0990(0.1686)	0.559	
Waist circumference (cm)	0.0070(0.0091)	0.442	
Body fat %	-0.0312(0.0151)	**0.042**	
Systolic BP (mmHg)	0.0169(0.0114)	0.142	
Diastolic BP (mmHg)	-0.0192(0.0118)	0.108	
HDL Cholesterol (mmol/L)	-0.6713(0.4165)	0.111	
Fasting plasma glucose (mmol/L)	0.3290(0.2122)	0.125	
Mean cIMT-for-height z-score (n=91)			8.95
Intercept	1.1366(1.4508)	0.436	
Age (y)	0.0002(0.0371)	0.995	
Family Hx of CVD	-0.1172(0.1777)	0.511	
Waist circumference (cm)	-0.0056(0.0087)	0.519	
Body fat %	-0.0105(0.0140)	0.456	
Systolic BP (mmHg)	0.0062(0.0113)	0.588	
Diastolic BP (mmHg)	-0.0089(0.0120)	0.460	
HDL Cholesterol (mmol/L)	-0.7634(0.4233)	0.075	
Fasting plasma glucose (mmol/L)	0.1790(0.2176)	0.413	

CHAPTER 4: DISCUSSION

The primary objective of this study was to characterize the relationships between CVRFs and specifically investigate the impact of dysglycemia on cIMT in children with obesity. Although we did not report any independent relationships between measures of glycemia and measures of mean cIMT, review of the literature highlighted methodological issues related to assessing cIMT in children, which may have implications on answering the proposed research questions and interpreting results from this study. For this reason, findings from our second objective, aimed at comparing methodologies used assess mean cIMT in children, will be discussed first.

Comparability of mean cIMT. Mean cIMT (mm) was statistically different between measures (0.40 ±0.03mm vs. 0.39±0.03mm, $p<0.001$), where inclusion of all arterial segments and walls generated a higher mean cIMT (mm). Our findings support the study hypothesis and other adult studies that IMT (mm), using the mean of all 12 arterial segments, is greater than IMT (mm) limited to the CCA (Chambless et al., 2000; Urbina et al., 2002). We attribute these discrepancies in part to segment-specific differences in arterial thickening that is consistent with non-uniform development of atherogenic changes (Mackinnon et al., 2004). We show that children with obesity have greater thickening in the carotid bulb compared to the ICA and CCA, which has previously been shown in youth with prediabetes, healthy adults, and adults with coronary artery disease (Shah et al., 2014; Crouse et al., 1995; Hulthe et al., 1997; Chambless et al., 2000).

To further evaluate similarities of cIMT quantification methods, an intra-class correlation showed that measures of mean cIMT measured with 2 segment (far wall CCA only) and 12 segments (far and near wall of the CCA, carotid bulb, and ICA) revealed moderate resemblance between measures (ICC: 0.65. 95%CI: 0.55-0.73, see **Figure 11**). However, an average mean difference of 0. 012mm between both measures suggests that that inclusion of all arterial segments and walls (12-segment) increases mean cIMT (mm) measures by 0.012mm (or, that limitation of mean cIMT measures to the far wall of the CCA routinely underestimates thickness by 0.012mm) (see **Figure 12**). Additionally, the limits of agreement (or interval of agreement) from the Bland-Altman plot showed that 95% of mean differences between measures fell between: 0.032mm and -0.057mm. Given that reports from the Bogalusa Heart Study revealed a cIMT rate of progression of 0.017-0.020mm/year in healthy young adults (Stein et al., 2004), a difference of 0.089mm translates to advanced vascular aging of 4.5-5.2 years from chronological age and may have considerable clinical significance if misreported. Such differences between cIMT measures progression may be detrimental in cases where risk is underestimated using the 2-segment approach. Indeed, additional bias analyses revealed the presence of a positive proportional bias (0.19, 95%CI: 0.06 to 0.31, p=0.003) which indicates greater variability at higher ranges of cIMT, thus increasing chances of risk misclassification. Although measurement variability is dependent on degree of arterial thickness in adults (Stensland-Bugge et al., 1997), to the best of our knowledge, this has not been reported in the pediatric literature. Taken together, significant differences in mean cIMT (mm) between

methodologies warrant caution when interpreting results and suggest that the 12 segment and 2 segment approach should not be used interchangeably.

Comparability of cIMT correlates. Noting the methodological differences shown in mean cIMT (mm), we also compared correlates of each measure. We found that age, sex, waist circumference, SBP and FPG were only related to 12-segment mean cIMT ($p<0.05$) and not to 2-segment mean cIMT ($p>0.05$) in univariable analyses. As previously mentioned, the carotid bulb is consistently thicker than other segments of the carotid artery and prone to vascular remodeling (Chambless et al., 1997, 2000). Given that the carotid bulb has been proposed as the earliest site of detectable subclinical atherosclerotic changes in children and adults (Urbina et al., 2013; Urbina et al., 2002; Chambless et al., 1997), exclusion of this segment when measuring cIMT may underestimate important structural changes associated with the onset of disease progression. Our analyses of segment-specific correlates showed that the carotid bulb was related to more CVRFs than ICA or CCA segments, which is consistent with our study hypothesis. Indeed, older age, male sex, and greater height, waist circumference, body fat %, and SBP were all significantly related to greater bulb thickness (inclusive of the far wall only, see **Appendix C5**), whereas IMT limited to the far wall of the CCA was only related to height, body fat %, and HDL-cholesterol (see **Table 5**). Inclusion of the near wall to CCA IMT showed that family history and FPG were also related but the relationship to HDL was abolished. Our results are consistent with and adds to the few studies in youth and young adults with obesity and dysglycemia (Shah et al., 2014; Urbina et al., 2013) that

have reported on individual arterial segments with different correlates of across the CCA, ICA and carotid bulb. These findings support the study hypothesis that a greater number of CVRFs are correlated to mean cIMT when all arterial segments were included in analyses, in both univariate and multivariate analyses, compared to mean cIMT (mm) limited to the far wall of the CCA. These relationships were mainly driven by areas of the carotid artery that are sensitive to segmental atherosclerosis and for this reason, inclusion of all arterial segments should continue to be considered the gold standard for assessing cardiovascular risk in the carotid artery (Touboul et al., 2012).

Use of cIMT z-scores. As a growing population, age and height are strong correlates of cIMT in children. We sought to explore the use of cIMT-for-age and cIMT-for-height z-scores as primary outcome variables in hopes of reducing the number of covariates in our analyses and further examine comparability of 12-segment mean cIMT (mm) to and 2-segment cIMT with the use of z-scores. These z-scores were calculated based on sex-specific age and height of reference data for the far wall of common carotid segments in children (Doyon et al., 2013). In our univariable analyses, only family history of CVD and parental history of premature CVD were related to cIMT z-scores (see **Table 6 and 7**) and no modifiable CVRF was significantly related to either cIMT-for-age or for-height z-scores. Interestingly, no CVRF was consistently related to all measures, when age, height and sex were controlled for in multivariable analyses, which support previous studies that demonstrate the considerable impact of non-modifiable CVRFs (age, sex, height, family history of CVD) to cIMT in children with obesity (Morrison et al., 2010;

Mittelman et al., 2010; Beauloye et al., 2007). However, inconsistent correlates among measures of cIMT in our study suggest that measures of far wall CCA (mean cIMT (mm), cIMT-for-age, cIMT-for-age) are not directly comparable to measures of mean cIMT when all arterial segments are included. These findings further emphasize the importance considering carotid segment selection in measures of arterial thickness. For this reason, although sex-specific cIMT-for-age and for-height z-scores allow comparison with a normative population, migration to the use of sex-specific cIMT-for-age and for-height z-scores for individual CVD risk assessment in children with obesity, especially when limited to far wall CCA measures, may be premature and warrant further investigation.

Measures of Glycemia and Carotid Intima-Media Thickness. Acknowledging the aforementioned concerns, we herein discuss results from the current study and highlight the importance of consistent methodologies for the characterization of CVRFs related to cIMT in children with obesity. To speak to our first study objective, in univariable analyses, we showed that FPG was significantly related to cIMT when all arterial segments of the carotid artery were included but not when limited to the far wall CCA segment. Contrary to the study hypothesis, no measure of glycemia (FPG, 2hr PG, or HbA1c) was independently related to any measure of cIMT ($p>0.05$) unlike other previous reports in children with obesity (Reinehr et al., 2006; Chen et al., 2014). The lack of significant relationships reported may be attributed to the extent of impaired glucose metabolism in study cohorts. Although Reinehr and colleagues found that FPG explained 5% of variance in children with obesity (n=96) (2006) and that IGT, as a group

variable, predicted increased IMT (≥0.7 mm) in youth with overweight (n=461) (2013), their cohorts demonstrated higher degree of glucose disturbances. Mean FPG and 2hr PG values were 4.9 mmol/L and 6.5 mmol/L, respectively, which are considerably higher than that in the current study (FPG: 4.74mmol/L; 2hr PG: 5.92 mmol/L). Similarly, Chen et al. (2014) reported an independent association between HbA1c and increased cIMT (≥75th centile for age and sex, Bohm et al., 2009) in a group of boys with obesity (n=354) with overall worse glucose parameters (mean HbA1c: 5.7-5.9%, mean FPG: 5.31-5.37 mmol/L, mean 2hr PG: 6.80-6.85mmol/L) compared to our participants (mean HbA1c: 5.19-5.79, mean FPG: 4.71-4.96 mmol/L, mean 2hr PG: 5.75-6.77 mmol/L). Our studies also differed in methodology; Reinehr et al. (2006, 2008, 2013) selected the greatest of 4 measures of mean cIMT for statistical analyses rather than taking the mean of the 4 measurements taken and details on the length of carotid segment measured are not clear. As a result, cIMT values from this group are at the higher end of (0.6mm) of cIMT measurements published in the pediatric obese population (0.4-0.7 mm) (Simsek et al., 2010; Yilmazer et al., 2010) and may not be representative of the thickness across entire CCA segment. Chen et al. (2014) unfortunately provided limited details of their measurement protocol and for these reasons, lack of significant associations of glycemic measures and cIMT in this present study may be due to differences related to extent of glycemic alterations and highlight the differences in cIMT methodologies used in the literature.

Prediabetes and Carotid Intima-Media Thickness. Prediabetes prevalence was less than previously published in this demographic (Morrison et al., 2012), with a total of 17 participants with at least one glycemic measure meeting prediabetes criteria (ADA, 2010) Participant cIMT measures were comparable between groups, suggesting that at this stage in life, cIMT is not significantly impacted by prediabetes. Our results are similar to those previously reported in the prediabetes adolescent population with no differences seen in mean cIMT (mm) when limited to the far wall of the CCA (Eklioglu et al. 2016; Shah et al., 2014). Interestingly, Shah et al. (2014) reported that youth with obesity and prediabetes (n=102) had higher ICA IMT compared to youth with obesity and normal glycemia (n=139). This was not seen in our current study (**Appendix C4**), which again may be related to differences in study population and methodologies. Shah et al. (2014) assessed maximum carotid thickness, which is routinely higher than measures of mean cIMT (Morrison et al., 2010) and our study population was considerably younger (mean age: 12.6-13.7y vs. 18.0-18.3y). Despite comparable CVRFs between children with and without prediabetes in our study, Shah et al (2014) demonstrated that youth with obesity and prediabetes had worse cardiometabolic profiles. In their study, youth with prediabetes were significantly heavier and had higher SBP, 2hr PG and HbA1c compared to those with normal glycemia. These differences in CVRFs were not seen in our study or others of similar age (Eklioglu et al, 2016), which may suggest that cumulative length of exposure to CVRFs in youth with prediabetes impacts arterial thickness.

Classification of Prediabetes. Of 17 participants met prediabetes criteria according to American Diabetes Guidelines (2010), and the 64.6% were classified using solely HbA1c criteria (≥5.7%, n=11). Acknowledging that there are limitations of HbA1c, it is still clinically accepted as a measure of glycemic status. If the higher Canadian diagnostic criteria were used to classify prediabetes (which excludes the use of HbA1c), only 6 individuals would be included in group analyses (see **Appendix C1 and C2**). Although the use of HbA1c is not recommended for diagnosis of type 2 diabetes in children (Canadian Diabetes Association, 2013) due to its discordance with OGTT and FPG tests (Kapadia & Zeitler, 2012), HbA1c ≥5.7% has high specificity to discriminate against false negatives (Morrison et al., 2012). Although there is limited data in the pediatric cohort to validate these HbA1c standards to long term health outcomes (Forouhi et al., 2006; Kapadia & Zeitler, 2012), Sjaarda and colleagues (2012) demonstrated that adolescents with obesity (n=160) with HbA1c ≥5.7% (prediabetes) had impaired beta-cell function, overall lower peripheral and hepatic insulin sensitivity but only 41% dysglycemia in overweight subjects were detected using traditional glycemic tests (IFG and IGT). Thus, despite its discordance with other glycemic measures, HbA1c ≥5.7% may be useful to screen for beta cell dysfunction and other measures associated with progression to type 2 diabetes in this population (Sjaarda et al., 2012; Chen et al., 2014).

Other Determinants of Carotid Intima-Media Thickness. Similar to published cross-sectional studies in children and youth with obesity, we demonstrated that age, height, and male sex were prominent correlates of cIMT in children with obesity in univariable

analyses. However, although age has been reported as an independent determinant of cIMT in children (Morrison et al., 2010; Wiegman et al., 2004b; Urbina et al., 2009), recent reports have suggested that this effect is mediated through body dimension (Weberrub et al., 2015, Doyon et al., 2013). This may explain the lack of an independent relationship between age and measures of cIMT in multivariable models when waist circumference, body fat % or height were included in the model. Given that increased cIMT in children with obesity, compared to children with normal weight, may be reflective of increased somatic growth associated with increased adiposity (Chowdhury et al., 2014), the role of body size and dimension may also explain why height was an independent correlate of cIMT-for-age z-score but age was not for cIMT-for-height z-score in the present study (**Table 7**).

Furthermore, we assessed the role of various measures of adiposity on cIMT. We found that despite a high overall adiposity in this study population, BMI z-score was not correlated or predictive of cIMT (p=0.844 for 2-segment mean cIMT (mm); p=0.993 for 12-segment mean cIMT (mm); p=0.31 for cIMT-for-age z-score, p=0.829 for cIMT-for-height z-score; see **Tables 4-7**). Waist circumference was related only to mean cIMT (mm) when all arterial segments were included (p=0.005), similar to that reported in a population sample of 11-13-year-old 385 children (Melo et al., 2014). Although Melo and colleagues (2014) reported an independent relationship between waist circumference and cIMT, we found that this relationship was abolished in the multivariable analyses. This may be due to their inclusion of lean participants in regression analyses, which give more

variation in measures of adiposity (Skinner et al., 2015; Beauloye et al., 2007). Our findings support the concept that adiposity is related to cIMT in children (Park et al., 2015). Interestingly, body fat % was the only measure of adiposity demonstrated an independent relationship to any measure of cIMT, however the direction of the relationship was negative. It is possible that this is reflective of the clinical population sampled in that those with greater risk factor load may seek medical attention and thus are more frequently under the care of a trained medical team. This in turn may reduce the presence of other CVRFs that impact cIMT (Lorenz et al., 2007), thereby diminishing or shifting the relationship between measures of adiposity and cIMT. It has been speculated that atherogenic changes are mediated through CVRFs present in high risk cohorts (e.g. children with obesity) rather than obesity itself (Reinehr & Wunsch, 2011). In a structural equation model in a population sample of children and young adults (n=784), the effect of BMI z-score, although large, was indirect and mediated through blood pressure, non-HDL cholesterol, and measures of blood glucose (Gao et al., 2015). Given the limited prevalence of abnormal blood pressure, non-HDL, and glycemic levels present in our study population, it is possible that the role of adiposity is not as great as that reported by studies with greater degree of metabolic disturbances (Reinehr et al., 2013; Urbina et al., 2009a). Furthermore, studies inclusive of normal weight children consistently demonstrate greater variability of adiposity within study population, which may not reflect the relationship between measures of obesity and cIMT (Melo et al., 2014; Ryder et al., 2016; Mittelman et al., 2010; Vercoza et al., 2009).

Although systolic blood pressure has repeatedly been related to cIMT in children with obesity, our multivariable analyses did not reveal a significant relationship ($p>0.1$). Mean SBP was slightly lower in this study population compared to other studies in obese cohorts reported by Urbina et al. (2009a) and Reinehr et al. (2006). Although both studies highlight the prominent effect of SBP to predict cIMT in children with obesity, there was a higher prevalence of hypertension in their cohorts (21.3-45.0%) compared to ours. In the current study, abnormal SBP and DBP was only present in 6.9% and 8.2% of participants (see **Figure 5**) and when measures of blood pressure were dichotomized based on pediatric cutoffs (normal vs. abnormal) neither abnormal measures were related to cIMT. The limited degree of hypertension in our cohort suggests that the impact of SBP on cIMT is not specific to children with obesity but to elevated blood pressure as seen in other pediatric studies of children with hypertension and without obesity (Di Salvo et al., 2006; Gao et al., 2015; Urbina et al., 2011).

Regression analyses also revealed that HDL cholesterol was the only lipid related to measures of mean cIMT in this study. 24.7% of participants had low HDL (<1.0mmol/L) (Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents, 2011) and mean values were similar to some reports (Reinehr et al., 2013; Shah et al., 2014) but slightly lower than others (Morrison et al., 2010). The protective effect of HDL on cIMT has previously been reported in children with normal weight (Caserta et al., 2010) and youth with obesity (Mittelman et al., 2010). Although not statistically significant ($p=0.075$), our findings are similar to those of Civilibal and

colleagues (2014) that reported an independent relationship between HDL and cIMT-for-height SDS in children with metabolic syndrome (n=52, mean age: 12.9y). Given that a z-score ≥1 is considered abnormal (Dalla Pozza et al., 2015, Bohm et al., 2009), the trend in our study (p=0.075-0.111) may have clinical significance in children with obesity, as each mmol/L decrease in HDL-cholesterol was related to approximately 0.76 increase in cIMT-for-height z-score. Total cholesterol, LDL cholesterol, nor non-HDL cholesterol were predictive of cIMT measures, however this may be due to limited prevalence of abnormal values (9.7%, 9.0%, 18.6%, respectively).

Lastly, family history of cardiovascular disease was inversely related to measures of cIMT in univariable analyses. These findings are different than those previously published by this group (Morrison et al., 2010), however, this may be due differences in study populations. Overall, there was a lower prevalence of family history reported (44.4% vs. 68.2%) and higher number of children of obesity included (n= 162 vs. n=44) in the present study. Differences in variable coding may also explain this unexpected finding, in that our current dataset was unable to differentiate between premature CVD and presence of CVD in some family members as gender of second-degree relatives was not available.

Limitations. One main limitation of this project is the inability to propose temporal relationships. The cross-sectional nature of this project limits the capability to assess the influence of length of risk factor exposure on cIMT. Additionally, data for this project

was taken from the CANPWR and cIMT Sub-study dataset, which was collected from participants' patient charts housed in the Children's Exercise and Nutrition Centre. These data are collected via prospective chart review as part of the CANPWR Protocol by qualified research personnel (MSc student or Research Co-ordinator) and were subject to data within clinical patient charts. This protocol was developed as part of the CANadian Pediatric Weight Management Registry (CANPWR) Study that was designed to assess clinical practices relevant to childhood obesity treatment in Canada. For this reason, classification of dysglycemia was limited to available measures and at a single time point (contrary to the recommended repeat testing by the CDA 2013 Guidelines). Missing values were also problematic in that 51.8% of participants did not have OGTT values, and 17.3% and 10.4% of the study population did not have HbA1c or FPG values, respectively. Although OGTTs are not routinely done in children <10y, 45.3% of children ≥10y did not have OGTTs, which may represent a potential problem with clinical practice. Additionally, approximately 10% of lipid values were missing whereas only 1.3% of blood pressure values were missing.

Although a strength of this study is that it included only 1 reader for cIMT measurements (thereby limiting inter-observer variability), variability within the reference population dataset was not ideal. Despite high reproducibility, variability was reported up to 9% (Doyon et al., 2013), which may have masked any ability to detect differences in cIMT z-scores between groups. Additionally, these reference values were limited to measures of mean, but not maximal cIMT, which are more associated with CVRFs in children and

adults (Urbina et al., 2009b; Hurwitz & Netterstrom, 2001). Consequently, maximal measurements are more representative of the focal thickening associated with atherosclerosis in adults, particularly as arterial thickening is not equally distributed across all segments (Wunsch et al., 2006; Touboul et al., 2012). However, this dataset was the only sizeable reference group available at time of study commencement and provided an opportunity to minimize the number of potential confounders in the relationship between CVRFs and cIMT in children. Unfortunately, the use of cIMT normative values (and z-scores) is relatively new within the pediatric literature and this dataset has only been used previously by one study (Civilibal et al., 2014). Thus, it is important to note that normative cIMT has yet to be validated through longitudinal studies or followed long term to identify potential cut off values associated with increased CV risk.

Furthermore, although the high proportion of Caucasian subjects in this current study was comparable to that of Doyon and colleagues (2013), the limited ethnic diversity in our study restricts the ability to extrapolate to other populations where others have reported a direct relationship between race and cIMT in children and youth with obesity (Gao et al., 2015; Urbina et al., 2009a; Shah et al., 2014). Given that the majority of participants with prediabetes were not of Caucasian descent and the overall small proportion of individuals with dysglycemia in this study population, our results may not be entirely representative of all children with obesity and prediabetes. Furthermore, the clinical sample in this study

may be subject to selection and referral bias of those enrolled in weight management and thus not entirely generalizable to all children with overweight or obesity.

Lastly, given the limited amount of thickness seen in the present study, the use of automated edge-detecting measurements for small distances may have been useful (Dalla Pozza et al., 2015). Although the protocol used in our study is highly reproducible, semi-automated programs allow tracking of frames for measurement of multi-frame images instead of single-frame measures (Selzer et al., 2001) and may reduce variability associated with image quality (Urbina et al., 2009b).

Hemodynamic-induced Arterial Thickening. Although cIMT has consistently been used as cardiovascular endpoints, it is important to note that cIMT is only a surrogate marker of subclinical atherosclerosis. It has been suggested that at lower degrees of cIMT, arterial thickening may be reflective of vascular remodelling to non-atherosclerotic stimuli such as increased tensile and shear stress and result in adaptive medial hypertrophy (Groner et al., 2006). Unfortunately, this thickening cannot be differentiated from disease-specific changes and hemodynamic stimulation (Wunsch et al., 2006). Given that progressive increases in blood pressure have been associated with increased cIMT, hemodynamic-induced remodelling may explain the reported relationship between SBP in hypertensive children (that are not overweight) (Urbina et al., 2011) and the lack of independent relationship in our relatively normotensive obese group. This highlights the direct role of blood pressure on cIMT and how the effects of adiposity and other

CVRFs may be mediated through SBP, as suggested by Gao and colleagues (2015). It is thus necessary to interpret results with caution and be cognisant of physiological limitations of cIMT measures.

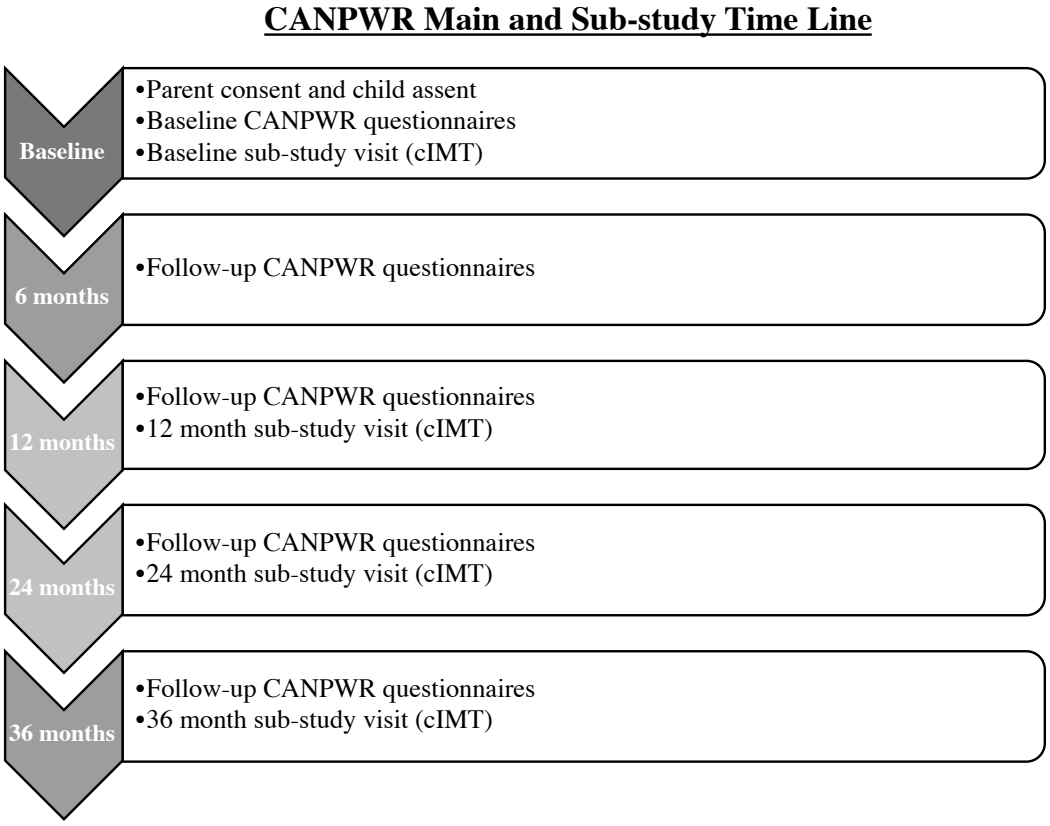
Future Directions. With the limited number of studies conducted in children and youth with obesity and prediabetes, it is important to continue identifying potential targets of cIMT progression in this high-risk cohort. The inclusion of functional and physiological changes within the vascular wall and endothelium would provide a more comprehensive understanding to vascular remodelling that is associated with accelerated atherosclerosis. As the pediatric literature lacks longitudinal normative reference values, the validation of cIMT cut offs and z-scores associated with increased risk of adult outcomes should be identified. Additionally, reference values for maximal cIMT measurements and other arterial segments would also be useful in understanding patterns of atherosclerotic development in the carotid artery as current normative measures are limited to only the far wall CCA. Future studies should also investigate how changes in notable predictors can reverse accelerated thickening with interventions in children with risk of premature CVD and efforts should be made to explore other novel contributors of cIMT throughout childhood. As the number of studies utilizing this measurement increases, it is important to standardize methodologies across studies to allow adequate comparison between studies.

Conclusions. In this current study, we set out to understand the impact of prediabetes on cIMT in children with obesity. We identified differences in the methodology used in the literature and therefore expanded our objectives to consider the influence of different cIMT measures. Measures of mean cIMT differed significantly, depending on the selection of arterial segments and walls used to assess cIMT. Children with obesity and prediabetes did not exhibit greater arterial thickness compared to children with obesity and without prediabetes. The investigation of determinants of cIMT in this study revealed that although FPG was related to increased cIMT in univariable analyses, no measure of glycemia was independently correlated to arterial thickness. By comparing determinants and absolute differences between measures of cIMT, this study emphasizes the heterogeneity of methodologies used to quantify cIMT that are currently used in the literature and highlights the need for normative values for all carotid segments. Additional methodological and longitudinal studies are warranted to elucidate the best measure(s) of cIMT that are reproducible, accurate, and validated with adult health outcomes. This current study adds to the limited number of reports on children with obesity and prediabetes and provides a good platform to build future studies investigating the relationship between CVRFs and early structural changes associated with atherosclerosis.

APPENDICES

Measure	Method of Data Collection
Consent	CANPWR Main and Sub-study Consent
Participant demographic	
Age	CANPWR Main Study Questionnaires
Gender	CANPWR Main Study Questionnaires
Race	CANPWR Main Study Questionnaires
Socioeconomic status	CANPWR Main Study Questionnaires
Participant anthropometry and physical characteristics	
Height	Chart Review: measured by Nurse Practitioner (APN) at initial medical assessment
Weight	Chart Review: measured by Nurse Practitioner (APN) at initial medical assessment
BMI	Calculated from height and weight from initial medical assessment
Body Fat %	Chart Review: measured by Nurse Practitioner (APN) via **4-point Bioelectrical Impendence Analysis** at initial medical assessment
Waist Circumference	Chart Review: measured by Nurse Practitioner (APN) at initial medical assessment
Blood Pressure	Chart Review: measured by Nurse Practitioner (APN) at initial medical assessment via **BPTru Blood Pressure Machine**
Participant Laboratory Assessment (Clinical Measures)	
Glycemia – Fasting blood glucose	Chart Review: from Routine Clinical Blood work
Glycemia – 2 hour OGTT	Chart Review: from Routine Clinical Blood work
Glycemia – HbA1c	Chart Review: from Routine Clinical Blood work
Lipids – fasting lipid profile	Chart Review: from Routine Clinical Blood work

APPENDIX A1: Data collection Summary. Data for this project was collected primarily as through CANPWR questionnaires and chart review from participants enrolled in the Growing Healthy Weight Management Program in the Children's Exercise and Nutrition Clinic (McMaster Children's Hospital), as per the CANPWR Main and Sub-Study protocol. Patient demographics were collected from the CANPWR Main Study Questionnaires. All participant information collected remained anonymous and confidential, with potentially identifiable information kept in a locked cupboard. Each participant was given a unique identification code upon enrollment.



APPENDIX A2: CANPWR Main Study and Sub-study Timeline. The CANPWR Main and Sub-study is 3-year prospective cohort study of children and youth enrolled in weight management. After participant consent at baseline, follow up data is collected at 6months, 12 months, 24 months, and 36 months from baseline. Participants enrolled in the study will be followed if they are no longer in the weight management clinic. CANPWR questionnaires report patient demographics, food behaviours and food frequency data, sleep and physical activity frequency as well as family medical history (Morrison et al., 2014). As a cross-sectional study, data for this project was taken from baseline (or, time of entry to weight management).

APPENDIX A3: SUMMARY OF CROSS-SECTIONAL cIMT STUDIES IN CHILDREN, YOUTH OR YOUNG ADULTS

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Alpsoy S, Akyuzs A, Akkoyun DC, Nalbantoglu B, Topcu B, Tulubas F, Demirkol M, Donma MM (2013) Is overweight a risk of early atherosclerosis in childhood? Angiology. doi:10.1177/0003319713476134	67 OW 115 control 6y-16: mean age 9y	-	Both	Far wall CCA 3 images per side, averaged	Mean	– At baseline, OB had greater: height, weight, BMI, SBP, DBP, TG, HDL, CRP, HOMA, fasting insulin and cIMT – cIMT: OB (R 0.58mm; L 0.56mm) > control (R 0.44mm; L 0.44mm) o univariate: serum insulin for both left (r=.521) and right (r=.502) o multiple stepwise regression: found that **age, WC, and TG** were associated with IMT – FMD: OB had lower FMD compared to control (also was associated with serum insulin but inversely) – OVERALL: **age, WC, and TG** were independent predictors of IMT; and **serum insulin** was positive correlation with increased IMT
Beauloye, V., Zech, F., Tran Thi Mong, H., Clapuyt, P., Maes, M., & Brichard, S. M. (2007). Determinants of early atherosclerosis in obese children and adolescents. *The*	104 OW/OB (recruited from Ped OB clinic) 93 control (recruiting from other surgical	-	Both	4 sites (far wall): 1cm and 2cm proximal and distal from carotid bulb	Max	– At baseline, OB had greater: weight, LDL, ApoA, IR, fasting glucose, adiponectin, leptin and sE-selectin and cIMT – cIMT: higher in OB (0.47mm) compared to control (0.438mm)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Journal of Clinical Endocrinology & Metabolism, *92*(8), 3025-3032.	clinics – dentistry, etc) 8-18y: mean age 13					○ univariate: associated with BMI, SBP, resistin, fasting insulin and HOMA-R; inverse association b/t adiponectin and cIMT ○ multiple regression: all remained significant after adjusting for sex and puberty BUT only adiponectin remained significant after adjusting for BMI also ○ Note: no sig correlation with *classic* CV RFs (WC, TG, ApoB, HDL, LDL, F Hx) or inflam markers (hsCRP, E-selectin, CAMs) ▪ When IMT levels were stratified: hsCRP was highest in high cIMT group → would suggest that inflam may only be associated with more severe stage of athero ▪ E-selectin was higher in OB children like adults; authors suggest that OB promotes early endo activation – OVERALL: **weight** (r=.323)is more important of predictor that sex of puberty; when all 3 are adjusted for, only **adiponectin** levels is an inverse independent

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						predictor of increased cIMT (r=-.290) → hypoadiponectinemia in adults predicts CAD and MI
Chen, L. H., Zhu, W. F., Liang, L., Yang, X. Z., Wang, C. L., Zhu, Y. R., & Fu, J. F. (2013). Relationship between glycated haemoglobin and subclinical atherosclerosis in obese children and adolescents. *Archives of disease in childhood*, archdischild-2013.	524 OB (354 boys, 170 girls) mean age: 10.14-10.84y	-	Both	Far wall CCA: - 3 measures were averaged	Mean	PreDM based on ADA guidelines. Stratified into boys and girls, then into normal or increased cIMT (>75th%tile for age and sex – Bohm et al, 2009): BASELINE DIFFERENCES: Boys with increased cIMT had: higher WC z-score and W:H z-score; and HbA1c (5.97% vs. 5.76%) Girls with increased cIMT had: higher WC indices only - Univariate: in boys, OR: 2.735 (1.664-4.495) - Multivariate logistic regression: HbA1c was independently correlated with increased cIMT in obese boys – for every 1% increase in HbA1c, OR for increased cIMT: o 2.702 (1.640-4.452) when adjusted for all CVRFs o 2.744 (1.666-4.521) when adjusted for age, BP, WC z-score, HbA1c, TG, ApoB, ApoA1, ALT, AST, uric acid
Ciccone, M. M., Faienza, M. F., Altomare, M., Nacci, C., Montagnani, M.,	35 OW/OB - 22 pre-pubertal	-	Both	Far wall CCA: - 3 measurements of each side	Mean	Mean cIMT: 0.47 +/- 0.06mm Pearson correlation to cIMT:

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Federica, V., ... & Leogrande, D. (2016). Endothelial and Metabolic Function Interactions in Overweight/Obese Children. *Journal of atherosclerosis and thrombosis*, (0).	mean age 9.53 +/- 3.35y					– age: r=.352, p=0.038 → independent determinant:r2=0.095, p=0.045) o 0.007mm/year – weight: r=0.329, p=0.054 – SBP: r=0.346, p=0.42
Eklioğlu, B. S., Atabek, M. E., Akyürek, N., & Alp, H. (2016). Prediabetes and Cardiovascular Parameters in Obese Children and Adolescents. *Journal of clinical research in pediatric endocrinology*, *8*(1), 80.	198 OB (81 PreDM, 117 Normogly) 6-18y: mean age: 11.8y-11.9y	Manual	Both	Far wall CCA	Mean	PreDM defined using ADA guidelines Baseline differences: - PREDM had higher: BMI (but not BMI z-score), DBP, Fasting insulin, and lower: HDL - No difference in CCA IMT between groups
Elkiran, O., Yilmaz, E., Koc, M., Kamanli, A., Ustundag, B., & Ilhan, N. (2013). The association between intima media thickness, central obesity and diastolic blood pressure in obese and owerweight children: A cross-sectional school-based	67 OB 24 OW 32 control 11-16y (mean age: 13.3y)	-	Left	CCA (no mention of far or near wall)	Mean	– OB and OW had higher: % BF, fat mass, SBP, DBP, cIMT, hsCRP o OB (only) had lower HDL – cIMT: was higher in OB (0.53mm) and OW (0.52mm) compared to control (0.36mm) – univariate analysis showed associations with: BMI, fat mass %, WC, DBP, gluc, hsCRP in OB group – multivariate analysis (of variables above mentioned):

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
study. *International journal of cardiology*, *165*(3), 528-532.						**WC** and **DBP** were only variables to be significant in OB group – OVERALL: WC and DBP have relationship with cIMT in OB children (WC should be used to evaluate CV risk factors in OB children)
Gao, Z., Khoury, P. R., McCoy, C. E., Shah, A. S., Kimball, T. R., Dolan, L. M., & Urbina, E. M. (2016). Adiposity has no direct effect on carotid intima-media thickness in adolescents and young adults: Use of structural equation modeling to elucidate indirect & direct pathways. *Atherosclerosis*, *246*, 29-35.	784 subjects (253 T2DM, 256 OB, 275 control) 10-24y - mean age: 18	Manual	Both	Far wall of: CCA (1cm prox to bulb), bulb, and internal: "multiple digital image loops" were taken	Max	used structural equation modelling (SEM) to extend general linear modelling - SEM adjusted error in latent variables (those variables prone to error) to reduce bias and to allow assessment of mediation and moderation (i.e. indirect effects) Latent variables: cIMT (calculated from all 3 segments), BP (from SBP and DBP z-scores), glycemia (BGC - FPG and HbA1c) - age, sex, and SBPz-score were significant determinants of all segments - direct relationship between non-HDL and cIMT Direct effects on cIMT: BP latent, BGC latent, age, non-HDL, race, sex Indirect effects on cIMT: BGC latent, CRP, insulin, BMI z-score, age, race, sex Total effect (top 5): 1. Age (B=0.3934)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						2. Sex (B=0.3622) 3. BP latent (B=0.2278) 4. BGC latent (B=0.2097) 5. BMI z-score (B=0.3622) *exclusion of t2DM: similar results but no relation bt insulin/BGC with BP **OVERALL: no direct relationship between BMI and cIMT → but it exerts its influence on other traditional CVRFs (magnitude of BMI effect on cIMT (although indirect) is greater than non-HDL**
Giurgea, G. A., Nagl, K., Gschwandtner, M., Höbaus, C., Hörtenhuber, T., Koppensteiner, R., ... & Schlager, O. (2015). Gender, metabolic control and carotid intima-media-thickness in children and adolescents with type 1 diabetes mellitus. *Wiener klinische Wochenschrift*, *127*(3-4), 116-123.	73 T1DM 243 control 8-16y: mean age 15y	"standardized scanning protocol"	both	Near and far wall CCA (1-2cm from bulb) - 3 angles: post-lateral, lateral and anterolateral views averaged 24 images	Mean	- T1DM had greater: weight, BMI, DBP, BP, FG, TC; lower HDL - cIMT: **No different between T1DM** (0.302mm) and control (0.301) - SEX effect: Girls > boys in T1DM but boys>girls in control - In whole group: gender, age, height, weight, BMI, SBP and DBP - In T1DM: **gender** and Hb1Ac - In boys: gender, age, height, weight, BMI, SBP, DBP, FG o DM boys: only weight - In girls: weight, BMI and SBP; no independent correlative in DM girls - **Multivariate** analysis showed: in above variables in: o model 1: all – 12.7% variance attributed to **BMI, gender, DBP**

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						○ model 2: control – 18.7% attributed to **gender, BMI, SBP** ○ model 3: T1DM – 9.4% attributed to **HbA1c** – OVERALL: **BMI, gender, SBP** and **DBP** are RF for early structural changes in control and some T1DM; but really **HbA1c** is only independent RF for T1DM (even though no diff between DM and control)→ suggests that control of glucose plays important role; sex differences were opposite between controls (M>F) and T1DM (F>M)
Gül, K., Üstün, İ., Aydin, Y., Berker, D., Erol, K., Ünal, M., ... & Güler, S. (2010). Carotid intima-media thickness and its relations with the complications in patients with type 1 diabetes mellitus/Tip 1 diyabetes mellituslu hastalarda karotis intima-media kalinligi ve komplikasyonlarla iliskisi. *Anadulu Kardiyoloji Dergisi: AKD*, *10*(1), 52.	113 T1DM 59 control 15-64y: mean age 27y	-	Both	Far wall of CCA, carotid bulb, and internal CA averaged 10 images	Max	– T1DM had higher smoking ratio, TG and cIMT – cIMT: higher for smokers in both control and T1DM and men with T1DM ○ those with DM complications are had higher cIMT ○ cIMT in T1DM group correlated with: age, duration of DM, # micro complications, and UAE ○ cIMT in control correlated with: age, TC, LDL ○ cIMT was higher in patients with microalbumuria than those without nephropathy (not diff between

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						microalbuminuria and macroalbuminuria) – After adjusting for age and sex in T1DM, cIMT was associated with duration of DM, UAE, Micro and macroalbuminuria and retinopathy – OVERALL: cIMT was higher in T1DM and was associated with microvascular complications; **duration and age** were most common predictors of cIMT
Iannuzzi, A., Licenziati, M. R., Acampora, C., Salvatore, V., Auriemma, L., Romano, M. L., ... & Trevisan, M. (2004). Increased carotid intima-media thickness and stiffness in obese children. *Diabetes care*, *27*(10), 2506-2508.	100 OB 47 age matched controls (recruited from Ped clinic) 6-14 y: mean age 10y	"following standardized protocol"; no description	Both	Far and near wall CCA	Max	– OB imt (0.55mm) > control (0.48mm) – Note: all lab measures were also different when compared to controls (i.e. SBP, DBP, TC, TG, FPG, insulin, HOMA, CRP) – When ANCOVA model was adjusted for confounders (age, sex, TC, HDL, TG), IMT was still significantly higher in OB compared to controls; addition of CRP in model did not change this – When **SBP** was added to model, association lowered but still remained significant – When **HOMA** was added into model, difference between OB and control did not reach significance → suggesting that **IR may play a role** in the relationship between OB and increased IMT

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						– OVERALL: OB is powerful determinant of early manifestation of structural changes in vessels; possibly mediated by **SBP** and **IR** (as measured by HOMA)
Jarvisalo MJ, Jartti L, Nanto-Salonen K, Irjala K, Ronnemaa T, Hartiala JJ, Celer- majer DS, Raitakari OT: Increased aortic intima-media thickness: a marker of pre- clinical atherosclerosis in high-risk children. *Circulation* 104:2943–2947, 2001	16 HyperChol (11 FH) 44 T1DM 28 control mean age: 11y	Manual	Both	Far wall CCA 2 angles: anterior oblique and lateral	Mean	– HC group had higher chol indices; DM had higher HbA1c at baseline – AIMT > cIMT in DM and HC group → more pronounced increase – Abnormal AINT and abnormal cIMT> abnormal AIMT and normal cIMT > normal AIMT and abnormal cIMT – cIMT: DM and HC > control (but no diff bt HC and DM); no correlates in control – In DM: **serum TC, LDL, SBP and DBP** – In HC: **age** – Multivariate analysis confirmed independent role of **DBP** and group (**presence of disease**) – OVERALL: **DBP** was strongest predictor of cIMT in all groups → potential role in SMC proliferation in early thickening of arterial wall
Krebs, A., Schmidt-Trucksäss, A., Alt, J., Doerfer, J., Krebs, K., Winkler, K., & Schwab, K. O. (2009). Synergistic effects of	60 FH 40 healthy control	Automatic	Both	Far wall CCA - Averaged 100+ images - 2 angles: posterior	Mean and max	– Stratified both healthy and FH groups into SBP < or ≥ 90th %tile: – Mean IMT: FH + SBP (0.594mm) > FH without SBP >90th%tile (0.545mm)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
elevated systolic blood pressure and hypercholesterolemia on carotid intima–media thickness in children and adolescents. *Pediatric cardiology*, *30*(8), 1131-1136.	5.8-17.9y: mean age 9-13 y			oblique and lateral		– Mean IMT: FH + SBP (0.712mm) > FH without SBP >90th%tile (0.687mm) – Univariate: in FH cohort (no significant relationship in healthy control) – mean cIMT and SBP index (SBP divided by 90th%tile) were correlated, r=0.358, p=0.011 - (i.e. if SBP index > 1, then you are higher than 90th centile for age and height and sex → abnormal) – max cIMT and SBP index were correlated, r=0.341, p=0.015 – OVERALL: lack of relationship between SBP and healthy controls but in FH sample suggests synergistic effect of elevated BP and hypercholesterolemia
Leite, A., Santos, A., Monteiro, M., Gomes, L., Veloso, M., & Costa, M. (2012). Impact of overweight and obesity in carotid intima-media thickness of portuguese adolescents. *Acta Paediatrica*, *101*(3), e115-e121.	50 OB 50 OW 50 control mean age: 12.9y	Manual	Both	Far wall CCA Averaged 3 measurements per side	Mean	– OB had higher: WC, BMI, SBP, DBP, TC, LDL, TG and lower HDL compared to control – More OW and OB had "borderline or abnormal" lipid profile (lower HDL and higher TG) – cIMT: higher in OB (0.465mm) and OW (0.46mm) compared to control (0.409mm) – MetS (n=11) had highest cIMT (0.465mm)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						– When adjusted for SBP, HDL, LDL and TG, all categories were still significantly higher than control – Correlations: cIMT was positively correlated with BMI (r=.439), WC (r=.301), and DBP (r=.266) – Multivariate analysis showed that only **BMI** (B=.004) was a significant predictor of IMT after adjusting for DBP, WC, and BMI – OVERALL: increases in cIMT can occur in **OW** (**not just OB**) independent of aging, BP, low HDL and high TG
Mittelman, S. D., Gilsanz, P., Mo, A. O., Wood, J., Dorey, F., & Gilsanz, V. (2010). Adiposity predicts carotid intima-media thickness in healthy children and adolescents. *The Journal of pediatrics*, *156*(4), 592-597.	599 all healthy (307 female, 292 male) 6-20y: mean age 14y	Automated program determined cIMT values in frames of each cycle, and 3 readings of systolic and 3 diastolic frames were used for analysis	Left	Far wall CCA 3 averaged images	Mean	– Sex differences: males were: taller, heavier, had greater WC and SBP, lower DBP and greater cIMT o Bc there were sex differences in cIMT, data was stratified by sex o Females: significant inverse correlation between HDL and cIMT o For both sexes: cIMT was correlated with weight, BMI z-score, SBP, TC:HDL, WC, HipC, and W:H when all pts were included; when only >85th %ile, lost ass of SBP,

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						TC:HDL and W:H in females; WC, hipC, W:H and TC:HDL in males) ▪ Not correlation with age, DBP or fasting TF, TC, LDL o Race: had higher cIMT, but there was not difference between whites, Hispanics and Asians – Multiple regression showed that BMI (even after excluding OB), WC, and race (black) independently predicted cIMT in both M and F; even after controlling for age, weigh, hipC, BP and fasting lipids o Female: association between race and IMT was lost if only analyzing <85th %percentile – OVERALL: **sex, race and BMI** were independent predictors of cIMT; regardless of being OB/OW → risk of CVD increases linearly with **BMI regardless of if OB** or not – Would also suggest that since LDL did not affect cIMT, LDL only plays a role if other known CVD RFs are present

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Morrison, K. M., Dyal, L., Conner, W., Helden, E., Newkirk, L., Yusuf, S., & Lonn, E. (2010). Cardiovascular risk factors and non-invasive assessment of subclinical atherosclerosis in youth. *Atherosclerosis*, *208*(2), 501-505.	51 elevated LD 44 OW 53 control	Manual	Both	Near and Far wall of ICA, CCA, bifurcation Minimum of 3 mean measurements and 3 maximal measurements per segment were taken to calculate respective average mean or average max	Max and mean	– Baseline differences: LDL group had more F Hx and cIMT compared to control; OW and LDL had higher BMI, %BF, TC, LDL, lower HDL, higher apoB, TG, insulin, HOMA IR; OW alone had higher apoA1 and SBP – cIMT: was only higher in LDL group (0.403mm) compared to OB (0.392mm) and control (0.387mm) o Age affect: increased IMT was only see in >10y; seen in all group o univariate analysis: cIMT was associated with age, height, weight, WC, DBP, apoA1 and apoB1, and TC:HDL o multivariate: **age** was strongest, F Hx and apoB were weaker predictors OVERALL: cIMT was only increased in **FH group**, which may be predicted by **age**, and partially by **family hx**
Oren, A., Vos, L. E., Uiterwaal, C. S., Grobbee, D. E., & Bots, M. L. (2003). Cardiovascular risk factors and increased carotid intima-media thickness in healthy young adults: the	750 health adults (352 male, 398 female) 27-30year: 28.4y	automatic	Both	Far wall and near CCA: x4 angles per side: (maximum 16 measurements)	Mean	Baseline differences: - Mean had higher: CCA IMT, SBP, DBP, LDL, FBG, TG and lumen diameter Correlates: – univariate: CCA IMT was related to BP, male sex, age, W:H, TC, LDL, TG, lumen diameter of CCA and # pack-years smoking

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Atherosclerosis Risk in Young Adults (ARYA) Study. *Archives of Internal Medicine*, *163*(15), 1787-1792.						– Multivariate model: r^2 : 0.36 o CCA IMT was independently related to: age, BMI, pulse pressure, sex, and LDL ▪ Age and BMI had largest contribution (as determined by per 1SD change in CVRF)
Pacifico, L., Anania, C., Martino, F., Cantisani, V., Pascone, R., Marcantonio, A., & Chiesa, C. (2010). Functional and morphological vascular changes in pediatric nonalcoholic fatty liver disease. *Hepatology*, *52*(5), 1643-1651.	250 OB (100 with NAFLD) 150 normal control mean age: 10.7-11.4y	-	Both	Far wall CCA 4 images were measured on each side	Mean and Max	MEAN cIMT: - NAFLD+OB cIMT(0.47mm) > OB cIMT(0.44mm) > Normal (0.40mm) MAX cIMT: - NAFLD+OB cIMT(0.55mm) > OB cIMT(0.52mm) > Normal (0.47mm) Univariate in OB only: did odds ratio adjusting for age, gender, tanner stage (used >90t healthy control as cutoff for "increased cIMT") - increased max cIMT associated with: BMI SDS, WC, high glucose, IR, CRP, MetS, NAFLD Multi-variate: variables include – age, sex, tanner stage, and either met s as group, individual and when IR replaces high gluc – **MET** as group variable, **High gluc** when MetS was separated, **IR** in separate model when replacing high gluc

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Pacifico, L., Cantisani, V., Anania, C., Bonaiuto, E., Martino, F., Pascone, R., & Chiesa, C. (2009). Serum uric acid and its association with metabolic syndrome and carotid atherosclerosis in obese children. *European Journal of Endocrinology*, *160*(1), 45-52.	120 OB (>97th%ile) 50 control 9-11y (mean age 10y)	-	Both	Far wall CCA 4 images were measured on each side	Mean and max	– OB had higher: gluc, insulin, HOMA-IR, TG, aLT, GGT, creatinine, UA, and BP; lower HDL o 27.5% has MetS – cIMT: was higher in OB (0.49mm; 0.55mm) compared to control (0.42mm; 0.42mm) in mean and max; **MetS** had higher max cIMT (0.60mm) compared to those without and increased cIMT by # of syndrome components – univariate: cIMT was associated with BMI-SDS, SBP, TG, ALT, GGT, gluc, insulin, HOMA-IR and UA in all participants after adjusting for age, sex and puberty o in control: cIMT was associated with just UA o in OB: cIMT was associated with **SBP, ALT, insulin, HOMA-IR** and **UA** o among the Obese, **MetS** had independent association with cIMT – multivariate linear (max IMT): after adjusting for age, gender, puberty, creatinine, and MS, **UA** and cIMT were significantly associated – multivariate logistic (max IMT): after adjusting for the variables

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						above but individual MET components, **UA** and **HOMA-IR** were significantly related to cIMT – OVERALL: **UA** and MetS (esp IR indices: **HOMA-IR, insulin**) are significantly associated with cIMT in OB population; UA was also significant in control pop
Pacifico, L., Bonci, E., Andreoli, G., Romaggioli, S., Di Miscio, R., Lombardo, C. V., & Chiesa, C. (2014). Association of serum triglyceride-to-HDL cholesterol ratio with carotid artery intima-media thickness, insulin resistance and nonalcoholic fatty liver disease in children and adolescents. *Nutrition, Metabolism and Cardiovascular Diseases*, *24*(7), 737-743.	391 OB/OW (282 boys) based on IOTF 6-16y: median age: 10.1	-	Both	Far wall CCA: 4 images were measured on each side	Mean and max	stratified based on TG:HDL - cIMT increased per tertile o Mean IMT: 0.42 < 0.43 < 0.45 o Max: 0.5< 0.5< 0.54 - Prevalence of CVRF increased per tertile; as did prevalence of cIMT >0.50mm - Independent predictors of cIMT: OR, adjusted for age, gender, puberty, WC or BMI, hsCRP o **Elevated BP: 5.13, p=0.046** o **IR: 2.16, p=0.003** o **NAFLD: 2.7 p=0.003** – **TG:HDL ratio:1.81, p=0.**025
Reinehr, T., Kiess, W., de Sousa, G., Stoffel-Wagner, B., & Wunsch, R. (2006). Intima media thickness in childhood	96 OB 25 normal weight control	-	Both	Far wall CCA: 4 measurements on each side	Max	– Baseline differences – OB had greater: BMI/weight/BMI z-score, % body fat, SBP, DBP, Insulin, hsCRP and IMT

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
obesity: relations to inflammatory marker, glucose metabolism, and blood pressure. *Metabolism*, *55*(1), 113-118.						○ OB IMT (0.6mm) > Lean IMT (0.4mm) ○ When stratified by increasing cIMT: increasing degree of overweight, SBP, DBP, glucose and hsCRP were significantly associated using Kruskal-Wallis test. – In OB, univariate analysis found that IMT was correlated to: BMI, BMI z-score, % body fat, SBP, DBP, glucose, hsCRP – multiple backward linear regression: r^2 for model = 0.21) ○ **BMI, SBP, glucose and hsCRP** were significant independent correlates of cIMT in a model including: LDL, age, sex, pubertal stage, SBP, DBP, HDL, TG and insulin
Reinehr, T., Wunsch, R., De Sousa, G., & Toschke, A. M. (2008). Relationship between metabolic syndrome definitions for children and adolescents and intima-media thickness. *Atherosclerosis*, *199*(1), 193-200.	264 overweight (all Caucasian) 7-16y: median age 11y	-	Both	Far wall CCA: - 4 measurements on each side	Max	- looked at diff definitions of MetS and compared to see which one predicated cIMT best: - overall IGT > Mets definitions to predict 0.7mm cIMT (top quartile) with 96%PPV whereas Met S definitions only predicted <50% increased cIMT – - OVERALL: IGT better predictor of cIMT and should be evaluated for CVRF

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Reinehr, T., Wunsch, R., Pütter, C., & Scherag, A. (2013). Relationship between carotid intima-media thickness and metabolic syndrome in adolescents. *The Journal of pediatrics*, *163*(2), 327-332.	461 OW (all Caucasian) 10-18y: median age 12.1	-	Both	Far wall CCA: - 4 measurements on each side	Max	- Multivariate: **WC, BMI, SBP, 2hrPG** (adjusted for age, sex, pubertal status) - BMI has protective effect? (OR for IMT >0.7mm = 0.92; beta for continuous cIMT = -1.19 - best model to predict >0.7mm cIMT was sum of quantitative Met S measures – OVERALL: use individual quantitative measures within METs to assess CVRF instead of dichotomous MetS variable (yes/no) – MetS group variable not as useful to predict cIMT
Ryder, J. R., Dengel, D. R., Jacobs, D. R., Sinaiko, A. R., Kelly, A. S., & Steinberger, J. (2016). Relations among adiposity and insulin resistance with flow-mediated dilation, carotid intima-media thickness, and arterial stiffness in children. *The Journal of pediatrics*, *168*, 205-211.	252 healthy (ranged from normal to OW/OW) 8-20y: mean age: 14.9-15.3y	Semi-automated Repeatability: 0.02 +/- 0.03mm	Left	Far wall CCA: measurements were taken "proximal to carotid bulb over a period of 10s"	Mean	cIMT: OB cIMT > normal weight cIMT Divided each dependent measure into tertiles: - IR: most IR had higher cIMT (high > low, p=0.05, high > med, p=0.059 - BF%: no diff but trending towards significance between high and low tertiles (p=0.086) - VAT: high > med > low Multiple linear regression: - VAT: was predictive of cIMT (r2=0.08, p=0.002) – BF%: was predictive of cIMT (r2 = 0.048, p=0.028)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Schiel, R., Beltschikow, W., Radon, S., Kramer, G., Perenthaler, T., & Stein, G. (2007). Increased carotid intima-media thickness and associations with cardiovascular risk factors in obese and overweight children and adolescents. *Eur J Med Res*, *12*(10), 503-508.	81 OB/OW (hospitalized for weight reduction; recruited from Diabetes Dept; completed structured treatment and teaching program, 36 days) mean age: 13.6y	*only 29/81 had IMT done (all OB)	Both	CCA (no mention of far or near wall) Averaged 5 images from each side	Mean	– mean IMT: 0.48 – stratified data based on low (<0.45mm) or high IMT (>0.45mm) – higher IMT patients also had after univariate analysis: higher BMI/BMI-SDS, fat mass and %fat, SBP and DBP, and serum uric acid – after multivariate analysis, only **BMI at onset of STTP** was significant – after correlation analysis: BMI (r=0.482), SBP and DBP (spontaneous) (r=0.359 both), and 24h SBP (r=0.344) had highest correlation – OVERALL: **BMI** is powerful predictor of pathological changes as seen in increased IMT
Schwab, K. O., Doerfer, J., Krebs, A., Krebs, K., Schorb, E., Hallermann, K., ... & Winkler, K. (2007). Early atherosclerosis in childhood type 1 diabetes: role of raised systolic blood pressure in the absence of dyslipidaemia. *European journal of pediatrics*, *166*(6), 541-548.	94 T1DM 40 control median age: 12.3y	Automatic	Both	Far wall CCA - Averaged 100+ images 2 angles: posterior oblique and lateral	Mean	– T1DM had high: HbA1c, weight, BMI/BMI sds, SBP, DBP, and bilateral IMT – cIMT: T1DM (0.571mm) > control (0.548mm) o In T1DM: (spearman rank correlation) bilateral IMT was associated with **DM duration, SBP, TC, ApoB** o In control, only **HbA1c** was correlated – Inflam markers: T1DM had higher: hsCRP, L-selectin, vWF,Plasmin/a2-antiplasmin

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						– OVERALL: T1DM had higher cIMT; when analyzed individually, **DM duration**, **SBP**, TC, APOB were associated with cIMT;
Shah, A. S., Gao, Z., Urbina, E. M., Kimball, T. R., & Dolan, L. M. (2014). Prediabetes: the effects on arterial thickness and stiffness in obese youth. *The Journal of Clinical Endocrinology & Metabolism*, *99*(3), 1037-1043.	102 OB with Pre-DM 129 OB (pre-DM: fasting gluc 100-125mg/dl, 2h OGTT 140-199mg/dl, or HbA1c 5.7-6.4%) 11-23y: mean age 18y	Automatic	Both	Far wall of CCA. Bulb and ICA:	Max	– OB+Pre-DM had higher: BMI-zscore, SBP, fasting insulin and gluc, HbA1c, 2h OGTT and internal cIMT o When dissecting within pre-DM group: only TG was different among groups (IGT >IFG>HbA1c → note over 50% of participants diagnosed with pre-DM met Hb1ac definition) – cIMT**: pre-DM** was significant determinant of internal cIMT (adjusted for age, race, sex, bmi zscore, lipids, bp fasting insulin, CRP and adiponectin o Also: Male Sex and race were also independent determinants (14% in internal, 12% in CCA) – Further regression that dissected pre-DM definitions showed that: *HbA1c* and abnormal *OGTT* were *trending* ($p<0.1$) towards association with internal cIMT (possibility of HbA1c contributing to false-positive reading; but reran regression without HbA1c and impaired OGTT was still trending toward association; and

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						pre-DM was still borderline significant) – OVERALL**: pre-DM** is independent RF for athero → provides additional influence on vascular not explained by traditional RFs; only affected internal cIMT (possibility that pre-DM only affects more peripheral vasculature)
Simsek, E., Balta, H., Balta, Z., & Dallar, Y. (2010). Childhood obesity-related cardiovascular risk factors and carotid intima-media thickness. *The Turkish journal of pediatrics*, *52*(6), 602.	75 OB (>97th%ile) 40 control (<95th%ile)	-	Both	Far wall CCA Minimum of 4 measurements on each side, only thickest one used for analysis	Max	– OB had higher:BP, WC, BMI, TG, LDL, TC, lower HDL, fasting insulin, HOMA-IR – cIMT: OB (0.52mm) > control (0.35mm) – BMI and IMT correlated: relationship was positively correlated with SBP, DBP, W:H, **WC** and HC, **TC**, LDL and TG in OB children – WC showed important correlation with – Multi stepwise regression showed that **BMI-SDS** (B=.071), **TG** (B=.001) **and QUICKI** (B=-1.35)(quantitative insulin-sensitivity check index) were independent predictors of cIMT; even after adjusting for SBP, lipids, fasting gluc and fasting insulin and HOMA IR o OVERALL: **BMI-SDS**, **TG** and **insulin**

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						sensitivity are predictors of IMT → should be used for screening
Tounian, P., Aggoun, Y., Dubern, B., Varille, V., Guy-Grand, B., Sidi, D., ... & Bonnet, D. (2001). Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *The Lancet*, *358*(9291), 1400-1404.	48 OB 27 control (recruited from outpatient clinic) 4-17y: mean age 12 OB, 12.6y control (note: all normotensive)	Semi-automatic	Right	Far wall CCA	Mean	– OB had higher TC, TG, lower HDL – Android/gynoid fat mass ratio: after univariate analysis, TG, fasting insulin and insulin AUC were significantly associated → greater truncal fat was positively correlated to plasma TG and measures of IR – cIMT: Found no difference between OB and control (OB 0.49mm, control 0.50mm;p=0.07); but there was diastolic wall stress was significantly increased → authors suggest that vascular remodeling in OB children may be enough to advance vessel stiffness but not enough to increase IMT – **Note**: main study cited for NS (>700 citations) BUT Lamotte 2011: suggested that it could be due to limited sample size – OVERALL: IR, TG and HDL are associated with **trucal fat** and that is associated with early changes in vascular function (flow mediated dilation, diastolic wall stress) but not structure (cIMT)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results

Urbina, E. M., Dabelea, D., D'Agostino, R. B., Shah, A. S., Dolan, L. M., Hamman, R. F., ... & Wadwa, R. P. (2013). Effect of Type 1 Diabetes on Carotid Structure and Function in Adolescents and Young Adults. *Diabetes Care*, *36*(9), 2597-2599.	403 T1DM
	206 control
	
	mean age 18.8-19.2y
		Manual	Both	Far wall CCA, ICA, Bulb
				6 different angles: 90, 120, 150, 210, 240, and 270 degrees
					Mean	- T1DM had greater: non-Hispanic whites, HR, TC, LDL, HDL, FG, HbA1c, and thicker bulb cIMT
						- cIMT: greater in T1DM (only in bulb) (0.461mm vs. 0.445mm)
						  - most consistent determinants of cIMT: age, male, adiposity, SBP
						  - internal: LDL
						  - CCA: TG
						  - bulb: TG; but sig was eliminated once HbA1c was adjusted for → suggests that glycemic control is important factor in T1DM for explain cIMT
						– OVERALL: **TG** and **LDL** were associated with cIMT in T1DM patients

Urbina, E. M., Khoury, P. R., McCoy, C., Daniels, S. R., Kimball, T. R., & Dolan, L. M. (2011). Cardiac and vascular consequences of pre-hypertension in youth. *The Journal of Clinical Hypertension*, *13*(5), 332-342	127 HTN 65 pre-HTN 531 control mean age: 18y	Semi-automatic	Both	Near and far wall of CCA, bifurcation and internal carotid	Mean	– CV profile worsened from: control < pre-HTN <HTN (i.e. T2DM, adiposity, activity levels, LVMI) – cIMT: was higher in HTN and pre-HTN in bifurcation and internal cIMT; CCA-IMT was only statistically higher in HTN o Multivariate analysis showed that **BP was independent predictor** of internal cIMT after adjusting for CV RFs (i.e. T2DM, BMI) and distending pressure - OVERALL: graded increased in cIMT linear to BP status; **BP was independent predictor** of internal

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						cIMT and should be used to monitor/prevent target organ damage
Verçoza, A. M., Baldisserotto, M., de los Santos, C. A., Poli-de-Figueiredo, C. E., & d'Avila, D. O. (2009). Cardiovascular risk factors and carotid intima-media thickness in asymptomatic children. *Pediatric cardiology*, *30*(8), 1055-1060.	93 asymptomatic children (recruited from regular clinic in hospital) (25% Ob, 19% OW) 4y-14y: mean age 8-9y	-	Both	ICA, Bulb, CCA (no mention of far or near wall)	Mean	– Girls had lower glucose and boys had higher cIMT (not affected by age) – OB/OW were correlated with: fibrinogen, TGL, SBP, DBP, cIMT, and negatively with HDL – cIMT: was correlated with SBP o after multiple linear regression that adjusted for age, gender, F Hx, TG, BMI-z score, and OW/OB, only **gender** and **OW/OB** were related to cIMT – OVERALL: males had higher cIMT which supports the association between cIMT and **gender; OW/OB and BMI**
Wiegman, A., de Groot, E., Hutten, B. A., Rodenburg, J., Gort, J., Bakker, H. D., ... & Kastelein, J. J. (2004b). Arterial intima-media thickness in children heterozygous for familial hypercholesterolaemia. *The Lancet*, *363*(9406), 369-370.	20 FH 80 unaffected siblings 8-18y	Manual	Both	Far wall ICA, Bulb, and CCA	Mean	Mean cIMT was higher in FH (0.494mm) than control (0.472mm); remained significant after adjusting for family hx, HDL and TG – Sib-pair differences (64 pairs): Mean cIMT diff of 0.022m (similar to entire study); FH siblings had at least 5x more rapid increase in cIMT/year – cIMT: was correlated with age, male sex, and HDL o multivariate analysis showed that age, male sex and LDL

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
						were independent predictors of cIMT o when analyzed separately, boys had thicker IMT and were partially due to LDL and age – OVERALL: important role of **LDL** in FH (which we know is main factor that increases athero burden); age and sex were also important predictors of cIMT; **age** is also important predictor
Yamasaki, Y., Kawamori, R., Matsushima, H., Nishizawa, H., Kodama, M., Kajimoto, Y., ... & Kamada, T. (1994). Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high-resolution B-mode imaging. *Diabetes*, *43*(5), 634-639.	105 T1DM (13y) 529 T2DM (60y) 104 controls (51 youth-19y; 53 adults- 45y)	-	Both	"1 cm upstream and 1 cm downstream from the site of the greatest thickness." - 3 Angles: anterior-oblique, lateral and posterior-oblique	Max	– T1DM youth (<10y no difference in cIMT but there was a sig among 10-19y group (0.52mm>0.44mm) – cIMT in T1DM children: o Univariate: linear relationship with **age** and **duration of DM**; confirmed with multi variant analysis o Multi analysis also showed weak (P<0.1) association with HbA1c and **sex** – OVERALL: 10-19y IDDM had higher cIMT but not 10y; since **age** and **duration of DM** were RF for cIMT, lack of ass in <10y group may be explained by shorter DM duration
Yilmazer, M. M., Tavli, V., Carti, Ö. U., Mese, T., Güven, B., Aydın, B., ... & Tavlı, T. (2010).	77 OB (recruiting from outpatient clinic of	-	Right	Far wall CCA Averaged 3 images	Max	– OB: had higher SBP, DBP, cIMT, TC, LDL, TG, insulin – cIMT: was higher in OB (0.57mm) compared to control (0.45mm)

Author, Year, Journal	Subjects	cIMT method	Left or Right	Segments	Mean or Max	Results
Cardiovascular risk factors and noninvasive assessment of arterial structure and function in obese Turkish children. *European journal of pediatrics*, *169*(10), 1241-1248.	Cardiology dept) 40 control (recruited from clinic investigating heart murmur) 7-16y: mean age 11.5y OB, 9.75y control					○ univariate analysis showed associations with BMI, WC, SBP, DBP, HTN, TG, Insulin ○ stepwise multi regression showed that **BMI** and **hyperTG** were correlated to high cIMT ▪ in hyperlipidemic patients: hyperTG was positively correlated with cIMT – Note: cIMT, FMD, and CAC were not affected by pubertal status – Interestingly increased cIMT and decreased CAC were associated with degree of OB – OVERALL: OB patients have higher cIMT, lower FMD and lower CAC; cIMT may be predicted by **BMI** and **TG**

APPENDIX B1: SAMPLE SIZE CALCULATIONS FOR MULTIVARIABLE MIXED MODELLING

Sample size estimation to examine association between cIMT and CVRFs among children and youth enrolled in a weight management program

Study Design: Cross-sectional

Outcome: cIMT (mm)
cIMT z-score

Proposed Test Variables (10): Measures of glycemia (HbA1c, FPG, 2hr PG)
BMI z-score
Age
Height
Family Hx of CHD
Systolic BP
HDL cholesterol
Non-HDL cholesterol or LDL cholesterol

Model: Multiple linear regression with fixed predictors

Estimated sample sizes

Level of significance (α %)	Power (%)	No. of predictors in the full model	No. of test predictors	R^2 for full model (%)	R^2 difference (full vs. reduced model)	Estimated sample size	Non response rate, missing values (%)	Sample size[1]
5	80	10	5	20	3	348	10	387
5	80	10	5	20	4	263	10	293
5	80	10	5	20	5	211	10	235
5	80	10	5	20	7	153	10	170
5	80	10	5	20	10	109	10	122

[1]After accounting for non response, missing values

APPENDIX B2: SAMPLE SIZE CALCULATION FOR GROUP MEAN DIFFERENCES:

$$n = \left({\delta_1}^2 + {\delta_0}^2\right) \times \left(\frac{z_{\frac{\propto}{2}} + z_\beta}{d}\right)^2$$

${\delta_1}^2 + {\delta_0}^2$: standard deviations of variable in each group
$z_{\frac{\propto}{2}}$: value of error (95% confidence = 1.96)
z_β: value of error (80% power = 0.85)
d: minimum difference between group mean values

Based on Marini et al, 2012 that saw a relative difference of 6.6% between those with and without prediabetes (as classified by HbA1c: 5.7-6.4%):

${\delta_1}^2 + {\delta_0}^2$: 0.04mm for PreDM, 0.03mm for Normal glycemia
d: was calculated by the mean cIMT as seen in our group (0.39mm) X relative difference seen in adult prediabetic cohorts (6.6%) = 0.0257

$$n = \left(0.04mm^2 + 0.03mm^2\right) \times \left(\frac{1.96 + 0.85}{0.0257}\right)^2$$

$$n = \left(0.0016 + 0.0009\right) \times \left(\frac{1.96 + 0.85}{0.0257}\right)^2$$

$$n = (0.0025) \times (109.17)^2$$

$$n = (0.0025) \times (11{,}917.76)$$

$$n = 29.79 = 30 \text{ participants per sub} - \text{group}$$

Therefore, in order to see a relative difference of 6.6% between prediabetics and normal glycemic groups, a minimum of 27 participants per sub-group is required, as per non-paired sample size calculations for quantitative variables.

APPENDIX C1: PARTICIPANT CHARACTERISTICS BY GROUP (IFG, IGT ONLY)

Variable	All	Normal Glycemia	Prediabetes (IFG or IGT)	p-value
N	162*	129	6	
Participant Demographics				
Age(y), n	12.77±2.95,162	12.57±3.00,129	12.11±2.18,6	0.710
Sex: Male (%), n	73(45.06),162	55(42.64),129	1(16.67),6	0.400
Race: Caucasian (%), n	122(75.31),162	101(78.29),129	4(66.67),6	0.614
Annual Household Income: < $50,000/year (%), n	41(25.31),162	33(25.58),129	1(16.67),6	1.000
Female Caregiver Education: attended university/college (%), n	125(77.16),162	102(79.07),129	6(100.0),6	0.599
Male Caregiver Education: attended university/college (%), n	110(67.90),162	89(68.99),129	4(66.67),6	1.000
Weight (kg), n	83.69±25.71,161	80.87±24.50,128	74.03±25.73,6	0.506
Height(m), n	1.57±0.14,161	1.56±0.14,128	1.55±0.08,6	0.902
BMI (kg/m2), n	33.03±6.20,161	32.47±5.89,128	30.24±8.27,6	0.374
BMI z-score, n	3.37±1.01,161	3.33±1.01,128	2.84±1.28,6	0.254
Waist Circumference (cm), n	96.56±14.66,107	95.30±14.71,88	84.00±7.21,3	0.190
Body fat %, n	43.71±7.04,140	43.48±7.16,111	43.08±7.72,5	0.904
Participant Cardiovascular Risk Factors				
Family Hx of CVD (%), n	72(44.44),162	53(41.09),129	3(50.00),6	0.692
Parental Hx of premature CVD (%), n	13(8.02),162	8(6.20),129	0(0.00),6	1.000
Systolic BP (mmHg), n	110.27±11.38,160	109.70±11.05,128	111.98±16.52,6	0.631
Diastolic BP (mmHg), n	69.57±9.11,160	69.06±9.12,128	69.60±11.10,6	0.888
Total Cholesterol (mmol/L), n	4.28±0.68,145	4.26±0.69,128	4.32±0.72,4	0.881
Triglycerides (mmol/L), n	1.36±0.67,146	1.31±0.65,129	1.41±0.20,4	0.756
HDL Cholesterol (mmol/L), n	1.15±0.21,146	1.16±0.21,129	1.24±0.24,4	0.439
LDL Cholesterol (mmol/L), n	2.54±0.62,145	2.53±0.63,128	2.43±0.61,4	0.734
Non-HDL Cholesterol (mmol/L), n	3.12±0.69,145	3.10±0.70,128	3.07±0.56,4	0.938

Variable	All	Normal Glycemia	Prediabetes (IFG or IGT)	p-value
TC/HDL ratio, n	3.83±0.90,145	3.77±0.85,128	3.50±0.46,4	0.536
Fasting Plasma Glucose (mmol/L), n	4.74±0.39,145	4.71±0.37,128	5.28±0.49,6	**<0.001**
2-hr OGTT Glucose (mmol/L, n	5.92±1.22,78	5.75±0.97,65	9.20±1.24,4	**<0.001**
HbA1c (%),n	5.26±0.31,134	5.19±0.25,118	5.74±0.38,5	**<0.001**
Participant cIMT measurements				
Average Mean cIMT, 12-segments (mm), n	0.40±0.03,162	0.40±0.03,129	0.40±0.02,6	0.999
Average Mean cIMT, 2-segments (mm), n	0.39±0.03,162	0.39±0.03,129	0.38±0.03,6	0.342
Mean cIMT-for-age z-score, n	0.10±0.72,161	0.13±0.72,128	-0.09±0.81,6	0.464
Mean cIMT-for-height z-score, n	0.07±0.72,160	0.10±0.71,127	-0.15±0.75,6	0.407

All measures are presented as mean±SD unless otherwise stated.

*n=16 participants did not have any glycemic measures

APPENDIX C2: Prediabetes Classification: ADA VS CDA

	American Diabetes Association (2010)	**Canadian Diabetes Association (2013)**
IFG (FPG: 5.6-6.9 vs 6.1-6.9 mmol/L)	3	0
IGT (2hr PG: 7.8-11.0 mmol/L)	4	4
HbA1c (HbA1c: 5.7-6.4 vs 6.0-6.4 %)	13	2
Total	17	6

Values presented are of number frequencies of individuals classified as prediabetic based on ADA 2010 and CDA 2013 guidelines

APPENDIX C3: Mean cIMT z-score Skewness Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Z*
Mean cIMT-for-age z-score	161	-1.34	2.40	.1038	.72177	.451	.191	2.36
Mean cIMT-for-height z-score	160	-1.32	2.47	.0691	.72203	.611	.192	3.18

*standardized z-distribution scores of skewness did not in exceed z=3.29 (Tabachnick & Fidell), and thus did not require data transformation.

APPENDIX C4: Mean cIMT measures by arterial segment

	Variable	All	Normal Glycemia	Prediabetes	p-value
Far and Near walls	**N**	**162**	**129**	**17**	
	Average Mean cIMT, 12-segments (mm), n	0.40±0.03, 162	0.40±0.03, 129	0.40±0.03,17	0.754
	CCA IMT (mm), n	0.40±0.03, 162	0.40±0.03, 129	0.40±0.03,17	0.967
	Bulb IMT (mm), n	0.44±0.04, 162	0.44±0.04, 129	0.43±0.04,17	0.759
	Internal IMT (mm), n	0.36±0.03, 145	0.36±0.03, 119	0.35±0.03,13	0.465
Far wall only	Average Mean cIMT, 2-segments (mm), n	0.39±0.03, 162	0.39±0.03, 129	0.39±0.04,17	0.940
	CCA IMT (mm), n	0.39±0.03, 162	0.39±0.03, 129	0.39±0.04,17	0.940
	Bulb IMT (mm), n	0.43±0.04, 162	0.43±0.04, 129	0.42±0.04,17	0.375
	Internal IMT (mm), n	0.36±0.03, 145	0.36±0.03, 119	0.35±0.04,13	0.460

All measures are presented as mean±SD unless otherwise stated.

*n=16 participants did not have any glycemic measures

Appendix C5a: Univariable Regression Analyses of Segment-specific cIMT (Common Carotid Artery, Carotid Bulb)

	CCA: far wall only			CCA: far and near wall			Carotid Bulb: far wall only			Carotid Bulb: far and near wall		
	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	p-value	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	p-value
Age (year)	0.0016 (0.0009)	2	0.072	0.0016 (0.0008)	2.18	0.061	0.0047 (0.0009)	14.12	<0.001	0.0042 (0.0009)	11.91	<0.001
Sex (Male)	0.0094 (0.0051)	2.1	0.066	0.0082 (0.0050)	1.65	0.103	0.0150 (0.0057)	4.15	**0.009**	0.0169 (0.0055)	5.65	**0.002**
Race: Caucasian	-0.0003 (0.0059)	0	0.955	-0.0040 (0.0058)	0.3	0.49	-0.0045 (0.0067)	0.28	0.501	-0.0025 (0.0065)	0.09	0.698
Height(cm)	0.0004 (0.0002)	3.2	**0.023**	0.0005 (0.0002)	4.73	**0.006**	0.0009 (0.0002)	13.48	**<0.001**	0.0010 (0.0002)	14.92	**<0.001**
Parental Hx of Premature CVD	-0.0163 (0.0093)	1.87	0.082	-0.0134 (0.0091)	1.32	0.145	-0.0023 (0.0107)	0.03	0.828	-0.0050 (0.0103)	0.15	0.625
Family Hx of CVD	-0.0099 (0.0051)	2.29	0.054	-0.0115 (0.0049)	3.28	**0.021**	-0.0030 (0.0058)	0.17	0.606	-0.0046 (0.0056)	0.42	0.415
Annual Household Income (<$50,000/year)	0.0026 (0.0059)	0.12	0.664	0.0050 (0.0057)	0.48	0.383	0.0008 (0.0067)	0.01	0.901	0.0019 (0.0064)	0.05	0.771
Female Caregiver Education: attended university/college	0.0060 (0.0061)	0.61	0.322	0.0054 (0.0059)	0.51	0.365	0.0016 (0.0069)	0.04	0.812	-0.0003 (0.0067)	0	0.966
Male Caregiver Education: attended university/college	-0.0052 (0.0055)	0.57	0.341	-0.0025 (0.0053)	0.14	0.637	-0.0023 (0.0062)	0.09	0.712	-0.0023 (0.0060)	0.09	0.701
BMI z-score	-0.0005 (0.0025)	0.02	0.844	-0.0008 (0.0025)	0.07	0.735	-0.0027 (0.0029)	0.55	0.351	-0.0019 (0.0028)	0.29	0.5

Waist Circumference (cm)	0.0002 (0.0002)	1.18	0.265	0.0003 (0.0002)	2.38	0.113	0.0005 (0.0002)	4.02	**0.038**	0.0005 (0.0002)	4.73	**0.024**
Body Fat %	-0.0009 (0.0004)	3.97	**0.018**	-0.0008 (0.0004)	3.27	**0.032**	-0.0010 (0.0004)	4.03	**0.017**	-0.0011 (0.0004)	4.75	**0.01**
Systolic BP (mmHg)	0.0002 (0.0002)	0.47	0.39	0.0003 (0.0002)	1.31	0.15	0.0006 (0.0003)	2.91	**0.031**	0.0007 (0.0002)	4.86	**0.005**
Diastolic BP (mmHg)	-0.0000 (0.0003)	0	0.939	-0.0000 (0.0003)	0.02	0.867	0.0005 (0.0003)	1.67	0.104	0.0007 (0.0003)	3.24	**0.023**
Total Cholesterol (mmol/L)	-0.0003 (0.0041)	0	0.943	0.0025 (0.0040)	0.28	0.524	-0.0011 (0.0045)	0.04	0.81	-0.0017 (0.0043)	0.11	0.689
HDL Cholesterol (mmol/L)	-0.0292 (0.0126)	3.57	**0.022**	-0.0158 (0.0126)	1.09	0.21	-0.0214 (0.0142)	1.54	0.135	-0.0251 (0.0136)	2.3	0.068
LDL Cholesterol (mmol/L)	0.0043 (0.0044)	0.64	0.338	0.0063 (0.0043)	1.45	0.15	0.0026 (0.0050)	0.19	0.606	0.0021 (0.0048)	0.14	0.657
Non-HDL Cholesterol (mmol/L)	0.0025 (0.0040)	0.27	0.535	0.0040 (0.0039)	0.72	0.311	0.0011 (0.0044)	0.04	0.811	0.0008 (0.0042)	0.03	0.845
TC/HDL ratio	0.0046 (0.0030)	1.55	0.136	0.0039 (0.0030)	1.19	0.191	0.0030 (0.0034)	0.56	0.372	0.0037 (0.0032)	0.9	0.257
Triglycerides (mmol/L)	0.0004 (0.0041)	0.01	0.924	0.0011 (0.0040)	0.06	0.779	0.0003 (0.0046)	0	0.947	0.0031 (0.0044)	0.34	0.487
Fasting Plasma Glucose (mmol/L)	0.0106 (0.0070)	1.59	0.131	0.0161 (0.0068)	3.75	**0.02**	0.0116 (0.0077)	1.53	0.138	0.0098 (0.0075)	1.2	0.19
2-hr OGTT Glucose (mmol/L)	-0.0030 (0.0030)	1.33	0.314	-0.0016 (0.0031)	0.36	0.602	-0.0020 (0.0030)	0.61	0.498	-0.0007 (0.0031)	0.07	0.815
HbA1c (%)	-0.0074 (0.0092)	0.49	0.419	-0.0072 (0.0090)	0.48	0.425	-0.0109 (0.0099)	0.92	0.271	-0.0102 (0.0095)	0.86	0.287

Appendix C5b: Univariable Regression Analyses of Segment-specific cIMT (Internal Carotid Artery)

	ICA: far wall only			ICA: far and near wall		
	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	p-value
Age (year)	0.0025 (0.0009)	5.66	**0.004**	0.0026 (0.0007)	8.8	**<0.001**
Sex (Male)	0.0083 (0.0052)	1.76	0.111	0.0087 (0.0043)	2.78	**0.045**
Race: Caucasian	0.0014 (0.0059)	0.04	0.816	0.0018 (0.0050)	0.1	0.711
Height(cm)	0.0004 (0.0002)	3.58	**0.023**	0.0005 (0.0001)	7.54	**0.001**
Parental Hx of Premature CVD	0.0005 (0.0091)	0	0.954	-0.0003 (0.0076)	0	0.97
Family Hx of CVD	-0.0069 (0.0052)	1.2	0.19	-0.0063 (0.0044)	1.44	0.15
Annual Household Income (<$50,000/year)	0.0062 (0.0061)	0.72	0.309	0.0023 (0.0051)	0.14	0.652
Female Caregiver Education: attended university/college	-0.0005 (0.0062)	0.01	0.931	0.0005 (0.0052)	0.01	0.922
Male Caregiver Education: attended university/college	-0.0048 (0.0055)	0.52	0.387	-0.0074 (0.0046)	1.8	0.108
BMI z-score	-0.0021 (0.0026)	0.45	0.425	-0.0018 (0.0021)	0.51	0.395
Waist Circumference (cm)	0.0001 (0.0002)	0.23	0.635	0.0002 (0.0002)	1.34	0.254
Body Fat %	-0.0006 (0.0004)	1.71	0.139	-0.0007 (0.0003)	3.52	0.033
Systolic BP (mmHg)	0.0003 (0.0002)	1.15	0.199	0.0004 (0.0002)	3.05	**0.036**
Diastolic BP (mmHg)	0.0001 (0.0003)	0.05	0.788	0.0003 (0.0002)	0.99	0.234

Total Cholesterol (mmol/L)	0.0029 (0.0040)	0.4	0.47	0.0035(0.0033)	0.85	0.29
HDL Cholesterol (mmol/L)	-0.0095 (0.0128)	0.42	0.457	-0.0110 (0.0107)	0.8	0.306
LDL Cholesterol (mmol/L)	0.0069 (0.0044)	1.91	0.114	0.0076 (0.0036)	3.29	0.038
Non-HDL Cholesterol (mmol/L)	0.0036 (0.0039)	0.67	0.35	0.0044 (0.0032)	1.38	0.177
TC/HDL ratio	0.0021 (0.0030)	0.38	0.479	0.0032 (0.0025)	1.22	0.205
Triglycerides (mmol/L)	-0.0029 (0.0040)	0.38	0.479	-0.0008 (0.0034)	0.04	0.813
Fasting Plasma Glucose (mmol/L)	0.0103 (0.0070)	1.64	0.145	0.0130 (0.0059)	3.67	**0.028**
2-hr OGTT Glucose (mmol/L)	0.0004 (0.0042)	0.01	0.928	0.0012 (0.0035)	0.17	0.73
HbA1c (%)	-0.0139 (0.0093)	1.81	0.138	-0.0106 (0.0078)	1.52	0.174

APPENDIX C6a: Multivariate Regression Analyses of Segment-specific cIMT (Common Carotid Artery, Carotid Bulb)

	CCA: far wall only			CCA: far and near wall			Carotid Bulb: far wall only			Carotid Bulb: far and near wall		
	B	Std. Error	Sig.	B	Std. Error	Sig.	B	Std. Error	Sig.	B	Std. Error	Sig.
(Constant)	0.444	0.071	<00.1	0.392	0.068	<0.01	0.429	0.076	<0.01	0.38	0.07	<0.01
Sex (male)	-0.004	0.008	0.563	-0.003	0.007	0.633	-0.008	0.008	0.315	-0.006	0.007	0.417
Age (years)	1.11E-05	0.002	0.995	0	0.002	0.863	-0.001	0.002	0.727	-0.001	0.002	0.517
Height (cm)	0	0	0.274	0	0	0.785	0	0	0.558	0	0	0.275
Family Hx of CVD	-0.003	0.008	0.736	-0.008	0.007	0.266	-0.002	0.008	0.83	-0.004	0.008	0.605
Waist Circumference (cm)	0	0	0.354	0	0	0.329	0	0	0.277	0	0	0.43
Percent body fat (%)	-0.001	0.001	**0.043**	-0.001	0.001	**0.038**	-0.001	0.001	0.056	-0.001	0.001	0.074
Systolic Blood Pressure (mmHg)	0.001	0.001	0.149	0.001	0	0.102	0	0.001	0.769	0	0	0.763
Diastolic Blood Pressure (mmHg)	-0.001	0.001	0.121	-0.001	0.001	**0.038**	0	0.001	0.75	0	0.001	0.601
HDL(mmol/L)	-0.034	0.018	0.067	-0.015	0.017	0.382	-0.025	0.02	0.202	-0.024	0.018	0.181
Fasting Plasma Glucose (mmol/L)	0.014	0.01	0.152	0.013	0.009	0.15	0.006	0.01	0.567	0.011	0.009	0.246

Appendix C6b: Multivariate Regression Analyses of Segment-specific cIMT (Internal Carotid Artery)

	ICA: far wall only			ICA: far and near wall		
	B	Std. Error	Sig.	B	Std. Error	Sig.
(Constant)	0.414	0.075	<0.01	0.371	0.058	<0.01
Sex (male)	-0.009	0.008	0.297	-0.006	0.006	0.337
Age (years)	-0.001	0.002	0.551	-0.001	0.001	0.553
Height (cm)	0	0	0.612	0	0	0.758
Family Hx of CVD	0.001	0.008	0.937	-0.003	0.006	0.637
Waist Circumference (cm)	0	0	0.65	0	0	0.581
Percent body fat (%)	-0.001	0.001	0.218	-0.001	0.001	0.138
Systolic Blood Pressure (mmHg)	0	0.001	0.62	0	0	0.368
Diastolic Blood Pressure (mmHg)	-0.001	0.001	0.249	0	0	0.281
HDL(mmol/L)	-0.025	0.02	0.203	-0.025	0.015	0.105
Fasting Plasma Glucose (mmol/L)	0.012	0.01	0.256	0.01	0.008	0.195

Appendix C7. Univariable Regression of Abnormal CVRFs and measures of cIMT

	Mean cIMT 2-segment, mm			Mean cIMT 12-segment, mm			Mean cIMT-for-height z-score			Mean cIMT-for-age z-score		
	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	p-value	Reg Coeff (SE)	R-Square (%)	P-value	Reg Coeff (SE)	R-Square (%)	P-value
Abnormal Systolic BP	0.0101 (0.0102)	0.62	0.323	0.0155 (0.0086)	2.02	0.074	0.2404 (0.2262)	0.72	0.29	0.2397 (0.2262)	0.71	0.291
Abnormal Diastolic BP	0.0028 (0.0094)	0.06	0.768	0.0092 (0.0080)	0.83	0.253	0.0809 (0.2102)	0.09	0.701	0.0499 (0.2103)	0.04	0.813
Abnormal Total Cholesterol	-0.0057 (0.0093)	0.26	0.543	-0.0069 (0.0080)	0.53	0.385	-0.0801 (0.2056)	0.11	0.697	-0.0834 (0.2054)	0.12	0.686
Abnormal HDL Cholesterol	0.0048 (0.0063)	0.4	0.446	0.0034 (0.0054)	0.26	0.538	0.0309 (0.1419)	0.03	0.828	0.0254 (0.1404)	0.02	0.857
Abnormal LDL Cholesterol	0.0084 (0.0096)	0.53	0.383	-0.0014 (0.0082)	0.02	0.861	0.1855 (0.2123)	0.54	0.384	0.1858 (0.2120)	0.54	0.382
Abnormal Non-HDL Cholesterol	0.0007 (0.0071)	0.01	0.919	0.0002 (0.0060)	0	0.968	0.0026 (0.1562)	0	0.987	0.0004 (0.1560)	0	0.998
Abnormal TC/HDL ratio	0.0099 (0.0108)	0.59	0.36	0.0066 (0.0093)	0.35	0.481	0.1776 (0.2392)	0.39	0.459	0.2343 (0.2387)	0.67	0.328
Abnormal Triglycerides	-0.0007 (0.0058)	0.01	0.904	0.0016 (0.0049)	0.07	0.746	-0.0482 (0.1278)	0.1	0.706	-0.0495 (0.1275)	0.11	0.699
Abnormal Fasting Plasma Glucose (IFG)	-0.0102 (0.0193)	0.2	0.596	-0.0078 (0.0165)	0.16	0.636	-0.1278 (0.4274)	0.06	0.765	-0.1370 (0.4280)	0.07	0.749
Abnormal 2-hr Plasma Glucose (IGT)	-0.0230 (0.0164)	2.53	0.165	-0.0021 (0.0145)	0.03	0.883	-0.4766 (0.3735)	2.1	0.206	-0.4388 (0.3853)	1.68	0.258
HbA1c (>=5.7%)	0.0072 (0.0097)	0.42	0.457	0.0043 (0.0083)	0.21	0.602	0.0580 (0.2145)	0.06	0.787	0.0136 (0.2139)	0	0.949

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