Excess Adiposity in Youth: Subclinical Cardiovascular Disease and Future Implications

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"Why do we love our teachers so much? Why, years later, do we think of them with such gratitude? I think it's because they come along when we need them most, when we are young and vulnerable and are tentatively approaching this craft called science that we are beginning to love. They have so much power. They could mock us, disregard us, use us to prop themselves up. But our teachers, if they are good, instead do something almost holy, which we never forget: they take us seriously. They accept us as new members of the guild. They tolerate our under-wonderful scientific ideas, the dopy things we say, our shaky-legged theories, our posturing, because they have been there themselves.

We say: I think I might be a scientist.

They say: Good for you. Proceed."

Adapted from an article in the New Yorker by George Saunders, October 2015, "My Writing Education: A Time Line."

Dedication

To my parents

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Introduction

1. Burden of Cardiovascular Disease and Adiposity in Pediatric Populations

1.1 General Overview

In the United States, 83.6 million adults (≥ 30%) are estimated to have one or more types of cardiovascular disease (CVD);[1] for both men and women, CVD is the leading cause of death in the United States.[2] Development of CVD risk factors beings early in life, therefore public health efforts have focused attention on CVD risk factor prevention in children.[3] Recent studies using the National Health and Nutrition Examination Survey (NHANES) from 1999-2012 found that pediatric overweight obesity trends had increased in the 14 years of follow-up, though may have stabilized between 2009-2010 and 2011-2012.[4,5] More recent evidence has suggested that prevalent overweight and obesity in youth continues to rise.[6] NHANES data through 2014 indicated that 33% of children 2-18 years of age were overweight,[5,6] and 17% were obese.[6] Furthermore, adiposity is known to track into adulthood more strongly than other risk factors; most children do not outgrow their adiposity status (Figure 1.1).[7–11] Steinberger and colleagues reported that BMI at 13 years of age correlated strongly with BMI at 22 years of age (r=0.67, p=0.0001).[11] Collectively, this evidence suggests that the rise in obesity prevalence is not a problem isolated to adults, and that CVD risk factor burden in childhood merits considerable concern.

1.2 Influence of Adiposity on Cardiovascular Disease

Numerous cardiovascular (CV) risk factors influence the progression of CVD in adults and children. Results from the Framingham Heart Study suggest, however, that adiposity is a foundational risk factor for adult cardiometabolic disturbances.[12,13] Importantly, adult adiposity and the cardiovascular and metabolic consequences have origins in childhood.[14] Not only is adiposity in childhood predictive of adiposity in adulthood,[11] but children with overweight and obese parents are more likely to be overweight or obese as adults.[15] Furthermore, overweight in adolescence has been associated with mortality in adulthood; the Harvard Growth Study found a 2.3 times higher risk of death from coronary heart disease (95% CI: 1.4-4.1) and 1.8 times higher risk of all-cause mortality (95% CI: 1.2-2.7) in adulthood when compared with lean counterparts.[16,17] Given the prevalence of overweight and obesity in children, considerable attention has been paid to the role of adiposity as a precursor to the

development of adult CVD.[18–20] Identifying children based on adiposity who are at risk for clinically evident CVD later in life may be an important strategy for CVD prevention.[20,21] Accordingly, throughout this Introduction substantial emphasis will be given to the consequences of overweight and obesity in childhood and adolescence.

2. Cardiovascular Disease Risk Factors in Pediatric Populations

2.1 Cardiovascular Disease Risk Factor Overview

Overweight and obese children and adolescents have a higher prevalence of numerous CVD risk factors compared to their normal weight counterparts,[18,19,22] including systolic blood pressure, HDL, and atherogenic lipids.[23–27] In addition, childhood overweight and obesity are positively associated with insulin resistance[28–31] and inflammation, including C-reactive protein, inflammatory cytokines, and regulation of metabolic gene expression.[18,32–36] The longitudinal Bogalusa Heart Study emphasized that BMI in children is the best predictor of fasting insulin levels, establishing the temporal relationship between BMI and fasting insulin.[37] Finally, the Minneapolis Childhood Cohort Studies found that adiposity at a mean age of 13 years was correlated with adiposity ($r=0.67$, $p=0.0001$), insulin resistance ($r=-0.5$, $p=0.006$), and LDL cholesterol ($r=0.48$, $p=0.01$) at a mean age of 22 years.[11]

In addition, the Bogalusa Heart Study evaluated CV risk factor clustering; risk factors (lipids, insulin, and blood pressure) were measured concurrently with adiposity in children. The investigators estimated that 70%, 39%, and 18% of children with a BMI $\geq$ 95th percentile had at least 1, 2, or 3 CV risk factors respectively; 65% had an adult BMI $\geq$ 35 kg/m^2. In the severely obese group, those with a BMI $\geq$ 99th percentile, 84%, 59%, and 33% had at least 1, 2, or 3 CV risk factors; 88% had an adult BMI of $\geq$ 35 kg/m (Figure 1).[2,9,38] Further evidence supports clustering of CV risk factors, often labeled metabolic syndrome, with increasing adiposity.[14,19,39–41] Data from NHANES III suggests 28.7%, 6.8%, and 0.1% of adolescents with a BMI $\geq$ 95th, $\geq$85th to <95th, and <85th percentiles respectively had prevalent metabolic syndrome.[14] These CV risk factor clusters have been shown to track into adulthood,[42] and increase future risk of CVD in adolescence[43–46] and adulthood.[47–51] Given the evidence supporting increased CV risk factor levels with childhood overweight and obesity, the concurrent secular trends of prevalent overweight, obesity, and severe obesity suggest a worsening of CVD risk profiles in pediatrics.

2.2 Cardiovascular Risk Factor Tracking

In addition to the increased burden of risk factors with overweight and obesity, both weight and CV risk factor status have been shown to track from childhood into adulthood. As mentioned above, childhood adiposity, in particular, has repeatedly been shown to be a predictor of BMI in adulthood.[8,9,11,17,52] The Bogalusa Heart Study found that children (mean age 11.4 years) with a BMI $\geq$ 99th percentile, 88% and 65% had an adult BMI $\geq$ 35 kg/m^2 and $\geq$ 40 kg/m^2 respectively.[9] According to a recent NHANES analysis in 12-21 year old adolescents, obese adolescents were substantially more likely to develop severe obesity in adulthood compared to overweight or normal weight adolescents (HR: 16.0, 95% CI: 12.4-20.5).[10] These studies also reinforce that adiposity in childhood is independently predictive of insulin resistance, dyslipidemia.[8,11] Finally, results from the Cardiovascular Risk in Young Finns Study over 27 years of follow-up found that BMI (r=0.43-0.46), systolic blood pressure (r=0.27-0.32), total serum cholesterol (r=0.50-0.52), LDL (r=0.52-0.56), HDL (r=0.46-0.51), and triglycerides (r=0.27-0.30) tracked from childhood to adulthood (all $p<0.0001$). In the same study, all subjects with high childhood risk factor levels had elevated odds of having abnormal CVD risk factors in adulthood; children and adolescents who were overweight or obese had a 5.7 (95% CI: 3.6-9.1) and 13.6 (95% CI: 8.3-22.3) higher odds of overweight or obesity in adulthood.[53]

2.3 Cardiovascular Disease Risk Factor Morbidity and Mortality

Cardiovascular risk factors in childhood are associated with adiposity, track over time, and have a tendency to cluster. Furthermore, these same CVD risk factors have been shown to be associated with subclinical vascular changes and CVD morbidity and mortality in adulthood.

2.3.1 Childhood Cardiovascular Risk Factors & Subclinical Atherosclerosis

Emphasis has been placed on subclinical atherosclerosis as it represents an intermediate step in the pathophysiology from the presence of CV risk factors to frank CVD. Broadly, subclinical atherosclerosis is defined as vascular damage that has yet to manifest into clinical signs or symptoms.[54–56] A number of landmark pediatric cohort studies have evaluated the impact of childhood risk factors and development of subclinical atherosclerosis.

In the Bogalusa Heart Study, Li et al. found that a 1 standard deviation difference in childhood LDL cholesterol (OR: 1.42, 95% CI: 1.14-1.78) and BMI (OR: 1.25, 95% CI: 1.10-1.54) were predictive of carotid intima media-thickness (upper quartile) in adulthood.[57] Evidence from the Muscatine and Young Finns studies found similar results, emphasizing the impact of childhood risk factor burden and future subclinical CVD.[7,58,59] In the Young Finns study, elevated adolescent (12-18 years) serum LDL, systolic blood pressure, BMI, and smoking were positively associated with increased carotid intima-media thickness (cIMT) in adulthood. When the investigators evaluated absolute number of risk factors in childhood (3-8 years), they found a weak, though statistically significant, relationship with increased cIMT in adulthood for men but not women. Concurrently, in youth (9-18 years) the absolute number of risk factors was positively associated with elevated cIMT.[60] In a separate analysis in the Young Finns cohort, childhood risk factors were positively associated with reduced arterial elasticity, but the effect was attenuated substantially when adjusting for risk factor levels in adulthood; systolic blood pressure was the only borderline significant risk factor for reduced arterial elasticity after adjusting for risk factors in adulthood ($p=0.06$).[61] Collectively, these studies provide strong evidence that risk factor burden in childhood has physiological CV implications in adulthood.

2.3.2 Childhood Cardiovascular Risk Factors & Atherosclerosis

Atherosclerosis, the underlying cause of coronary heart disease, is the leading cause of cardiovascular death in North America.[3] Atherosclerosis is well studied in adults but has been shown to be present in the young dating back to autopsies done on soldiers from the Korean War.[62] Broadly speaking, atherosclerosis begins with vascular fatty streaks, progresses to fibrous plaques, raised lesions (secondary to lipid deposition, smooth muscle and connective tissue proliferation), and culminates with lesion rupture (thrombosis), acute ischemia, or vascular rupture. Major clinical manifestations of atherosclerosis such as myocardial infarction and stroke occur in middle or late adulthood and are predominant public health concerns in their own right.[3,63,64]

More recent autopsy studies in childhood have confirmed that the atherogenic process begins in childhood.[43–46] The Pathological Determinants of Atherosclerosis in Youth (PDAY) study,[65] a multi-center study on black and white participants performed autopsies on 2876 subjects between 15 and 34 years of age who died of external causes from 1987-1994. The PDAY study reported that carotid intimal atherosclerotic

lesions were present in all of the aortas and over half of the right coronary arteries in the youngest participants (15-19 years of age). Prevalence and severity of atherosclerotic lesions in both locations increased with age. Furthermore, for every 30 mg/dL increase in non-HDL cholesterol, a visible increase in burden and severity of atherosclerosis was found; lack of risk factor burden was associated with the absence of atherosclerotic lesions.[66] The Bogalusa Heart Study, a multi-racial cohort, found that grouped CV risk factors measured in children and young adults were correlated ($r=0.70$, $p<0.001$) with the burden of lesions in the coronary arteries and the aorta; 19.1%, 30.3%, 37.9% and 35.0% of subjects with 1, 2, 3, and 4 risk factors had aortic fatty streaks (p test for trend=0.003).[43] To date, only two studies have examined the relation between CV risk factors in youth and vascular health outcomes in young adulthood; although informative, one was limited to a unique minority population[67] and the other had a small sample size.[68] Therefore, it is clear that risk factor burden in childhood is associated with initiation and progression of atherosclerotic development.

2.3.3 Childhood Cardiovascular Risk Factors & Clinical Cardiovascular Disease

Until recently, there were no prospective long-term studies evaluating the impact of childhood risk factor development and frank CV events in adulthood. Notably, a recent longitudinal study in Israel reported that cardiovascular mortality in adulthood was 3.5 times higher (95% CI: 2.9-4.1) and diabetes-related mortality was 17.2 times higher (95% CI: 11.9, 24.8) for individuals classified as having obesity as adolescents.[69,70] Furthermore, in adolescents with hypertension, incident stroke was 3.1 times higher (95% CI: 1.76, 5.54) than normotensive adolescents.[71] Nonetheless, it is important to consider how CV risk factors manifest into subclinical cardiovascular disease, an intermediate and modifiable stage in the development towards life threatening CVD outcomes. Compelling evidence can been found by looking at genetic disorders that accelerate the pathogenesis of CVD. Homozygous hypercholesterolemia, a condition that causes extremely high levels of LDL cholesterol levels starting in infancy (above 800 mg/dL), results in CVD events in the first decade of life and a reduced life expectancy. As would be expected, heterozygous hypercholesterolemia (generally LDL levels >200 mg/dL) results in a less extreme cardiovascular profile, nonetheless 25% of women and 50% of men have hard coronary events by 50 years of age.[3] In natural history studies of Type II diabetes mellitus (DMII), heart disease and stroke are the leading causes of death in DMII; adults diagnosed with DMII are 2 to 4 times more likely to have CVD or

stroke than non-diabetics.[1] Furthermore, in the PDAY study hyperglycemia was associated with advanced atherosclerosis of the coronary arteries.[44–46] Finally, in the Princeton Lipid Research Clinics Follow-up Study, metabolic syndrome (MetS) predicted CVD in adults after 25 years of follow-up (OR: 14.6, 95% CI: 4.8-45.3, p<0.0001).[72] Together, these data support the belief that risk factors in childhood impact clinical CVD events in adulthood.

2.3.4 M*ortality & Life Expectancy*

Numerous studies have shown the negative impact of overweight and obesity in childhood and adolescence with long-term morbidity and mortality.[73–76] in a cohort of Danish children 7 to 13 years of age, those with a higher BMI had an increased risk CHD morbidity and mortality in adulthood. The risk of a fatal or non-fatal CHD event increased linearly with age, and across the entire BMI distribution.[77] Franks *et al.* found that obesity, hypertension, and glucose intolerance in childhood (mean age of 11) were all positively associated with premature death. The death rates were more than double in children with the highest quartile of BMI compared to the lowest quartile (RR: 2.3, 95% CI: 1.46, 3.62), and 57% higher in children with hypertension (RR: 1.57, 95% CI: 1.10-2.24).[78] While CVD mortality rates continue to decline,[1] the increasing rates of life expectancy in the United States are attenuating.[79] Olshansky *et al.* argues that current life expectancy estimates may decrease by 2030 due to the impact of obesity on longevity.[80]

3. Reversibility of Cardiovascular Disease Risk Factors in Pediatric Populations

From a clinical perspective, it is fundamental to determine whether the associations between childhood risk factors and adult outcomes are due simply to tracking (i.e. childhood adiposity tracks to adulthood), or whether circumstances in childhood have permanent impacts independent of adult risk factor status. Current evidence suggests that many of the common CV risk factors present in childhood (LDL cholesterol, smoking status, and blood pressure) are associated with subclinical atherosclerosis *independent* of adult risk factor levels. Furthermore, clustering of CV risk factors in adolescence (i.e. having 1, 2, 3 or 4 CV risk factors) was associated with subclinical disease in stratified analyses in adults with different numbers of these same risk factors.[58,81] Interestingly, childhood adiposity status may be reversible. Overweight or obese children who maintained or increased their adiposity status as adults had increased cardiometabolic risks whereas the risks were absent in individuals who were normal weight as adults.

Despite this favorable outcome, 65% of overweight or obese children became obese as adults.[20] Therefore, multiple CV risk factors in childhood appear to have independent impacts on subclinical disease, and adiposity status though reversible is unlikely to occur in clinical practice.

4. Severe Obesity in Pediatric Populations

Though childhood obesity prevalence may be plateauing,[4,5] some evidence suggests it continues to rise.[6] Nonetheless, severe obesity appears to be the most rapidly rising category of adiposity.[6] Current NHANES and California Health Plan prevalence statistics estimate that between 4% and 6% of children are severely obese, with the highest prevalence of 11.9% in African American girls.[82–84] The American Heart Association (AHA) released a Scientific Statement dedicated to severe obesity in childhood, with clinical implications on the identification, associated health risks, and treatment options. The writing group defined severe obesity in childhood (≥2-18 years of age) as having a "BMI ≥120% of the 95th percentile or absolute BMI ≥35 kg/m^2, whichever is lower based on age and sex.[82,84]

These same nationally representative data suggest alarming trends for severe obesity: class 2 obesity has increased from 3.8% to 5.9% (p=0.04), and class 3 obesity has increased from 0.9% to 2.1% (p=0.002).[84,85] Childhood overweight and obesity increase CV risk in adulthood; severe obesity shows much stronger associations in the magnitude and severity of CV risk later in life. As mentioned previously, obesity in adolescence has been shown to be positively associated with obesity and severe obesity in adulthood; prevalence estimates project severe obesity will increase by 130% by 2030.[10] Furthermore, based on Behavioral Risk Factor Surveillance system data (BRFSS), obesity and severe obesity in adulthood are predicted to increase steadily through 2030. Finkelstein *et al.* estimate a 33% and 130% increase in the prevalence of obesity and severe obesity respectively; medical savings based on prevalence estimates remaining at 2010 levels suggest medical cost savings of $549.5 billion.[86] Furthermore, in the National Longitudinal Study of Adolescent Health cohort, obese adolescents had significantly higher risk of severe obesity (HR: 16.0, 95% CI: 12.4-20.5) in adulthood than normal or overweight adolescents after adjusting for race/ethnicity, age, and weighted for national representation.[10] Therefore, not only do researchers and clinicians

need to consider the intermediate implications of overweight and obesity in childhood. Attention to severe obesity and subclinical and overt atherosclerosis is compulsory.

5. Ideal Cardiovascular Health & American Heart Association 2020 Goals

In 2010, the American Heart Association (AHA) established strategic Impact Goals for 2020: "By 2020, to improve the cardiovascular health of all Americans by 20%, while reducing deaths from CVDs and stroke by 20%."[87] Seven health metrics have been established to define CV health – categorized as poor, intermediate, and ideal. Ideal CV health is defined as meeting all seven CV health metrics and the absence of CV disease or CV medication use. The seven cardiovascular health metrics are split into 4 health behaviors (smoking status, physical activity, dietary patterns, and BMI) and 3 health factors (optimal fasting total cholesterol, blood glucose, and blood pressure). Table 1.1 details specific definitions of poor, intermediate, and ideal CV health for children aged 12-19 years. Overall levels of ideal CV health are abysmally low; approximately 0.1% of American adults[88,89] and less than 1% of American children meet all 7 ideal CV health metrics.[90] Less than 50% of adolescents meet ≥5 of the 7 ideal CV health metrics.[91] According to these AHA metrics, children aged 12-19 years are generally in better CV health than adults in the United States. However, children fare modestly worse for physical activity (63.5% have poor or intermediate physical activity vs. 59.1% in adults) and dietary intake (99.9% have poor or intermediate dietary intake vs. 99.5% in adults). Prevalence trends from 2007-2008 to 2009-2010 indicate that ideal dietary patterns have decreased.[1] The low prevalence of meeting ideal dietary and physical activity will likely exacerbate the worsening outlook of obesity in childhood, adolescence and adulthood.[90,91]

Importantly, the number of ideal CV health metrics in childhood is positively associated with a lower risk of various CV outcomes in adulthood. A recent study in the Cardiovascular Risk in Young Finns prospective cohort study, children in ideal CV health had lower odds of: 1) hypertension (OR: 0.66, 95% CI: 0.52-0.85), 2) metabolic syndrome (OR: 0.66, 95% CI: 0.52-0.77), 3) elevated low-density lipoprotein cholesterol levels (OR: 0.66, 95% CI: 0.52-0.85), and 4) increased cIMT thickness (OR: 0.75, 95% CI: 0.60-0.94).[90,92] Prioritizing population-level ideal cardiovascular behaviors and metrics in childhood is paramount for public health efforts aimed at CVD prevention.

6. Relevance of Subclinical Cardiovascular Disease in Pediatric Populations

As previously discussed, CVD is a lifelong process with origins in childhood. Clinical CVD events or complications, however, arise many decades later in life.[43] Although diagnosis, management, and treatment of CVD has been evolving, the development of frank CVD is rarely seen in childhood.[93] Therefore, surrogate biomarkers or measurements of subclinical disease merit substantial consideration for primary CVD prevention in children and adolescents. Risk assessment measures to predict development of CVD have been developed, most notably the Framingham Risk Score from the Framingham Heart Study.[94,95] The need for the development of similar tools in a pediatric population is apparent, as the prevalence of overweight, obesity, and severe obesity has been increasing in recent decades.

Subclinical atherosclerosis offers a window of opportunity to recognize early CV pathophysiological processes. Classic clinical manifestations of CVD such as stroke and myocardial infarction are known to be a result of underlying atherosclerosis and disrupted vascular integrity, and adults with no CV risk factors and healthy CV behaviors have low rates of CVD.[96–99] Furthermore, evidence suggests that CV risk factors present in childhood independently predict subclinical atherosclerosis in adulthood.[57–59] Considering subclinical CVD in a pediatric population is relevant to CVD prevention longer term, but also to the timing of development of subclinical atherosclerosis. Timing of atherosclerosis occurrence is critical, particularly as it relates to when an intervention should occur and what type of intervention is most appropriate.

7. Pathophysiology of Subclinical Cardiovascular Disease

7.1 Vascular Structure & Function

The vascular system, composed broadly of arteries and veins, circulates continuously throughout the body. The structure and function of blood vessels, however, changes based on location. Interestingly, a consistent structural component of the vascular system is the endothelial cell; these cells comprise the single-celled innermost layer of all blood vessels, the endothelium.[100,101] For example, capillaries are comprised of only the endothelium, whereas larger arteries and veins include additional connective tissue and/or smooth muscle components.[101] The endothelium acts as a semipermeable barrier between the bloodstream and blood vessel, but also serves various functional roles: regulation of blood flow, vascular repair, and vascular tone.[102,103] One primary function of the endothelium involves nitric oxide secretion, which acts as a vasodilator.[104–106] Nitric oxide is known to be protective of vasculature, and has also

been established to have numerous anti-atherogenic properties: inhibition of leukocyte adhesion,[107] platelet aggregation,[108] and smooth muscle proliferation.[109]

Dysfunction of the endothelium within the arterial system is thought to be one of, if not the initial step, in the pathogenesis of atherosclerosis.[110] Endothelial and smooth muscle damage are generally initiated by classic CV risk factors, catalyzing the inflammatory and proliferative immune response indicative of vascular injury. This process takes decades to accumulate, and evaluation of subclinical atherosclerosis represents a possibility to measure and track the atherosclerotic process. Because atherosclerosis disrupts both the function and structure of the arterial system, numerous invasive and non-invasive imagine techniques have been developed to measure subclinical vascular disease burden.

7.2 Atherosclerosis & Subclinical Atherosclerosis

Subclinical atherosclerosis is most commonly identified as a systemic pathophysiological process affecting the entire vascular system, and plays a pivotal role in the etiology of coronary artery disease and other CVDs.[100,102] Atherosclerosis is a progressive disease involving the accumulation of fatty streaks, inflammation, infiltration of various immune cells, and development of scar tissue within the vessels. As a leading cause of coronary artery disease and CVD mortality, classic CVD risk factors and genetic predisposition contribute to atheromas in addition to a thickening and loss of elasticity of the arterial wall.[111] Atherosclerosis is often categorized as a subclinical disease because it is rarely diagnosed in absence of other cardiovascular events (i.e. heart attack or stroke). Atherosclerosis generally occurs in various types and sizes of arteries (primarily in the heart, brain, kidneys, and extremities), though is known to be present in some specific anatomical locations: the carotid arteries and bulb, and coronaries arteries.[112]

Atherosclerosis is a disease that disrupts both the function and structure of circulatory system,[100,113] and is a systemic process of the arterial system that involves chronic vascular inflammation, and accrual of lipids and immune cells within the arterial walls. It has been established that atherosclerosis is initiated by a synergistic accumulation of CVD risk factors that cause immediate injury to the endothelium and smooth muscle, thereby initiating an inflammatory process that catalyzes a thickening of the endothelium. With thickening, the vessel walls increase thereby decreasing the size of the vessel lumen (arterial diameter) and a reduction in blood flowing through the

artery. Although atherosclerosis may take decades to develop formally, it is commonly accepted that the initial processes take place earlier in life and develops over time.[1]

7.2 Measuring Subclinical Atherosclerosis

Two imagining techniques are most commonly cited for quantification of subclinical atherosclerotic disease burden: computed tomography (CT) for evaluation of coronary artery calcification (CAC) and B-mode ultrasound for evaluation of carotid artery intima-media thickness (cIMT). Early identification of subclinical atherosclerosis through diagnostic imaging is commonly utilized in clinical and research settings, though some evidence suggests limited utility in certain populations. High-resolution ultrasound measuring cIMT is the most commonly used technique to evaluate arterial structure because it: (i) does not expose subjects to radiation, (ii) is more appropriate for a low risk population whereas CAC is recommended for those at intermediate risk of heart disease (i.e. an estimated 10-year risk of 10-20%), and (iii) measurement variability with CAC can be problematic.[114] The common carotid artery is the anatomical site most frequently measured with high-resolution ultrasound because it is linked to CV risk factors,[115] subclinical atherosclerosis,[116] adverse cardiovascular events,[117,118] and it incurs minimal measurement error.[55]

7.3 Factors Impacting Subclinical Atherosclerosis

The primary factors impacting cIMT are: (i) aging, (ii) sex, (iii) adiposity, (iv) physical activity, and (v) smoking status.[55,100]

7.3.1 Age

As has been discussed, atherosclerosis develops over an extended period of time. Therefore, it is not surprising that evidence shows an increase in cIMT with aging.[119–121] It should be noted, however, that as cIMT is impacted by both adiposity and physical activity it remain unclear whether the increase in cIMT with time is due to aging directly or changes in lifestyle behaviors.

7.3.2 Sex

In general, men have higher cIMT values (in millimeters) than women.[122] It remains to be determined whether the sex difference in cIMT is driven by differences in CVD risk factors by sex, or by sex alone. Generally speaking, most differences in arterial function are attenuated when analyses adjust for baseline brachial artery diameter, evidence in regard to vascular structure (cIMT) is sparse.[123,124] Importantly, the impact of sex on cIMT has not been reported in children or adolescents (<18 years of age).[125]

7.3.3 Adiposity

Repeatedly, adult cIMT has been shown to be higher in individuals that are overweight or obese in comparison to their lean counterparts. These results hold even after adjusting for current levels of classic CVD risk factors.[126,127] Furthermore, obesity has also been shown to be inversely associated with endothelial function in adult populations.[128,129] In children, a positive association between obese and overweight and arterial structure (cIMT) has been reported,[130,131] whereas an inverse association between weight and arterial function has been identified.[132–134] the consistent evidence that both arterial structure and function are affected by adiposity provides reasonable evidence to suggest that improvements in vascular health could be achieved with weight loss interventions. One clinical trial, for example, found that a 1-year weight loss intervention reduced cIMT in children.[135] Results in adults have been similar but variable in magnitude, likely due to the population studied, amount of weight loss, and mechanisms of weight loss.[136]

7.3.4 Physical Inactivity

Cross-sectional studies examining leisure time physical activity and TV watching have suggested that no association exists between level of physical inactivity and cIMT.[137,138] Longitudinal studies have been mixed[139,140] and clinical trials have generally found no effect on exercise training and cIMT in adults.[138,141] Interestingly, in a pediatric population, the results are more conclusive; one clinical trial evaluating a 6-month aerobic exercise intervention demonstrated an improvement in both arterial structure and function in obese children.[131] Another study found improvement of endothelial function in an 8-week exercise intervention of overweight children and adolescents.[142] It is possible that chronological age impacts whether physical activity is able to alter arterial structure and function. Again, this information lends itself to a greater emphasis on public health efforts and interventions early in life.

7.3.5 Smoking Status

Smoking is a well-established risk factor for CVD, so it comes as no surprise that smoking has negative effects on vascular structure and function.[143] Numerous cross-sectional and longitudinal studies have identified that smoking is positively associated with an increased cIMT[143,144] and lower vascular function.[145–147] Importantly, inhalation of passive smoke is also damaging to both arterial structure and function. In one study, dose-response relationship was found between exposure to second-hand smoke and

cIMT thickening.[146] Furthermore, EDD was found to be lower in both individuals exposed to second hand smoke currently and formerly compared to nonsmokers. It is unclear how permanent the effect of active or passive smoking are on vascular health.[148]

8. Subclinical Cardiovascular Disease in Pediatric Populations: Gaps & Future Implications

As summarized above, current pediatric prospective cohort studies such at Young Finns, Bogalusa, and Muscatine offer considerable evidence regarding the associations of risk factors in childhood and development of subclinical atherosclerosis and CVD in middle adulthood. Precise and reliable measurement of subclinical atherosclerosis in childhood in conjunction with longer term follow-up provides an excellent opportunity to predict future risk of: (i) CV outcomes such as myocardial infarction and stroke later in life, and (ii) subclinical disease itself.

Subclinical disease measurement could potentially help improve risk stratification and prediction in a pediatric population, where frank CV events rarely occur. Furthermore, the ability to categorize children and adolescents according to their future risk of subclinical disease could provide: (i) improvement in our understanding of *when* risk factor burden has the biggest impact on vascular health (i.e. childhood, pre-adolescence, adolescence, etc.), (ii) information for development of risk prediction of subclinical or early CVD, and (iii) help target appropriate timing of interventions and treatments (i.e. providing treatments or interventions earlier in the disease process or when behavioral change may be more malleable). Continued efforts to treat and prevent primary or secondary cardiovascular events is of paramount importance. Simultaneously, a more targeted consideration and evaluation of subclinical disease in youth and young adulthood is warranted, particularly considering the increased prevalence of overweight, obesity, and severe obesity in pediatric populations.

In conclusion, excess adiposity remains a serious public health threat in childhood and adolescence; obesity in adolescence is associated with increased CVD morbidity and mortality later in life. Subclinical atherosclerosis is a critical factor for future development of overt CVD, and thus warrants attention for CVD prevention in earlier periods of life. Little is known about subclinical atherosclerosis in early adulthood; this dissertation aims to address this gap in two ways. First, by developing a model that uses risk factors in childhood and adolescence to predict subclinical atherosclerosis in

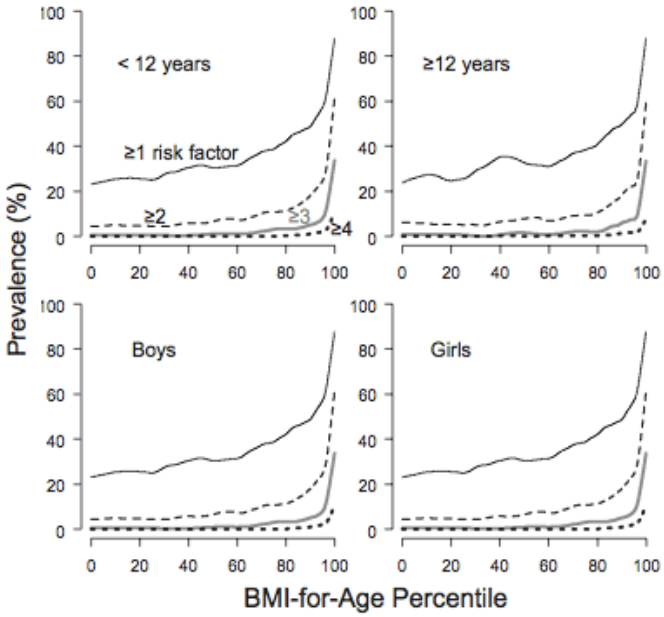
middle adulthood, and second by examining the association between CVD risk factors in adolescence and subclinical atherosclerosis in early adulthood. Lastly, in an effort to prioritize CVD prevention, the American Heart Association developed the ICH metric as a tool to promote cardiovascular health and reduce future CVD. No data exist on the prevalence of ICH across levels of adiposity, particularly severe obesity, in a pediatric population. Thus, the final aim of this dissertation was to examine the distribution of ICH metrics by adiposity status.

Table 1.1. Definitions of poor, intermediate, and ideal cardiovascular health according to AHA 2020 goals: health behaviors and risk factors for children 12-19 years of age.

Metric	Poor	Intermediate	Ideal
Smoking status	Tried > 30 d ago	…	Never tried; never smoked whole cigarette
BMI	>95th percentile	85th- 95th percentile	<85th percentile
Physical activity level	None	>0 and <60 min moderate or vigorous activity every day	≥60 min moderate or vigorous activity every day
Healthy Diet Score*	0-1 components	2-3 components	4-5 components
Total Cholesterol	≥200 mg/dL	≥170 - <200 mg/dL	<170 mg/dL
Blood Pressure	>95th percentile	90-95th percentile	<90th percentile
Fasting Blood Glucose	≥126 mg/dL	100 - 125 mg/dL	<100 mg/dL

BMI indicates body mass index.
*The Healthy Diet Score is based on adherence to the following dietary recommendations: fruits and vegetables, ≥4.5 cups per day; fish, 2 or more 3.5-oz servings per week; sodium, <1500 mg/d; sugar-sweetened beverages, ≤450 kcal (36 oz) per week; and whole grains, ≥3 servings a day scaled to a 2000-kcal/d diet. Reprinted with permission Circulation. 2016;134: e236-e255

Figure 1.1. The relation of BMI-for-age and the proportion of children with ≥1, ≥2, ≥3, and ≥4 risk factors; stratified by age (top panels) and sex (lower panels).[10]



Paper 1: Risk Prediction and Vascular Health

Introduction

Atherosclerosis begins in childhood, and is a critical component to the future development of cardiovascular disease (CVD).[1,149] Evidence of advanced coronary atherosclerosis in young adults has been accumulating since autopsy studies performed on Korean and Vietnam War casualties.[62] Using additional autopsy evidence, the Pathological Determinants of Atherosclerosis in Youth (PDAY) Study reinforced these findings in the second half of the 20th century.[66] The PDAY investigators estimated that 5% of men 25-29 years of age and 20% of men 30-34 years of age had advanced coronary artery atherosclerotic lesions (≥40% stenosis). Established adult CVD risk factors for coronary heart disease such as adiposity, dyslipidemia, hyperglycemia, and smoking status were found to be cross-sectionally associated with prevalence and severity of atherosclerosis in the PDAY cohort.[44–46] Subclinical cardiovascular outcomes such as carotid intima-media thickness (cIMT) have become widely accepted as relevant to earlier stages of CVD.[55] Identical CVD risk factors have been studied in numerous pediatric longitudinal cohort studies, and consistently are associated with subclinical atherosclerosis in middle adulthood (mid-30's).[57–59]

Based on post-mortem evidence of atherosclerotic development early in life, the PDAY investigators proposed a cross-sectional risk score using CVD risk factors to predict coronary atherosclerosis in early adulthood (all assessments: 15-34 years).[150] This risk score closely resembles the Framingham Risk Score for coronary heart disease prediction.[12,151] Though innovative, this risk prediction score has a number of limitations. First, it used post-mortem, cross-sectional data on individuals who died accidentally. Post-mortem evaluation provided concrete evidence of atherosclerosis, but required unconventional methods for risk factor measurement. For example, presence of hypertension was determined based on renal artery intimal thickness demonstrating an average blood pressure of ≥ 110 mm Hg.[152] In contrast, in a living study population, brachial artery blood pressure would generally be assessed using a clinical grade blood pressure device or mercury sphygmomanometer.[153] Finally, cross-sectional data may not lend themselves to future risk prediction. Importantly, predicting future risk could help identify individuals who could benefit from interventions thereby preventing future development of disease. Furthermore, the age range of participants in PDAY (15-34

years) included only a small portion of adolescence and focused more heavily in young adulthood.[12,151]

Although the association between childhood risk factors and increased cIMT in middle adulthood (mid-30's) has been established,[58,154,155] the application of this information in a risk prediction model has yet to be achieved using longitudinal data in a pediatric population. Risk prediction models quantify the impact of numerous risk factors (both innate and modifiable) on the development of various diseases. In general, pediatric risk prediction models have focused on common clinical ailments such as asthma[156] and fever management.[157] Previously considered diseases of adulthood, prediabetes[158,159], type 2 diabetes[160,161], dyslipidemia[25,162,163], and hypertension[164,165] have been increasing in adolescence, primarily in parallel with the obesity epidemic.[166,167] Thus, risk prediction models for subclinical atherosclerosis in early adulthood may be more relevant than when these diseases were less prevalent. To the best of our knowledge, no studies to date have developed a model predicting subclinical atherosclerosis at any stage in life. Thus, the objective of this study was to develop a risk prediction model that predicted cIMT in middle adulthood using relevant cardiovascular risk factors in adolescence.

Methods

Cohort Description

The Prevention of High Blood Pressure in Children (PHBPC) study is a longitudinal cohort study initiated at the onset of the 1977-78 school year in Minneapolis Public Schools; the sampling and measurements have been previously described.[168,169] Basic anthropometric data were collected twice annually until high school, and annually through the end of high school. Three post-high school visits occurred: (i) visit 2 (1989-91), (ii) visit 3 (1991-95), and (iii) visit 4 (2007-11).[170] The cohort was randomly selected from all incoming students in 1st through 3rd grades that participated in a school-wide blood pressure (BP) screening program (n=2641). In an effort to enroll children with a higher future CV risk, a cohort was selected that included: (i) all children at the upper and lower 5th percentiles of the normal systolic BP distribution, (ii) 50% of the African American children that remained, and (iii) 10% of the white children that remained. At each post-high school study visit, participants underwent extensive clinical examinations; cIMT was assessed at visit 4. For the current analysis, we used visit 3 as baseline

(mean age: 23 years) and visit 4 as follow-up (mean age: 38 years). The University of Minnesota Institutional Review Board approved this study and all exams were conducted with written informed consent (from parents when children were below 18 years old, with verbal assent from the children; from the participants themselves after they came of age).

Cardiovascular Disease Risk Factor Assessment

Subsequent to arriving at the Masonic Clinical Research Unit (MCRU) at the University of Minnesota, all participants provided written informed consent. All participants were also examined according to a standardized protocol developed by study investigators and approved by the University of Minnesota Institutional Review Board. Relevant predictors were determined through an extensive literature review and availability in the PHBPC cohort. Protocols for data collection were similar across visits unless otherwise noted.

Anthropometrics & Blood Pressure

Two seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were taken in the right arm using a random zero sphygmomanometer; an average of these two measurements were used for all analyses. Weight was measured using a medical grade balance scale without shoes; height was measured using a standing stadiometer to the nearest centimeter. BMI was calculated as weight (kilograms; kg) divided by height (meters; m) squared. BMI calculations and height measurements were transformed into age- and sex-specific percentiles based on Centers for Disease Control and Prevention guidelines.[171]

Sociodemographics

Age, race, and sex were collected at all visits according to standardized research questions. Age was asked to the nearest year, race was categorized by the following: white, black, Native American, Asian, Hispanic, or other. Sex was assessed as male or female.

Carotid Intima-media (cIMT) Assessment

All vascular health assessments were performed in a quiet room at a comfortable temperature (22-23°C) at the final study visit (mean age: 38 years). Participants were asked to refrain from caffeine and medication consumption prior to their scheduled clinic visit. After lying in a supine position for 15 minutes, a standard high-frequency

ultrasound device (Image Point HX, Philips Medical) using a 7.5 MHz linear array probe was used to capture B-mode images of the common carotid artery (carotid intima-media thickness; cIMT). The transducer was held at a constant distance from the skin, and over a fixed point on the carotid artery using a stereotactic arm. All images were recorded, saved, imported, and stored on a separate computer system for storage and future analysis.

Appropriate software for high-frequency ultrasound imaging of vasculature (CVI Analysis, Information Integrity, Boston, MA) was used for measuring resting longitudinal images of the common carotid artery (1-2 cm proximal to the carotid bulb). Images were recorded directly onto the hard drive of a PC computer with the aforementioned software. Arterial images and measurements (arterial lumen diameter, intima-media thickness, and distensibility) were collected at 30 Hz for the first 475 ms of the cardiac cycle to acquire both the maximal and minimal lumen diameters (media-adventitia border of the near wall to the intima-lumen surface of the far wall). From preliminary data, within person analyses estimated a mean difference (± SD) of 0.02 ± 0.03%.[100,113] Higher cIMT indicates lower vascular health (i.e. lower cIMT is better).

Exclusions and Missing Data

Of the 2641 children randomly selected to participate in the cohort, 1207 met inclusion criteria and provided written informed consent for participation using University of Minnesota Institutional Review Board approved documents. These 1207 children were examined twice yearly until high school; at which time they were examined annually. A post-high school examination was conducted on average 2 years after graduation (mean age of 19 years); at this exam 817 individuals participated. There were no baseline characteristics that differed between the 817 individuals who participated and the 319 individuals lost to follow-up. At the most recent clinic visit conducted in 2007-11, when participants were on average 38 years of age, a more complete cardiovascular profile and cIMT was collected on 444 participants; our final sample size was 444 observations.

Statistical Analysis

Descriptive statistics are presented as mean ± standard deviation (SD) or as a frequency distribution (%) by cIMT quartile. Of the 444 participants at the most recent clinic visit, we did not exclude any individuals because all 444 participants had at least

one previous clinic visit with a complete risk factor profile. Participants were measured multiple times throughout the study period, thus general estimating equations (GEE) regression models were used to estimate differences in cIMT in models including known CVD risk factors.[172] This modeling approach can be used in contexts with repeated exposure measurements on the same individual at different time points (i.e. multiple informants).[173] GEE model specifications included an independent working correlation matrix, a robust standard error variance estimator, a Gaussian distribution, and a canonical identity link. This analytic approach requires meeting the assumption that any missing data are missing completely at random (MCAR); no missing data were present in our current data based on our criteria of: 1) presence of valid data on the outcome of interest (cIMT), and 2) at least one clinic visit prior to the final visit with complete risk factor assessment.

Our candidate variable list was limited to the following demographic and anthropometric variables: age, race, sex, BMI percentile, height percentile, SBP, and DBP. To improve ease of interpretation, all variables were modeled using a one-unit difference with the exception of percentile variables (BMI and height) and blood pressure variables (SBP and DBP) which were converted to a 10 percentile and 10 mm Hg unit difference, respectively. We did not perform a formal candidate variable selection process because all aforementioned variables were reasonable to include based on literature review of CVD risk factors, and were consistently associated with cIMT in middle adulthood.[58,59,174] To determine our final risk prediction model, we fit three GEE models: 1) Model 1 adjusted for baseline age, sex, race, BMI percentile, SBP and DBP, and 2) Model 2 additionally adjusted for height percentile. We considered a third model to account for potential interactions and non-linear associations. Interactions were initially tested for all variables in a stepwise fashion; results were highly dependent on the order in which variables were added to the model.[175] Thus, we chose to model interactions that included BMI percentiles or SBP, which would be expected based on current literature.[176–178] Interaction terms were included in our final model if they were statistically significant using the traditional cutoff of $p<0.05$. Thus, Model 3 additionally adjusted for cubic and quadratic terms for SBP and two-way interaction terms that included BMI percentile with age and with SBP. We had originally hypothesized that, similar to annual well child visits, risk predictions models would be most relevant to specific chronological ages. Nonetheless, using an independent group t-test, no

differences were found when comparing CVD risk factor beta coefficients in GEE models modeling: 1) chronological age (year), or 2) developmental age according to the American Academy of Pediatrics definitions of early (11-14 years), middle (15-17 years), or late adolescence (18-21 years).[179] Consequently, our final GEE risk prediction model included age as a continuous variable.

Model performance was evaluated using: (i) graphical representation comparing observed and predicted cIMT decile means as a measure of calibration (consistency between predicted and observed mean cIMT), and (ii) Spearman's correlation coefficient of observed and predicted cIMT means as a global measure of discrimination (the ability of the prediction model to differentiate individuals with and without subclinical atherosclerosis).[180,181] In sensitivity analyses, quadratic and cubic terms were included for all CVD risk factors to determine if calibration was improved by adding non-linear terms that were significantly associated with cIMT with $p<0.05$. In the absence of an external validation sample, we used bootstrapping with replacement (n=1000 replicates) for optimism correction that would be expected when generating a risk prediction model on the estimation sample.[181] Spearman correlation coefficients were generated for each bootstrapped replicated and plotted to examine the overall distribution; a 95% confidence interval was estimated by identifying values at the 2.5% and 97.5% of the distribution. All analyses were performed using Stata 14 and R software packages.[182,183]

Results

Table 2.1 describes the baseline characteristics of study participants (n=444, mean age: 12±3 years) by cIMT quartile. Mean cIMT was 642±74 µm and 437±24 µm in quartiles 4 and 1, respectively. Individuals in the highest quartile of cIMT were more likely to be male, black, and have a higher BMI and weight compared to the lowest quartile. A modest increase in SBP was seen across quartiles; mean DBP was consistent across quartiles. Differences in cIMT by cardiovascular risk factor are reported in Table 2.2. In Model 2, female sex was associated with a lower cIMT (-27.3 µm; 95% CI: -42.9, -11.7), whereas black race (37.6 µm; 95% CI: 16.1, 59.1), higher BMI percentile (0.24 µm; 95% CI: -0.27, 0.51, per 10% increase), and SBP (7.6 µm; 95% CI: 1.9, 10.3, per 10 mmHg increase) were all associated with an increased cIMT. Despite the fact that age, DBP, and height did not meet standard thresholds for statistical significance, we included them in our final risk prediction model based on *a priori*

literature review and because we had a limited number of variables to consider. We chose Model 3 as our final model due to significant non-linear associations (SBP) and interaction terms (BMI percentile*age; BMI percentile*SBP).

To examine model performance, we plotted observed and predicted mean cIMT decile values to assess calibration (Figure 2.1), and the distribution of Spearman's correlation coefficients between observed and predicted cIMT by individual clinic visit to examine discrimination (Figure 2.2). Overall calibration of our risk prediction model in the estimation sample was poor, as depicted by the discrepancy between what would be perfect calibration (i.e. a slope of 1.0) and the observed versus predicted mean cIMT decile values (Figure 1). Though both quadratic and cubic terms for SBP met thresholds for statistical significance, the calibration plot remained unchanged when the non-linear terms were added to the model. When assessing discrimination, Spearman correlation coefficients did not change dramatically by clinic visit, and were also weak (range: 0.33-0.43; Figure 2.2). Using bootstrapped replicates of the estimation sample, the distribution of correlation coefficients between observed and predicted mean cIMT values was normally distributed (mean 0.32; 95% CI: 0.30, 0.34) (Figure 2.3).

Discussion

Although longitudinal associations were observed between most CVD risk factors in adolescence and increased cIMT in middle adulthood, our risk prediction model evaluating the ability of CVD risk factors in adolescence to predict cIMT in middle adulthood performed poorly based on calibration and discrimination metrics. Nonetheless, predicting subclinical CVD based on risk factors in youth is important in that it facilitates assessment of risk for individuals who would benefit from earlier or specific treatments, and provides an easy tool for clinicians and researchers to use to determine appropriate treatments (behavioral, pharmacological, etc.).

In particular, it is unclear why BMI percentile had a small magnitude of effect when we examined the association between CVD risk factors in adolescence and cIMT in middle adulthood. The prevalence of obesity in childhood is high, with an overall prevalence in 2013-2014 of 17.4% in 6-11 year olds and 20.6% in 12-19 year olds.[5] Childhood obesity tracks into adulthood,[11,184,185] and is associated with insulin resistance,[30,186,187] impaired glucose,[188] dyslipidemia,[189] and blood pressure.[58] Furthermore, Mendelian randomization studies confirm that adiposity is a causal risk

factor for cardiovascular risk factors and atherosclerosis in adulthood.[190,191] A recent longitudinal study in 2.3 million obese adolescents reported a 3.5 times higher lifetime risk of CVD mortality (95% CI: 2.9-4.1),[69] and a 17.2 times higher risk of diabetes mortality in midlife (95% CI: 11.9-24.8).[70] Finally, numerous longitudinal pediatric cohort studies have reported and association between BMI in youth, and cIMT in middle adulthood.[57–59] It is possible that, on average, the participants in the PHBPC cohort had a healthy weight status; even in the highest quartile of cIMT the mean BMI and BMI percentile were 21±5 kg/m^2 and 68±25th percentile, respectively (Table 2.1). In the Young Finns study, for example, baseline BMI was 26±4 for males and 25±4 for females. If few PHBPC participants had overweight or obesity, it would be difficult to assess the impact of excess adiposity on cIMT. Finally, BMI percentile is the preferred indicator for adiposity in childhood in adolescence;[192,193] other pediatric cohorts commonly used BMI as a measure of adiposity[57–59] which could have resulted in biased estimates.[194]

Two recent longitudinal studies in adolescents are the first to support an association between cardiovascular risk factors in adolescence and increased cIMT early in life, and may offer context for some of our results.[67,68] The first, in 313 Aboriginal Australian children and adolescents (mean age: 11 ears) with metabolic syndrome, found that cIMT increased by 22 µm over 6 years (p=0.0007) but only in participants with C-reactive protein above the median.[67] Using logistic regression, the authors found that an interaction between metabolic syndrome and C-reactive protein was responsible for increased cIMT at follow-up. The impact of inflammation on vascular disease has been previously described,[195,196] data examining inflammatory risk factors in youth with subclinical vascular outcomes in adulthood, however, are lacking. In the current study, inflammation was not assessed; since excess adiposity catalyzes an inflammatory response,[196] systemic inflammation may not have been extensive enough in PHBPC participants for subclinical cardiovascular disease to be present. In the second study examining risk factors and cIMT in youth, 42 Swedish adolescents (mean age: 14 years; 28 and 14 obese and lean participants, respectively) reported that radial-carotid IMT increased by 20 µm in participants with obesity, over only 5 years (p=0.04).[68] Although informative, these results were unadjusted for other CVD risk factors so may be confounded, and were in the radial versus common carotid artery. Though one was in a minority population[67] and the other had a small sample size,[68] taken together these

results support the premise that metabolic dysfunction and excess adiposity have an impact on cIMT in adolescence. In the current analysis SBP had the largest magnitude of effect; these results are consistent with previous literature supporting an independent effect of SBP on cIMT in youth[197] and adulthood.[58,198]

Although we included what we believed to be the primary CVD risk factors for increased cIMT in middle adulthood, it is likely that our risk prediction model was lacking important candidate variables that are associated with, or are potentially relevant to cIMT in the literature. Primarily, low-density lipoprotein cholesterol,[57,58] smoking,[58] dietary intake,[199,200] physical activity,[201,202] socioeconomic status,[203,204] pubertal timing,[205,206] and baseline arterial diameter in adolescence.[55] Based on findings from two pediatric cohort studies, the presence of dyslipidemia and current smoking/secondhand smoke exposure were both independently associated with cIMT in middle adulthood.[57,58] Evidence is lacking on the impact of diet on cIMT early in life, but studies in adults indicate that both a Mediterranean diet[199] and a diet low in saturated fats and carbohydrates[200] are associated with lower cIMT. Literature examining the association between physical activity and cIMT in adolescence are mixed; in the European Youth Heart Study no association was found,[201] but the Brazilian Birth Cohort study reported that physical activity in young adulthood was inversely associated with pulse wave velocity, another indicator of vascular health.[202] Lower socioeconomic status in youth was associated with metabolic syndrome, higher impaired fasting glucose, and type 2 diabetes;[207,208] lower summary measures of psychological well-being, which included socioeconomic status, in childhood has been shown to be associated with increased cIMT[209]and coronary artery calcification[210] in middle adulthood. Evidence on the impact of pubertal status and cIMT is sparse; two studies that collected pubertal status did not find increases in cIMT during puberty,[205,206] though one was in children with Type 1 diabetes mellitus.[205] Finally, the protocol for cIMT measurements in our study protocol are known to be conservative; it is possible that the extent or severity of subclinical atherosclerosis was not fully captured by measuring mean cIMT of a single anatomical location (common carotid artery).[55] In adults, differential effects of CV risk factors on distinct segments of the carotid artery have been reported; blood pressure was more strongly associated with common cIMT variability than other segments, while LDL-C tended to correlate more with the bulb and internal segments.[211] It is unclear if these disparate effects exist in youth, nonetheless it is a possibility that warrants consideration.

This project has a number of limitations. First, not all candidate CVD risk factor variables were available in this cohort. The post-mortem PDAY risk prediction model in youth included age (5 years), male sex, non-HDL cholesterol, HDL cholesterol, smoking, hypertension, obesity, and hyperglycemia.[150] The PDAY risk score had reasonable discrimination (c-index: 0.78) and calibration (goodness-of-fit statistics).[150] Current evidence in longitudinal pediatric cohort studies suggests that BMI, SBP, and dyslipidemia have the greatest impact on development of subclinical atherosclerotic disease.[57–59] Although we included BMI percentile and SBP in our final model, total cholesterol or low-density lipoprotein cholesterol were not collected. Second, while the study was designed to obtain a racially diverse study sample with a wide distribution of blood pressure values, the results are likely not generalizable to a broader adolescent population. Unfortunately, we did not have access to another dataset to perform external validation. Nonetheless, we ran bootstrapped replicates in an effort to correct for expected optimism in our results. Third, given that losses to follow-up are notable, the possibility of selection bias is likely. Fourth, the clinical utility of cIMT is uncertain; questions remain regarding normative values and which populations would receive the greatest benefit from vascular imaging. Fifth, standardized quantitative approaches to assess model performance metrics (calibration and discrimination) in risk prediction models with continuous outcomes are lacking. For example, use of the c-statistic affords predetermined definitions of acceptable ($0.7 \leq c < 0.8$) or excellent ($0.8 \leq c < 0.9$) discrimination for dichotomous outcomes.[181] Finally, the proposed risk prediction model does not account for measurement error in: (i) risk factors used in the model, or (ii) subclinical vascular measurements. Strengths of this analysis include the collection of a CVD risk factor profile from adolescence through middle adulthood, a conservative estimate of cIMT by using mean cIMT of the entire area measured (versus maximum cIMT), and state-of-the-art software that provided improved resolution of cIMT and increased precision in measurement. To the best of our knowledge, this project is the first to evaluate a risk prediction model using CVD risk factors in adolescence and subclinical atherosclerosis is middle adulthood.

If a risk prediction model for subclinical atherosclerosis was developed that met appropriate model performance metrics, it could impact the management of children and adolescents at risk for development of subclinical atherosclerosis. First, individuals with high-risk scores could be differentiated from those with low-risk scores without having to

measure vascular pathology. As a result, treatment optimization could be more tailored for high-risk individuals. Second, risk factor treatment (behavioral or pharmacological) could be monitored over time, thereby allowing for a more personalized approach to prevention and management of atherosclerosis. The risk prediction model we evaluated did not meet standard criteria for appropriate model performance, and thus it is not worthwhile to consider for clinical utility. Nonetheless, it may be beneficial for future research efforts to consider risk prediction models for subclinical atherosclerosis in younger populations using longitudinal pediatric cohort studies with more robust CVD risk factor collection.

Table 2.1. Selected baseline participant characteristics by carotid intima-media thickness (cIMT) quartile, Prevention of High Blood Pressure in Children study (n=444).

	Carotid intima-media thickness Quartile (cIMT)			
Category	**Q1**	**Q2**	**Q3**	**Q4**
N (Total)	112	131	93	108
cIMT, *µm (SD)*	437 (24)	488 (15)	541 (16)	642 (74)
cIMT, *µm range*	320-460	470-510	520-570	580-930
Age, *years (SD)*	11.9 (3)	12.0 (3)	11.9 (3)	12.1 (3)
Sex, *female, N (%)*	73 (65)	71 (54)	35 (38)	46 (43)
Race, *white, N (%)*	77 (69)	96 (73)	59 (63)	59 (55)
BMI, *kg/m^2 (SD)*	19.0 (3.6)	20.0 (4.6)	19.4 (4.1)	21.1 (4.8)
BMI, *%* (SD)	51 (28)	58 (29)	56 (29)	68 (25)
Height, *cm (SD)*	149 (17)	152 (17)	151 (17)	153 (17)
Height, *%* (SD)	45 (30)	54 (30)	52 (27)	55 (27)
Weight, *lbs (SD)*	96 (35)	107 (42)	102 (40)	113 (42)
SBP, *mm Hg (SD)*	107 (10)	110 (11)	110 (11)	112 (10)
DBP, *mm Hg (SD)*	63 (13)	62 (14)	62 (14)	62 (14)

Abbreviations and units: Carotid intima-media thickness, cIMT; micrometers, µm; standard deviation, SD; body mass index, BMI; kilograms/meters squared, kg/m^2; centimeters, cm; percentile, %; millimeters of mercury, mm Hg; systolic blood pressure, SBP; diastolic blood pressure, DBP.

Table 2.2. Differences in carotid intima-media thickness (cIMT) by cardiovascular risk factor, Prevention of High Blood Pressure in Children study (n=444).

	Difference in cIMT, in µm (95% confidence interval)		
	Model 1	**Model 2**	**Model 3**
Intercept	435 (379, 490)	435 (380, 492)	1765 (616, 2913)
Age, *years*	-0.18 (-1.0, 0.62)	-0.10 (-0.92, 0.72)	-2.89 (-5.01, -0.77)
Sex, *female*	-27.3 (-42.8, -11.7)	-27.3 (-42.9, -11.7)	-27.4 (-42.9, -12.0)
Race, *black*	38.1 (16.6, 59.5)	37.6 (16.1, 59.1)	36.9 (15.5, 58.2)
BMI, *%*	0.28 (0.19, 0.54)	0.24 (-0.27, 0.51)	1.19 (-0.65, 3.0)
SBP, *mm Hg*	8.4 (2.7, 10.4)	7.6 (1.9, 10.3)	-36.0 (-66.6, -5.4)
DBP, *mm Hg*	-2.3 (-5.2, 0.5)	-2.3 (-5.2, 0.5)	-2.2 (-5.0, 0.7)
Height, *%*	-	0.15 (-0.095, 0.40)	0.17 (-0.79, 4.2)
SBP^2	-	-	0.34 (0.065, 0.62)
SBP^3	-	-	-0.0001 (-0.002, -0.0002)
BMI%*age	-	-	4.5 (1.5, 7.5)
BMI%*SBP	-	-	-1.4 (-3.2, 0.46)

Abbreviations and units: Carotid intima-media thickness, cIMT; micrometers, µm; standard deviation, SD; body mass index, BMI; kilograms/meters squared, kg/m^2; centimeters, cm; percentile, %; millimeters of mercury, mm Hg; systolic blood pressure, SBP; diastolic blood pressure, DBP; quadratic term for systolic blood pressure, SBP^2; cubic term for systolic blood pressure, SBP^3; interaction term for BMI percentile and age, BMI%*age; interaction term for BMI percentile and SBP, BMI%*SBP.
Unit interpretation: All coefficients are presented as a one unit change with the exception of: 1) BMI and height percentile variables: 10 percentile unit change, and 2) SBP and DBP: 10 mm Hg unit change.
Model 2: Model 1 + height %
Model 3: Model 2 + SBP^2 + SBP^3 + BMI%*age + BMI%*SBP

Figure 2.1. Observed versus predicted mean cIMT (µm) in estimation sample (calibration).

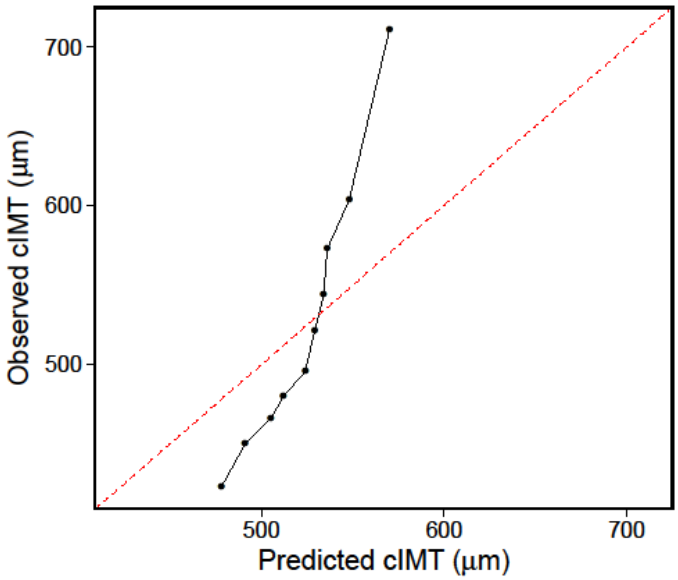


Figure 2.2. Spearman's correlation coefficients between observed and predicted cIMT by individual clinic visit in estimation sample (discrimination).

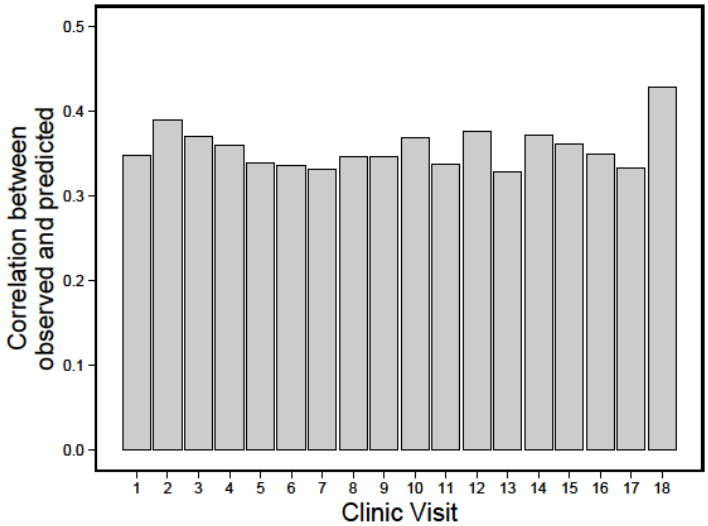
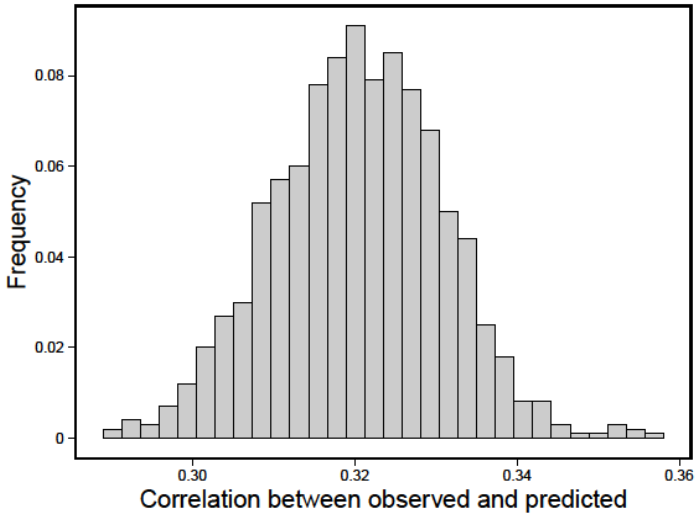


Figure 2.3. Distribution of Spearman's correlation coefficient[1] between observed and predicted mean cIMT from bootstrapped replicate samples (n=1000).



[1]95% CI: 0.30-0.34

Paper 2: Cardiovascular Risk Factors in Adolescence and Young Adulthood and Common Carotid Artery Intima-Media Thickness in Early and Middle Adult Life

Introduction

Atherosclerosis has been shown to begin early in life,[1] most often with the presence of fatty streaks in the intimal layer of the arteries.[212] Aortic fatty streaks have been identified as early as 3 years of age[213] and increase with age.[214] In addition, coronary artery atherosclerotic plaques have been documented during adolescence.[215] Cross-sectional autopsy results from the Pathological Determinants of Atherosclerosis in Youth Study confirm that atherosclerosis is present in childhood; cardiovascular risk factor burden is associated with the extent and severity of atherosclerosis in youth.[44–46]

Cardiovascular risk factors such as obesity, Type 2 diabetes mellitus, and hypertension are strongly associated with adult cardiovascular disease (CVD).[1,94,216] Previously considered diseases of adulthood, prediabetes[158,159], Type 2 diabetes[160,161], dyslipidemia[25,162,163], and hypertension[164,165] have been increasing in adolescence, primarily in parallel with the obesity epidemic.[166,167] A recent study in 2.3 million adolescents found that adolescent obesity was associated with a 3.5 times higher lifetimes risk of CVD mortality (95% CI: 2.9-4.1);[69] obesity in adolescence was also associated with a 17.2 times higher risk of diabetes mortality in midlife (95% CI:11.9-24.8).[70] Overt CVD events, however, are rare in childhood.[1,9] Consequently, subclinical cardiovascular outcomes such as carotid intima-media thickness (cIMT) have become widely accepted as relevant to earlier stages of CVD.[55] Several cohort studies have measured cardiovascular risk factors in children and evaluated cIMT in midlife (33-39 years of age).[57–59] They consistently found that presence of CVD risk factors in childhood and adolescence were associated with increased cIMT in middle adulthood.[58,61,122,220] A recent cross-sectional study found that presence of obesity or diabetes mellitus in adolescence was associated with an increased internal cIMT compared to their lean non-diabetic counterparts.[219]

Although the association between childhood CVD risk factors and increased cIMT in adults in their mid-30's has been established, only two longitudinal studies have examined vascular health outcomes in early life. In a recent study of 313 Aboriginal Australian adolescents 9 to 13 years old with metabolic syndrome, cIMT increased by 22 µm over 6 years (p=0.0007).[67] A second study of 42 obese Swedish adolescents (mean

age: 14 years) documented a 20 µm increase in cIMT over only 5 years (p=0.04).[68] Although informative, one was limited to a unique minority population[67] and the other had a small sample size.[68] Therefore, our objective was to add to the existing literature by: (i) to examining the association between CVD risk factors in adolescence and cIMT in young adulthood (early 20's) in a larger bi-racial cohort study, and (ii) to evaluate the association between CVD risk factors in young adulthood (mid 20's) and cIMT in middle adulthood (mid-30's). We hypothesized CVD risk factors at all ages would be associated with increased cIMT in early (mid-20's) and middle adulthood (mid-30's).

Methods

Cohort Descriptions

Insulin Study (InS)

The Insulin Study (InS) is a longitudinal prospective cohort study initiated at the onset of the 1995-96 school year in Minneapolis Public Schools (Table 3.1).[220] Three follow-up exams were conducted: (i) visit 2 (1997-98), (ii) visit 3 (1999-2000), and (iii) visit 4 (2004-09). The cohort was randomly selected from all incoming students in 5th through 8th grades who participated in a school-wide blood pressure (BP) screening program (n=2,915). From the initial BP screening, strategic attempts were made to acquire a higher proportion of children with the potential for future CV risk. The cohort was stratified by sex, ethnicity (non-Hispanic white and black), and 75th systolic blood pressure percentile cut point (50% whose screening systolic blood pressure was above the 75th percentile of its observed distribution, and 50% whose screening systolic blood pressure was below this cut point). To increase sample size, siblings of all original participants were eligible to participate in the InS starting in 2000. Inclusion criteria for participation included: (i) probands: participated in the initial 1995-96 study exam, and (ii) siblings: siblings of the original participants. At each study visit, participants underwent extensive clinical examinations; cIMT was assessed at visit 4. For the current analysis, we used visit 2 as baseline (mean age: 15 years) and visit 4 as follow-up (mean age: 23 years).

Prevention of High Blood Pressure in Children (PHBPC)

The Prevention of High Blood Pressure in Children (PHBPC) study was previously described in Paper 1; Table 3.1 provides a brief summary of both cohort studies in the current analysis. In brief, PHBPC is a longitudinal cohort study that was initiated at the

onset of the 1977-78 school year in Minneapolis Public Schools. Basic anthropometric data were collected annually through high school; three additional visits occurred: (i) visit 2 (1989-91), (ii) visit 3 (1991-95), and (iii) visit 4 (2007-11).[170] The cohort was randomly selected from all incoming students in 1st through 3rd grades (n=2641). In an attempt to enroll participants at increased risk of future CVD, study investigators used the followed selection criteria: (i) all children at the upper and lower 5th percentiles of the normal systolic BP distribution, (ii) 50% of the African American children that remained, and (iii) 10% of the white children that remained. Comprehensive clinical exams were completed at visits 2-4; cIMT was assessed at visit 4. For the current analysis, we used visit 3 as baseline (mean age: 23 years) and visit 4 as follow-up (mean age: 38 years).

The University of Minnesota Institutional Review Board approved both cohorts and all exams were conducted with written informed consent (from parents when children were below 18 years old, with verbal assent from the children; from the participants themselves after they came of age). All methods of exposure, outcome, and covariate assessment were identical in both studies unless otherwise noted.

Exclusions and Missing Data

Insulin Study (InS)

Baseline exclusion criteria included: (i) currently pregnant, (ii) Type 1 diabetes mellitus, (iii) kidney dialysis or end-stage renal disease patients, (iv) cancer patients, or (v) other chronic diseases that have an impact on the study measurements as deemed appropriate by study investigators.[220,221] Of the original 2915 participants randomly selected, 401 children provided written consent or parental assent. Of these 401 participants, 357 remained after exclusions (44 were excluded for the following reasons: 25 withdrew from participation, 2 were ineligible due to chronic disease, 17 were too technically difficult to establish a venous catheter for the clamp studies at the University of Minnesota Clinical and Translational Science Institute). Siblings were recruited for participation and examined only in visits 3 and 4, and had a slightly broader age range (11-35 years of age). Siblings that matched the baseline proband age range were included in the analysis. For the present study, we used visit 2 (n=512; proband n=357, sibling n=155) as baseline and visit 4 (n=317; proband n=215, sibling n=102) as the follow-up visit. The final sample size consisted of 317 participants.

Prevention of High Blood Pressure in Children (PHBPC) study

PHBPC exclusions and missing data were previously described in Paper 1. In brief, of the 2641 children randomly selected to participate in the cohort, 1207 met inclusion criteria and provided written informed consent for participation using University of Minnesota Institutional Review Board approved documents. Participants were examined twice yearly until high school; at which time they were examined annually. A post-high school examination was conducted on average 2 years after graduation (mean age: 19 years); at this exam 817 individuals participated. Baseline characteristics did no differ between the 817 individuals who participated and the 319 individuals lost to follow-up. At the most recent clinic visit in 2007-11 (mean age: 38 years), a comprehensive cardiovascular profile that included cIMT was collected on 444 participants; our final sample size was 444 observations.

Cardiovascular Disease Risk Factors

Anthropometrics & Blood Pressure

Two seated systolic blood pressure (SBP) and diastolic blood pressure (SBP) measurements were taken at the right brachial artery using a random zero sphygmomanometer; an average of these two measurements was used for all analyses. Weight was measured using a medical grade balance scale without shoes; height was measured using a standing stadiometer to the nearest millimeter. Pulse pressure (PP) was defined as the difference between systolic and diastolic blood pressure.

Fasting Laboratory Studies (cholesterol, glucose, insulin, and HOMA)

Fasting blood samples were drawn using standard phlebotomy procedures to measure: total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, and glucose. Non-HDL cholesterol (non-HDL-C) was defined as the difference between total cholesterol and HDL-C. Whereas PHBPC measured fasting insulin, the InS collected euglycemic insulin clamp data, the gold standard for measuring insulin resistance.[222] Homeostasis model assessment (HOMA) was defined as: (glucose*insulin)/405.[223]

Sociodemographics

Age, race, and sex were collected by self-reported questionnaires. Age was asked to the nearest year; race was categorized as non-Hispanic white or African American. No measures were collected for parental socioeconomic status.

Pubertal Timing

Puberty was not assessed in PHBPC through the high school clinic visit. After the high school visit, participants were asked to report pubertal development using a visual scale that parallels classic Tanner development stages.[224,225] In the InS, board certified pediatricians conducted a physical exam to assess Tanner stages of pubertal development. Children were divided into Tanner stages according to pubic hair and breast development in girls, and pubic hair development in boys. In girls, whichever Tanner stage was higher (pubic hair or breast development) was used.[226]

Carotid Intima-media Thickness

All vascular health assessments were performed in a quiet room at a comfortable temperature (22-23°C); cIMT was collected at the final exam in both InS and PHBPC, 2004-09 (mean age: 23 years) and 2007-11 (mean age: 38 years) respectively at the University of Minnesota Clinical and Translational Science Institute. Participants were asked to refrain from caffeine and medication consumption prior to their scheduled clinic visit. After lying in a supine position for 15 minutes, a standard high-frequency ultrasound (Siemens, Sequoia 512, New York, NY) using an 8-15 MHz linear array transducer was used to capture B-mode images of the far wall of the left common carotid artery. Images for determining cIMT were obtained at end-diastole (gated by R wave on ECG). Measurements were obtained at the distal 10 mm of the common carotid artery as recommended by pediatric guidelines.[55] An electronic wall-tracking software program (Medical Imaging Applications, Coralville, IA) was used for the analysis of cIMT. Mean cIMT was calculated by averaging individual cIMT values over the entire length of the arterial measurement area. Our laboratory has previously documented satisfactory cIMT reproducibility, with the mean difference for repeated measurements on separate days in the same subjects of 0.02 ± 0.03%.[227]

Statistical Analysis

For both studies, descriptive statistics were calculated: (i) stratified by quartiles of cIMT, and presented as mean ± standard deviation (SD) or as a frequency distribution (%) of cardiovascular disease risk factor, and (ii): stratified categorically or by quartiles (continuous variables) of cardiovascular disease risk factor, and presented as mean ± standard deviation (SD) of cIMT. For both studies we examined the association between a one SD unit difference in CV risk factors and cIMT, adjusting for potential confounding variables. Consensus is lacking in regards to the best modeling approaches for vascular health measurements. We chose to model cIMT continuously; CV risk factors were

transformed into standardized z-scores to facilitate comparison between CV risk factors. For the InS, we used multivariable Generalized Estimating Equations (GEE) models to account for correlation between siblings. Siblings were included if they fell in the same age range as probands - 12 to 18 years of age (excluded: n=17, age 11; n=92 age 19 or older). GEE model specifications included a Gaussian family, a canonical identity link, an independent working correlation matrix and a robust standard variance estimator. For the PHBPC, no siblings were included; multivariable linear regression models were used. In all analyses, we fit two models: (i) Model 1 adjusted for baseline age, sex, race, Tanner stage (InS longitudinal analysis only), and each cardiovascular risk factor independently; (2) Model 2 adjusted for baseline age, sex, race, Tanner stage (InS longitudinal analysis only), and all cardiovascular risk factors concurrently. We excluded pulse pressure, LDL-C, triglycerides, glucose and insulin in Model 2 due to collinearity with other variables in the model. In both cohorts, cross-sectional and longitudinal analyses were conducted. Exploratory analyses examined results stratified by sex and race, both cross-sectionally and longitudinally. All analyses were performed using Stata 14.[182]

Results

Of the 317 InS and 444 PHBPC participants included in our analysis, mean cIMT was higher in PHBPC (527±32 µm) than in InS (444±22 µm), respectively. Table 3.2 shows study-specific descriptive statistics for baseline variables presented by cIMT quartile. By design, participants in InS were younger at baseline (mean age: 15 years) compared to the PHBPC (mean age: 23 years); baseline age was similar in cIMT quartiles 1 and 4 (Q1 and Q4) in both studies. Participants in InS and PHBPC were predominantly non-Hispanic white, with lower proportions of females and higher proportions of blacks in quartiles 3 and 4 of cIMT. In both studies, participants in cIMT Q4 had higher BMI percentile or BMI, systolic blood pressure (SBP), LDL-C, non-HDL-C, triglycerides, glucose, insulin, and HOMA than those in cIMT Q1 (Table 3.2).

In InS (Table 3.3), black participants had a 45.4 µm (95% CI: 13.7, 77.0; $p<0.005$) and 29.9 µm (95% CI: 13.7, 46.1; $p<0.0001$) higher cIMT than white participants in a demographic (Model 1) and fully adjusted model (Model 2), respectively. A one standard deviation unit difference in BMI percentile z-score (27.0%) at age 15 was associated with a 9.6 µm (95% CI: 4.8, 14.5; $p<0.0001$) higher cIMT at age 23 after

adjusting for demographics. The association was similar, though slightly attenuated, in a fully adjusted model (6.9 µm; 95% CI: 1.1, 12.6; p=0.02). After adjusting for demographics, a one standard deviation unit difference in non-HDL-C z-score (29.5 mg/dL) at age 15 was associated with a 5.9 µm (95% CI: 1.4, 10.4; p=0.01) higher cIMT at age 23; this association became slightly attenuated after adjusting for other cardiovascular risk factors (5.5 µm; 95% CI: -0.07, 11.0; p=0.05). An association between a one standard deviation unit difference in HOMA z-score (2.1) at age 15 and cIMT at age 23 was present (7.1 µm; 95% CI: 2.8, 11.5; p=0.001) after adjusting for demographics, but was also attenuated after further adjustment for cardiovascular disease risk factors (5.6 µm; 95% CI: -0.43, 11.5; p=0.07). No association was found between other cardiovascular risk factors z-scores and cIMT (SBP, DBP, or HDL-C) in the fully adjusted model.

In PHBPC (Table 3.4), a one standard deviation unit difference in age (1.5 year) was associated with a higher cIMT in a demographics adjusted model (5.9 µm; 95% CI: 0.89, 11.1; p=0.02); this association was attenuated after adjusting for cardiovascular risk factors (5.0 µm; -0.60, 10.6; p=0.08). Female participants had a lower cIMT (-26.7; 95% CI: -44.4, -9.1; p=0.003) and blacks had increased cIMT (45.3 µm; 95% CI: 24.8, 65.8; p<0.0001) in fully adjusted models. A one standard deviation unit difference in BMI z-score (5.8 kg/m^2) at age 23 was associated with a 10.9 µm (95% CI: 2.7, 19.0; p=0.009) higher cIMT at age 38 after adjusting for demographics; the association disappeared after adjusting for other cardiovascular risk factors was attenuated in a fully adjusted model (-1.2 µm; 95% CI: -11.5, 9.2; p=0.83).

SBP, however, was associated with cIMT; a one standard deviation unit difference in SBP z-score (10.8 mm Hg) was associated with a 17.0 µm higher cIMT in the demographics (95% CI: 8.8, 25.1; p<0.0001) and 21.0 µm higher cIMT in the fully adjusted model (95% CI: 10.3, 31.8; p<0.0001). After adjusting for demographics, a one standard deviation unit difference in HOMA z-score (4.4) at age 23 was associated with a 9.4 µm (95% CI: 0.82, 18.1; p=0.002) increase in cIMT at age 38; this association was attenuated in the fully adjusted model (5.8 µm; 95% CI: -3.9, 15.6; p=0.24). No other cardiovascular risk factors (DBP, HDL-C, non-HDL-C) were associated with increased cIMT. In both studies, cross-sectional results were nearly identical to longitudinal results (data not shown).

Discussion

In both the InS (Table 3.3) and PHBPC (Table 3.4), we found that black participants had a higher cIMT compared to white participants after adjusting for demographics and cardiovascular risk factors. In InS, a one standard deviation unit increase in BMI percentile z-score (27.0 %) at mean age 15 was associated with elevated cIMT at mean age 23 in a fully adjusted model. In PHBPC, a one standard deviation unit increase in BMI z-score (5.8 kg/m^2) at mean age 23 was associated with a higher cIMT at mean age 38 in a demographics adjusted model. When adjusting for additional cardiovascular disease risk factors, no association was found between BMI and cIMT. SBP, however, was associated with higher cIMT; a one standard deviation unit difference in SBP z-score (10.8 mm Hg) at mean age 23 and cIMT at age 38 persisted in the fully adjusted model. In InS, after adjustment for cardiovascular risk factors, a one standard deviation unit increase in a non-HDL-C z-score (29.5 mg/dL) at mean age 15 was marginally associated with increased cIMT at mean age 23; no association between non-HDL-C and cIMT was found in PHBPC. These results extend the newly emerging evidence that BMI percentile in adolescence is associated with increased cIMT earlier in life than was previously thought, and provides evidence that systolic blood pressure may mediate the association between BMI and cIMT in middle adulthood.

A longitudinal study in 2.3 million adolescents reported a 3.5 times higher lifetime risk of CVD mortality (95% CI: 2.9-4.1),[69] and a 17.2 times higher risk of diabetes mortality in midlife (95% CI:11.9-24.8) in obese participants.[70] Overt CVD events are uncommon in childhood.[1,9] Therefore, emphasis has been placed on subclinical atherosclerosis as it represents an intermediate step in the pathophysiology from the presence of CV risk factors to frank CVD.[55] Two recent studies are the first to support an association between cardiovascular risk factors in adolescence and increased cIMT early in life.[67,68] One, in 313 Aboriginal Australian children and adolescents (9 to 13 years) with metabolic syndrome, found that cIMT increased by 22 µm over 6 years (p=0.0007).[67] The second, in 42 obese Swedish adolescents (mean age: 14 years) reported that cIMT increased by 20 µm, over only 5 years (p=0.04).[68] Though one was in a minority population[67] and the other had a small sample size,[68] taken together these results support the premise that metabolic dysfunction and excess adiposity have an impact on vascular health in adolescence.

A number of well-known pediatric cohort studies have evaluated the impact of childhood risk factors and development of subclinical atherosclerosis in mid-adulthood. In the Bogalusa Heart Study, Li and colleagues found that a one standard deviation difference in childhood LDL cholesterol (OR: 1.42, 95% CI: 1.14-1.78) and BMI (OR: 1.25, 95% CI: 1.10-1.54) were predictive of increased carotid intima media-thickness (upper quartile) in adulthood (25-37 years of age).[57] Evidence from the Muscatine and Young Finns studies found similar results, emphasizing the impact of childhood risk factor burden on future subclinical CVD (age range: 33-42 years, and mean age: 31 years, respectively).[7,58,59] Collectively, these studies provide strong evidence that risk factor burden in childhood has vascular implications in middle adulthood. Our findings extend the understanding of the longitudinal impact of CV risk factors on vascular health to early adulthood (mean age: 23 years). In the current analysis, a one standard deviation unit difference in non-HDL-C was associated with increased cIMT in InS, but not in PHBPC. Furthermore, LDL-C and was not associated with increased cIMT in PHBPC, triglyceride and log transformed triglyceride (to normalize right skewed nature of natural triglycerides) levels were not associated with cIMT in either study, and HDL-C was not protective in either study. These results are consistent with a 2011 National Heart, Lung, and Blood Institute report on cardiovascular health in children and adolescents which concluded that non-HDL-C captures persistent dyslipidemia in adolescent populations more accurately than LDL-C or HDL-C levels.[228] SBP was only associated with elevated cIMT in PHBPC, and appeared to mediate the association between BMI and cIMT. It is possible that lipid and blood pressure changes are not as influential on vasculature earlier in life, that they require a longer duration to elicit an effect (i.e. changes in BMI status generally occur prior to increases in blood pressure or lipids and therefore have a longer period of time to impact vasculature), or that our sample size is insufficient to detect a smaller magnitude of effect in young adulthood. Only 1 of the 3 longitudinal pediatric cohort studies has reported an association between high childhood SBP and cIMT in middle life.[58] Nonetheless, two recent cross-sectional studies in adolescents found that elevated systolic blood pressure was independently associated with cIMT;[229,230] one concluded that only age and blood pressure were responsible for the direct effects on elevated cIMT.[229] A cross-sectional study in adults reported that hypertension status was most strongly associated with cIMT and degree of severity; an association was present, but to a lesser extent, with dyslipidemia and

diabetes and cIMT.[231] Furthermore, a longitudinal study in adults found that a 5 mm Hg increase in SBP was associated with and 8 µm increase in cIMT (95% CI: 2.5, 14.0).[232] In short, more evidence is needed in regard to the complex relationship between BMI, SBP, and cIMT in both adolescent and adult populations.

Recent evidence in adults provides insight into possible mechanisms by which childhood overweight and obesity may impact vascular health. Specifically, endothelial function appears to be a critical factor in predicting cardiovascular events, independent of conventional CV risk factors.[233–235] A meta-analysis in adults determined that a 1% decrease in flow-mediated dilation predicted a 13% higher risk of cardiovascular disease.[233] Furthermore, adipose tissue secretes pro-inflammatory cytokines such as interleukin-6, a known regulator of C-reactive protein, which is linked with inflammation and CVD.[236] A cross sectional study in healthy adults found that adiposity status (normal weight vs. overweight and obese) was positively associated with C-reactive protein,[237] and observational data in adults suggests that C-reactive protein predicted an increased risk of CVD.[238] Future examination of these factors in childhood and adolescent populations could be informative etiologically, particularly in the understanding of physiological mechanisms involved in subclinical CVD.

Strengths of the InS and PHBPC studies include collection of a comprehensive CV risk factor profile from adolescence through middle adulthood and an accurate estimate of cIMT by using: (i) mean cIMT of the entire area measured (versus maximum cIMT), and (ii) specialized software that allows greater resolution of arterial imaging. Importantly, InS measured cIMT in early adulthood (mean age 23 years) which is critical to establishing the impact of cardiovascular risk factor burden on vascular health in early adulthood. Finally, results from PHBPC are consist with other pediatric cohorts.[57–59] Limitations of both studies include potential unmeasured confounding (i.e. parental socioeconomic status, dietary intake, and physical activity levels), and the possibility of selection bias due to low response rates at the initiation of the study (despite a near complete census of school-aged children). Smoking status was assessed in PHBPC (current, former, never) but not in InS. Results were unchanged when we included smoking status in our PHBPC models, hence we excluded smoking status from our analyses for consistency. Results may not apply to other ethnic groups beyond non-Hispanic whites and African Americans. Assessment methods for insulin were different in PHBPC (fasting insulin) and InS (euglycemic insulin clamp – gold standard); PHBPC

insulin measurements are likely not as accurate as those performed in InS. Secondary to study design, age-matched siblings had short follow-up time (4.9 years) compared to probands (6.6 years), thus siblings may have had less follow-up time to accumulate changes in cIMT. Finally, the clinical utility of cIMT is uncertain; questions remain regarding normative values and which populations would receive the greatest benefit from vascular imaging. Epidemiological evidence suggests that elevated cIMT is associated with frank CVD events in adulthood; a meta-analysis in older adults reported that the age- and sex-adjusted risk of myocardial infarction and stroke were 1.15 (95% CI: 1.12, 1.17) and 1.18 (95% CI: 1.16, 1.21) per 0.10 mm change in cIMT, respectively.[239] There are no studies to date examining the relationship between childhood or adolescent cIMT and risk of future CVD events. In a cross sectional analysis examining adolescents with Type 1 diabetes, mean cIMT was 0.55 ±0.04 mm compared to 0.51±0.04 mm in healthy participants ($p<0.001$) suggesting that a difference in cIMT of approximately 0.04 mm may have clinical implications in adolescence.[240] Nonetheless, the magnitude of cIMT changes that have clinical implications in childhood and adolescence is uncertain, and presents a rich area for future research.

The implications of our results merit consideration of increased adiposity status in childhood and adolescence. National Health and Nutrition Examination Survey (NHANES) data suggest that 33% of children 2-18 years of age were overweight or obese; 17% were obese.[167] The prevalence of pediatric overweight and obesity has been steadily increasing over the past 30 years, tracks into adulthood, and is positively associated with risk factors that predict cardiovascular disease morbidity and mortality.[241–245] Furthermore, adiposity is known to track into adulthood more strongly than other risk factors; most children do not outgrow their adiposity status.[44–47] Robust cross-sectional evidence suggests that obese children and adolescents have lower arterial health and endothelial function than their healthy counterparts.[130,132,134,246,247] Longitudinal pediatric cohort studies have established that overweight and obesity have vascular consequences in adulthood;[57–59] our results support that these physiological implications are present earlier in adulthood than was known and that elevated adiposity is the biggest predictor of vascular dysfunction in adolescence. Preventive measures that improve vascular function may prevent future cardiovascular disease events by disrupting the development of atherosclerosis.[248]

Importantly, vascular changes have been shown to be reversible. In 2010, the American Heart Association (AHA) established strategic Impact Goals: “By 2020, to improve the cardiovascular health of all Americans by 20%, while reducing deaths from CVDs and stroke by 20%.”[87] Independently, the development of the Ideal Cardiovascular Health (ICH) metric by the AHA substantiates the importance of primordial CVD prevention.[249] Furthermore, using the AHA ICH metric, investigators from the Young Finns study demonstrated that a 1-unit increase in ICH in childhood (12-18 years) was associated with a 25% reduction in the odds of high carotid intima-media thickness (cIMT) (OR=0.75, 95% CI: 0.60-0.94, p=0.01). Notably, these results were independent of changes in ICH between childhood and adulthood.[92] Finally, exercise appears to positively impact vascular health in obese and overweight children. In otherwise healthy youth 9-12 years of age, cIMT was reduced in the group randomized to an exercise program indicating that vascular dysfunction is at least partially reversible.[133] A recent meta-analysis confirmed that exercise improves vascular function in overweight and obese children and adolescents, and that the magnitude of change was large enough to reestablish healthy vascular function.[250]

In conclusion, elevated BMI beginning in adolescence was associated with increased cIMT in young adulthood. In the mid-20’s, SBP appeared to be associated with cIMT in the mid-30’s. These results suggest that youth is a critical period for detection and intervention to preserve future cardiovascular health. Initiation of preventive strategies in adolescence and young adulthood, particularly in regard to adiposity and blood pressure, may reduce future subclinical atherosclerosis. Next steps include replication in other cohorts and further exploration of the most appropriate strategies for clinical adiposity management.

Table 3.1. Overview of the Insulin Study and Prevention of High Blood Pressure in Children (PHBPC) study.

	Insulin Study		PHBPC
General study characteristics	**Probands**	**Siblings**	
Type	Prospective cohort	Prospective cohort	Prospective cohort
Grade level[1]	5th-8th	5th-8th	1st-3rd
Location	Minneapolis, MN	Minneapolis, MN	Minneapolis & St. Paul, MN
Stratification sampling variables[2]	Blood pressure, sex, race	-	Blood pressure, race
Analys characteristics	**Probands**	**Siblings**	
Initial visit	1997-98	2000	1991-95
Follow-up visit	2004-09	2004-09	2007-11
CV risk factor assessment[3]	15 yrs	15 yrs	23 years
cIMT assessment[3]	23 yrs	23 yrs	38 years
Initial visit (*n*)	357	155	679
Follow-up visit (*n*)	215	102	444
Total sample size	317		444

Abbreviations: cardiovascular, CV; Carotid intima-media thickness cIMT.
[1] Matched sibling age to probands in analytic sample (12-19 years).
[2] Stratified sampling of near complete school census, then low response rates; stratified by sex, race (non-Hispanic white and black), and blood pressure (50% of children in upper 25th and 50% in lower 75th percentiles of normal systolic distribution).
[3] Mean; baseline age range: 12-19 years (Insulin) and 20-25 years (PHBPC); follow-up age range: 20-25 years (Insulin) and 38-40 years (PHBPC)

Table 3.2. Selected baseline participant characteristics; the Insulin Study and Prevention of High Blood Pressure in Children (PHBPC) study.

	Insulin Study				PHBPC			
	1997-2000; mean age 15				1991-95; mean age 23			
	n=317				n=444			
	Carotid intima-media thickness quartiles							
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
N	99	101	49	68	112	131	93	108
Mean, µm ± SD	375 (37)	433 (7)	455 (5)	511 (38)	437 (24)	488 (15)	541 (16)	642 (74)
Range, µm	220-410	420-440	450-460	470-640	370-460	470-510	520-570	580-940
Age, mean years ± SD	15.5 (1.0)	14.7 (1.3)	14.5 (1.0)	15.6 (1.1)	23.0 (1.0)	23.1 (0.94)	23.0 (0.91)	23.2 (0.93)
Female, n (%)	48 (49)	51 (51)	19 (39)	24 (35)	73 (65)	71 (54)	35 (38)	46 (43)
Black, n (%)	11 (11)	10 (10)	15 (31)	20 (29)	21 (19)	25 (19)	24 (26)	38 (35)
Tanner stage (1-4), n (%)	17 (17)	22 (22)	14 (29)	10 (15)	-	-	-	-
BMI[1]	61 (27)	59 (29)	70 (22)	78 (21)	24 (5)	26 (7)	26 (5)	27 (6)
SBP, mm Hg ± SD	108 (7)	107 (9)	108 (9)	111 (10)	107 (10)	110 (11)	113 (11)	115 (10)
DBP, mm Hg ± SD	58 (13)	58 (14)	58 (15)	56 (13)	70 (9)	70 (10)	72 (10)	72 (10)
PP, mm Hg ± SD	50 (15)	49 (15)	50 (18)	55 (17)	37 (8)	41 (9)	42 (9)	43 (9)
LDL-C, mg/dL ± SD	83 (25)	87 (26)	83 (25)	89 (29)	103 (27)	102 (25)	108 (33)	108 (27)
HDL-C, mg/dL ± SD	43 (9)	45 (9)	43 (9)	41 (8)	51 (13)	47 (12)	46 (11)	49 (11)
Non-HDL-C, mg/dL ± SD	105 (29)	106 (30)	99 (25)	112 (32)	121 (31)	121 (31)	128 (37)	127 (32)
Triglycerides, mg/dL ± SD	86 (37)	84 (45)	81 (32)	102 (53)	86 (44)	96 (63)	101 (56)	97 (66)
Glucose, mg/dL ± SD	85 (5)	86 (6)	85 (7)	88 (8)	85 (8)	89 (16)	89 (9)	90 (8)
Insulin, mU/L ± SD	10 (6)	12 (9)	11 (6)	13 (9)	19 (10)	19 (11)	20 (13)	22 (13)
HOMA ± SD	2 (2)	3 (2)	2 (1)	3 (3)	4 (2)	4 (3)	5 (4)	5 (3)

Abbreviations and units: quartiles 1, 2, 3 and 4, Q1, Q2, Q3, Q4, respectively; body mass index, BMI (kg/m^2); systolic blood pressure, SBP (mm Hg); diastolic blood pressure, DBP (mm Hg); pulse pressure, PP (mm Hg); low-density lipoprotein cholesterol, LDL-C (mg/dL); high-density lipoprotein cholesterol, HDL-C (mg/dL); non-high-density lipoprotein cholesterol, non-HDL-C [total cholesterol – HDL-C]; homeostasis model assessment [insulin*glucose/405], HOMA.

[1]BMI reported as age- and sex-adjusted percentile (SD) based on CDC growth curves in the Insulin Study and kg/m2 in the PHBPC.

Table 3.3. Differences in carotid intima-media thickness (cIMT, µm) by cardiovascular risk factor (z-score); Insulin Study (n=317).

		Model 1[1]		Model 2[2]	
		Mean ages: 15 yrs at baseline; 23 yrs at follow-up.			
	SD	Coef (95% CI)	*p*-value	Coef (95% CI)	*p*-value
Age	1.6	2.1 (-11.4, 7.2)	0.66	3.2 (-0.73, 7.1)	0.11
Sex, female[3]	-	-6.4 (-14.3, 27.1)	0.54	-7.7 (-18.4, 3.1)	0.16
Race, black[3]	-	45.4 (13.7, 77.0)	0.005	29.9 (13.7, 46.1)	<0.0001
Tanner stage		-2.2 (-15.6, 11.2)	0.75	-0.34 (-12.1, 11.4)	0.96
BMI %[4]	27.0	9.6 (4.8, 14.5)	<0.0001	6.9 (1.1, 12.6)	0.02
SBP	8.6	3.4 (-1.6, 8.4)	0.18	2.3 (-3.5, 8.1)	0.44
DBP	12.8	2.7 (-1.7, 7.1)	0.23	0.38 (-4.6, 5.3)	0.88
PP	15.2	-0.7 (-5.2, 3.9)	0.78	n/a[5]	n/a[5]
LDL-C	26.0	5.5 (1.0, 10.0)	0.02	n/a[5]	n/a[5]
HDL-C	9.2	-2.7 (-7.3, 1.9)	0.24	1.5 (-3.8, 6.9)	0.58
Non-HDL-C	29.5	5.9 (1.4, 10.4)	0.01	5.5 (-0.1, 11.0)	0.05
Triglycerides	53.0	5.2 (0.7, 9.8)	0.03	n/a[5]	n/a[5]
Glucose	7.7	6.0 (-2.0, 14.0)	0.14	n/a[5]	n/a[5]
Insulin	8.8	7.5 (3.3, 11.6)	<0.0001	n/a[5]	n/a[5]
HOMA	2.1	7.1 (2.8, 11.5)	0.001	5.6 (-0.4, 11.5)	0.07

Abbreviations and units: Body mass index percentile, BMI %; systolic blood pressure, SBP (mm Hg); diastolic blood pressure, DBP (mm Hg); pulse pressure, PP (mm Hg); high-density lipoprotein cholesterol, HDL-C (mg/dL); non-high-density lipoprotein cholesterol, non-HDL-C [total cholesterol – HDL-C]; and homeostasis model assessment [insulin*glucose/405], HOMA.

All models predicting cIMT at mean age 23 years in participants and like-aged siblings; cardiovascular risk factors measured at mean age 15.

[1] Model 1: adjusted for age, sex, race, tanner stage, and each cardiovascular risk factor independently.

[2] Model 2: adjusted for age, sex, race, tanner stage, and all cardiovascular risk factors concurrently.

[3] Referents = male and white for sex and race, respectively.

[4] BMI age- and sex-adjusted percentiles based on CDC criteria used to account for pubertal development during adolescence.

[5] Excluded in full model due to linear dependency on other variables in the model.

Interpretation: The change in cIMT (µm) observed from a one standard deviation unit change in cardiovascular risk factor z-score.

Table 3.4. Differences in carotid intima-media thickness (cIMT, µm) by cardiovascular risk factor (z-score); Prevention of High Blood Pressure in Children (PHBPC) study (n=444).

		Model 1[1]		Model 2[2]	
		Mean ages: 23 yrs at baseline; 38 yrs at follow-up.			
	SD	**Coef (95% CI)**	***p*-value**	**Coef (95% CI)**	***p*-value**
Age	1.5	5.9 (0.89, 11.1)	0.02	5.0 (-0.60, 10.6)	0.08
Sex, female[3]	-	-27.3 (-42.9, -11.8)	0.001	-26.7 (-44.4, -9.1)	0.003
Race, black[3]	-	43.3 (24.8, 61.8)	<0.0001	45.3 (24.8, 65.8)	<0.0001
BMI	5.8	10.9 (2.7, 19.0)	0.009	-1.2 (-11.5, 9.2)	0.83
SBP	10.8	17.0 (8.8, 25.1)	<0.0001	21.0 (10.3, 31.8)	<0.0001
DBP	9.8	3.7 (-4.5, 11.9)	0.38	-11.1 (-21.3, -0.90)	0.03
PP	9.0	15.0 (7.2, 22.9)	<0.0001	n/a[4]	n/a[4]
LDL-C	27.8	6.9 (-1.1, 15.0)	0.09	n/a[4]	n/a[4]
HDL-C	11.8	-4.2 (-12.6, 4.2)	0.33	1.1 (-8.1, 10.2)	0.82
Non-HDL-C	32.4	8.1 (0.06, 16.2)	0.05	7.8 (-2.2, 17.8)	0.13
Triglycerides	58.1	6.6 (-1.6, 14.9)	0.12	n/a[4]	n/a[4]
Glucose	11.2	9.0 (0.77, 17.3)	0.03	n/a[4]	n/a[4]
Insulin	19.8	8.5 (-0.19, 17.2)	0.06	n/a[4]	n/a[4]
HOMA	4.4	9.4 (0.82, 18.1)	0.002	5.8 (-3.9, 15.6)	0.24

Abbreviations and units: Body mass index, BMI (kg/m^2); systolic blood pressure, SBP (mm Hg); diastolic blood pressure, DBP (mm Hg); pulse pressure, PP (mm Hg); high-density lipoprotein cholesterol, HDL-C (mg/dL); non-high-density lipoprotein cholesterol, non-HDL-C [total cholesterol – HDL-C]; and homeostasis model assessment [insulin*glucose/405], HOMA.

All models predicting cIMT at mean age 38 years in participants; cardiovascular risk factors measured at mean age 23.

[1] Model 1: adjusted for age, sex, race, and each cardiovascular risk factor independently.

[2] Model 2: adjusted for age, sex, race, and all cardiovascular risk factors concurrently.

[3] Referents = male and white for sex and race, respectively.

[4] Excluded in full model due to linear dependency on other variables in the model.

Interpretation: The change in cIMT (µm) observed from a one standard deviation unit change in cardiovascular risk factor z-score.

Paper 3: Ideal Cardiovascular Health and Adiposity: Implications in Youth

Introduction

To a great extent, cardiovascular disease is preventable; individuals without cardiovascular risk factors have low rates of cardiovascular disease in adulthood.[1,99] In an attempt to prioritize cardiovascular health and shift towards primordial prevention, the American Heart Association (AHA) set 2020 Strategic Goals that included the concept of ideal cardiovascular health (ICH).[249] ICH incorporates seven health metrics (smoking, body mass index [BMI], physical activity, diet, blood pressure, total cholesterol, and glucose), and is characterized as poor, intermediate, or ideal based on the number of metrics an individual meets. ICH is defined as meeting the ideal definition for all 7 metrics. The Cardiovascular Risk in Young Finns Study reported a relationship between low ICH (below the median number of 'ideal' metrics) in childhood with cardiovascular disease in adulthood. A noteworthy finding was that no children in this study met all 7 metrics for ICH.[92,251] In the same cohort, individuals that changed their ICH status from low to high from childhood to adulthood had similar cardiometabolic health profiles as the participants originally classified as having high ICH as children.[252]

Excess adiposity remains a serious public health threat;[6,253] 33% of U.S. adolescents are classified as having overweight or obesity.[5,6] Despite a potential plateau in overall obesity rates, the prevalence of severe obesity in children and adolescents 2-19 years of age has increased from 4% in 1999-2004,[254] to over 8% in 2013-14.[6] Severe obesity in youth is associated with increased cardiometabolic risk factors,[164] vascular dysfunction,[255] increased oxidative stress,[256] and risk factors for chronic disease.[84] Recent evidence suggests that cardiovascular mortality in adulthood was 3.5 times higher (95% CI: 2.9-4.1) for individuals classified as having obesity as adolescents.[69] The AHA estimates that 33% of adolescents do not meet the ICH metric for ideal BMI (i.e. were classified as having overweight or obesity).[91] Given the prevalence of overweight and obesity in children, considerable attention has been paid to the role of adiposity as a precursor to the development of adult cardiovascular disease.[12,13,20] Mendelian randomization studies confirm that adiposity is a causal risk factor for cardiovascular risk factors and atherosclerosis in adulthood.[190,191] Therefore, identifying children based on adiposity who are at risk for clinically evident cardiovascular disease later in life may be an important strategy for cardiovascular disease prevention.[20,21]

Given that pediatric cohort studies report an absence of ICH in children[90,92] and secular trends show increases in severe obesity prevalence, the clinical utility of the ICH metric could be improved with a greater focus on adiposity status. Furthermore, it is unclear if categorizing biologically continuous risk factors for ICH estimation is the ideal approach.[257,258] To our knowledge, no data exist on the prevalence of ICH across levels of adiposity in a pediatric population, nor has an adiposity specific or continuous ICH metric been proposed. The objectives of the current study were twofold. First, we aimed to improve the understanding of the distribution of ICH metrics by adiposity status. Second, we examined the same ICH metrics by adiposity status using a continuous ICH sample z-score.

Methods

Study Population

This cross-sectional study included 300 children and adolescents aged 8-17 years from the greater Minneapolis and St. Paul metropolitan area recruited from 2011-2016. Enrollment was stratified by adiposity: normal weight, overweight/obese, and severely obese. Participants were recruited from various pediatric clinics, including the University of Minnesota Masonic Children's Hospital Pediatric Weight Management Clinic (participants with obesity or severe obesity only). Participants and parents provided assent and written informed consent, respectively. The study protocol was approved by the University of Minnesota Institutional Review Board.

Participants were excluded if they met any of the following criteria: (i) untreated obstructive sleep apnea (known to impact endothelial function), (ii) genetic causes of obesity (i.e., Prader-Willi), (iii) previous medical history of weight loss surgery, (iv) current medications known to affect the endothelium (i.e., statins, ACE inhibitors, PPAR-gamma agonists, metformin, and 3rd generation beta blockers), (v) current use of stimulants for Attention Deficit and Hyperactivity Disorder (e.g., methylphenidate), illness, hospitalization, or trauma in the previous 2 weeks, type I diabetes mellitus, familial hypercholesterolemia, chronic kidney disease or end-stage renal disease, Kawasaki disease (inflammatory disease of the vasculature), autoimmune inflammatory disease, or congenital heart disease.

Ideal Cardiovascular Health Metrics

In 2010, the American Heart Association (AHA) established the ICH metric.[249] Seven health metrics have been established to define cardiovascular health – categorized as poor, intermediate, and ideal (Table 1.1, as seen in Introduction). Ideal cardiovascular health is defined as meeting all seven cardiovascular health metrics and the absence of cardiovascular disease or cardiovascular medication use. The seven cardiovascular health metrics are split into 4 health behaviors (smoking status, physical activity, dietary patterns, and BMI) and 3 health factors (fasting total cholesterol, blood glucose, and blood pressure). Table 1.1 is reprinted with permission from the American Heart Association, and details specific definitions of poor, intermediate, and ideal cardiovascular health for children aged 12-19 years.

Clinical Ideal Cardiovascular Components: BMI, Blood Pressure, Total Cholesterol, and Glucose

Height was measured using a wall-mounted stadiometer; weight was measured using a medical-grade electronic scale. BMI was calculated and reported in kilograms per meter squared (kg/m^2). Centers for Disease Control and Prevention definitions of sex- and age-based BMI percentiles (2-20 years of age) were used.[259] Study BMI categories were defined as: normal weight: BMI < 85th percentile; overweight/obese: BMI 85th percentile to <1.2 times the 95th percentile, and severely obese: BMI ≥1.2 times the 95th percentile or BMI ≥ 35 kg/m^2.[84] Seated blood pressures were taken using a manual sphygmomanometer, using an appropriately-fitted cuff. Each participant sat quietly with legs uncrossed for 10 minutes prior to blood pressure measurement. Three consecutive blood pressure readings were taken at least 3 minutes apart. The average of the final 2 measurements was used. Fasting blood samples (>10 hr) were collected and total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides, glucose, and insulin were measured using standard methods by the Fairview Diagnostics Laboratories, Fairview-University Medical Center (Minneapolis, MN, USA) - a Center for Disease Control and Prevention certified laboratory.

Lifestyle Ideal Cardiovascular Health Components: Diet, Physical Activity, and Smoking Exposure

Dietary intake was measured using the Youth/Adolescent Questionnaire (YAQ), a semi-quantitative food frequency questionnaire. The YAQ asks participants about dietary and supplement intake in the previous year.[260,261] General physical activity was assessed

using a modified Godin Leisure-Time questionnaire[262,263] that assessed: (i) intensity (strenuous, moderate, mild) of physical activity in the previous week during free time, (ii) participation in organized sports in the previous years, and (iii) leisure time physical activity (at least 10 times in the previous year). Smoking exposure was evaluated by self-report questionnaire as never, current, former, previous, or secondhand smoke exposure.

Conversion from Study Instruments to Ideal Cardiovascular Health Components

BMI categorization and total cholesterol, blood pressure, and glucose thresholds were consistent between the study instruments and ICH guidelines; therefore, no modifications were made to these variables. Current (n=2) and former smokers (n=5) were categorized as meeting the definition of poor ICH, whereas never smokers (n=293) were classified as meeting ICH. The modified Godin Leisure-Time questionnaire assessed strenuous and moderate physical activity in the past week (times per week and minutes per session). Strenuous and moderate activity was defined as “heart beats rapidly” and “not exhausting,” respectively; both listed various activities that were relevant. For the ICH metric, we calculated minutes per day of moderate and vigorous physical activity using the following equation: (times per week x minutes per session)/7 days per week. The YAQ is a 152-item food frequency questionnaire that collects a comprehensive assessment of food intake in the past week or month. We utilized 45 questions that met the specified Dietary Approaches to Stop Hypertension (DASH) food categories for the ICH guidelines: fruits/vegetables (n=30), whole grains (n=10), fish (n=2), sugar sweetened beverages (n=3), and salt intake (micronutrient analysis). Nearly all food questions assessed frequency of intake using the following, or similar, answer options: 1) never/less than 1 per month, 2) 1-3 per month, 3) 1 per week, 4) 2-4 times per week, and 5) 5 times per week. We calculated frequency of intake by taking the midpoint (i.e. midpoint=2 servings per month if participant chose option #2 [1-3 per month]) or absolute value (i.e. serving=1 if participant chose option #3 [1 per week]) reported. We then calculated the total servings per unit of time for each relevant ICH dietary subcomponent: fruits and vegetables (≥4.5 cups per day), whole grains (≥3 servings/day), fish (≥ 2 3.5 ounce servings/week), sugar sweetened beverages (≤36 ounces/week), and salt (≤1500 mg/day) based on a 2000-kcal/day diet. For example, for fruit and vegetable consumption, each participant’s overall servings per day was a summary score of servings from the relevant 30 fruit/vegetable YAQ questions. This

process was repeated for whole grains, fish, and sugar-sweetened beverages. A summary salt intake was calculated using a proprietary micronutrient algorithm at the Harvard T.H. Chan School of Public Health Nutrition Department.

Sociodemographics

Age and race were determined based on self-report; race was categorized as non-Hispanic White and Black. Socioeconomic status was approximated by self-report of free or reduced lunch eligibility. Sex and pubertal stage were determined by a pediatrician or trained nurse using classical Tanner staging.[226]

Exclusions and Missing Data

Of the 309 participants, we excluded individuals for the following reasons: 1) no Tanner stage performed (n=5), and 2) participant declined venipuncture, unable to perform a successful venipuncture, or non-fasting (n=4). Our final sample size was 300 observations. Additional individuals would have been excluded based on missing dietary intake (n=154; new instrument version released mid-study, data unable to be processed off-site due to old versioning), physical activity (n=30), and eligibility for free or reduced lunch (n=183; collection began mid-study). To minimize selection bias, maximize the use of available information, and capture appropriate uncertainty estimates, we used multiple imputation by chained equations (MICE) with 25 repetitions to impute missing data for these individuals who would have been excluded in a complete case analysis based on missing data.[264] Because both dietary intake and eligibility for free lunch were dependent on chronological time of measurement, we operated under the assumption that the missingness mechanism was missing at random (MAR). Results between the complete case analysis and non-imputed data set were similar; we opted to use the imputed data set based on the aforementioned strengths of MICE.

Statistical Analysis

Baseline characteristics of participants are described using means (SD) and frequency (percentages) stratified by categories of adiposity (normal weight, overweight/obese, and severely obese). For the first study objective, prevalence of overall ICH and ICH subcomponents were calculated across levels of adiposity. For the second study objective, a continuous ICH z-score was generated, both as an overall ICH score and ICH subcomponent scores. The overall ICH score was calculated based on the average of the individual z-scores (standardized to the sample mean and standard deviation) of the ICH subcomponents (BMI, physical activity, healthy diet score, total

cholesterol, blood pressure, and glucose). BMI was converted to age and sex-standardized scores using Centers for Disease Control and Prevention growth charts.[265,266] For overall blood pressure and healthy dietary consumption z-scores, an average of the individual z-scores (SBP and DBP percentiles for blood pressure; fruits and vegetables, whole grains, fish, sugar sweetened beverages and sodium intake for diet) was used to calculate the overall z-score. To maintain consistency in sample ICH z-score interpretation (higher is better), all risk factors conferring cardiovascular risk were multiplied by -1 to flip the direction of the score (e.g., higher blood pressures have negative values to contribute to a lower overall ICH z-score). The z-score represents the number of standard deviations a measurement is from the mean, and allows comparison between variables measured on different scales, from different distributions. A z-score greater, equal to, or less than zero estimates that a particular variable is greater than, equal to, or less than the mean, respectively. [267,268] For our final study objective, multiple linear regression models were used to estimate differences in ICH z-scores by adiposity status after adjustment for demographics and Tanner stage (Model 1), and additional adjustment for reduced or free lunch eligibility (Model 2). Smoking was not included in our primary analysis because only 2 participants were current smokers, and 5 were former smokers. All analyses were be performed using Stata 14 and *R* software packages.[182,183]

Results

Table 4.1 shows descriptive statistics for baseline variables presented separately by adiposity level (normal weight, overweight/obese, and severely obese). In general, participants with severe obesity were more likely to be female, non-white, have a higher Tanner stage of pubertal development, be eligible for reduced or free lunch, and be above the 90th systolic blood pressure percentile. Individuals with severe obesity also had higher mean insulin, LDL-c, total cholesterol, and triglyceride levels than their normal weight counterparts. Conversely, HDL-c was lower in participants with severe obesity. Of the 300 participants included in our analysis, none met the AHA definition of ICH (Table 4.2). When examining the prevalence of ICH components by adiposity status (Table 4.2), very few participants were current smokers (n=2) or had elevated glucose levels (n=7). Only one participant met the criteria for ideal healthy diet score. Participants with severe obesity were more likely to fall in the ‘poor’ ICH category for physical activity

level (16% vs 1%), total cholesterol (16% vs. 4%), and blood pressure (30% vs. 1%). For overall ICH, all participants with severe obesity were in poor cardiovascular health; most of the participants with overweight/obesity (81%) and normal weight (80%) were also in poor cardiovascular health. The total number of ICH components increased with healthier adiposity status; 58% of normal weight participants met the definition of 5 ICH components, whereas only 2% of individuals with severe obesity met the same criteria (Figure 4.1).

For all results reporting continuous z-scores, a positive value indicates higher ICH sample z-score; a negative value represents a lower ICH sample z-score. Normal weight participants had a higher overall ICH sample z-score (0.80±0.9) than participants with overweight/obesity (-0.40±0.7) and severe obesity (-0.58±0.8) (Table 4.3). Sample z-scores for physical activity, SBP percentile, DBP percentile, overall blood pressure percentile, and glucose were all in the positive range for normal weight participants, and negative for participants with overweight/obesity and severe obesity. The overall healthy diet consumption z-score followed a similar pattern, but participants with overweight/obesity had positive subcomponent z-score values for fruits and vegetable, fish, and sodium (0.03±0.9, 0.16±1.1, and 0.07±1.1, respectively). More specifically, only 4%, 9%, and 2% of participants with normal weight, overweight/obesity, and severe obesity consumed the ICH recommended ≥ 2 servings of fish per week (Table 4.4). Few participants met the ICH sodium intake recommendations of ≤1500 mg sodium daily; 6%, 9%, and 23% for participants with normal weight, overweight/obesity, and severe obesity, respectively. Ideal fruit and vegetable consumption (≥4.5 servings daily) was relatively consistent across all adiposity levels; ideal whole grain intake was higher in participants with normal weight (48%) compared with overweight/obesity (30%) and severe obesity (18%). Ideal levels of sugar sweetened beverages (≤36 ounces weekly) were most prevalent; 63%, 72% and 56% in participants with normal weight, overweight/obesity, and severe obesity, respectively (Table 4.4). In a multiple linear regression model adjusting for demographics and eligibility for free or reduced lunch, compared to normal weight, the ICH z-score was lower for participants with overweight/obesity (-1.35; 95% CI: -2.3, -1.1) and severe obesity (-1.45; 95% CI: -2.9, -0.92) (Table 4.5). Results were similar for individual ICH metrics.

Discussion

In this study, we used the AHA's "Strategic Impact Goal Through 2020 and Beyond"[249] ICH metric to examine the prevalence of overall ICH and subcomponents stratified by adiposity in youth. In addition, we generated a continuous overall ICH sample z-score and ICH subcomponent z-scores across levels of adiposity for an internal comparison. To the best of our knowledge, this is the first study to examine ICH metrics by adiposity status, and to create a continuous ICH z-score that captures the continuous nature of the data. Overall, we found that no participants achieved ICH; 13% and 87% of study participants were in intermediate and poor cardiovascular health, respectively. Importantly, 80% of normal weight, 81% of overweight/obese, and all participants with severe obesity were in poor cardiovascular health. After adjustment for demographics and eligibility for reduced or free lunch, participants with overweight/obesity and severe obesity had lower ICH z-scores than normal weight participants.

Previous studies have found the prevalence of ICH to be low or absent in adolescent[15,39–40], young adult,[269] and adult populations.[272] Our findings are consistent with existing literature, and extend these concerning results to a younger population (mean age: 12.8 years; SD 2.7). Shay and colleagues found a lack of ICH in adolescents aged 12-19 years; less than 50% of adolescents had ≥ 5 ICH components.[91] In the Young Finns study, the largest pediatric cohort study to date, no participants met the criteria for ICH (mean age: 15.0 years; SD: 0.05).[270] Because children are generally considered to be born with ICH,[1] these collective results serve as a considerable reminder that ICH is being lost at younger ages, and that targeted intervention efforts to maintain ICH in early life may be beneficial to prevent the deterioration of ICH in childhood and adolescence. Given that the prevalence of ICH was absent in the current analysis, and that 87% of all participants were in poor cardiovascular health, the functionality of the ICH metric remains uncertain. Furthermore, secular trends for cardiovascular risk factor burden and overweight/obesity have increased or at best plateaued,[166,167] thus raising concerns that the prevalence of ICH is unlikely to increase in the immediate future. We found that nearly all participants had healthy fasting blood glucose levels and were nonsmokers, even with a large number of participants with severe obesity. Furthermore, only one participant met the ideal criteria for the healthy diet score. Previous studies have attempted to address this issue by dichotomizing the

number of ICH metrics (≤ 3 or ≥ 4 ICH metrics),[270] creating summary scores for total number of ICH metrics present (maximum of 7),[269,271] or generating a composite score (0, 1, or 2 assigned for poor, intermediate, and ideal cardiovascular health, respectively, for each of the 7 factors).[91] Alternatively, it may be beneficial to consider a continuous ICH metric which captures the dynamic physiological state of cardiovascular health.[273–275]

A recent AHA Scientific Statement was published in late 2016, with the intention of being a supplemental document to the "Strategic Impact Goal Through 2020 and Beyond."[249] Steinberger and colleagues specifically discussed challenges and opportunities for cardiovascular health in children and adolescents, and addressed potential modifications that could improve the ICH metric.[257] One of the primary recommendations was that future ICH tools could benefit from prioritizing continuous measures of risk factors. Furthermore, the ICH includes thresholds for behavioral and health factors that are based on percentiles calculated from population estimates. The authors argue that thresholds that are relevant to cardiovascular outcomes in adulthood would be more meaningful, but these data are generally nonexistent.[257] It has previously been shown that continuous cluster scores in childhood can be predictive of cardiometabolic dysfunction in adulthood.[51] In light of these considerations, our results include an evaluation based on comparisons using a continuous z-score in our study sample. After adjustment for demographics and Tanner stage, participants with overweight/obesity and severe obesity had lower overall ICH sample z-scores (-1.35 and -1.45, respectively) compared with normal weight participants; results were similar for ICH subcomponents. These results suggest that overweight/obesity and severe obesity are independent predictors of ICH, and the impact of adiposity on ICH may not be fully captured by weighting adiposity and other ICH metrics equally. In addition, prevalence estimates suggest that 80% and 81% of individuals with normal weight and overweight/obesity, respectively, met criteria for poor ICH (Table 4.2); ICH sample z-scores, however, suggest that individuals with overweight/obesity have a much lower ICH sample z-score (-0.40±0.9) than normal weight individuals (0.80±0.9) (Table 4.3). Thus, prevalence estimates alone indicate that individuals with normal weight and overweight/obese have similar ICH, whereas ICH sample z-scores suggest that the overweight/obese group had lower ICH than the normal weight group. Finally, youth with overweight/obesity and severe obesity are rich targets for primary prevention efforts;

additional research is needed on the short-term impacts of lifestyle improvements on ICH metrics in childhood and adolescence.

Examining the prevalence of ICH by adiposity merits independent attention for a variety of reasons. First, the prevalence of overweight and obesity in childhood and adolescence is 33% (17% and 15% are obese and overweight, respectively);[166] it is unclear if these numbers have plateaued or continue to rise.[166,167] Prevalent severe obesity in children and adolescents, however, has increased from 4% in 1999-2004,[254] to over 8% in 2013-14.[6] Second, adiposity in youth is a foundational risk factor subclinical cardiovascular disease,[7,57,59,276] future obesity[11], and cardiovascular disease and diabetes-related mortality in adulthood.[69,277] Third, Mendelian randomization studies support a causal role for obesity in abnormal cardiovascular disease risk factors the development of adult atherosclerosis.[190,191] Identifying children based on adiposity who are at risk for clinically evident cardiovascular disease later in life may be an important strategy for cardiovascular disease prevention.[20,21]

Strengths of this study include the comparison of ICH by adiposity status in a sample encompassing childhood and adolescence, the rare ability to examine this relationship in youth with severe obesity, comprehensive cardiovascular risk factor assessment, and the generation of continuous ICH sample z-score for use in our study sample (overall and subcomponent z-scores). In the current study, prevalent overweight/obese and severe obesity was higher, by design, than national estimates.[166,167] Nonetheless, our results are consistent with findings in the Young Finns study which reported that ICH was rare (1%), ideal fasting glucose and non-smoking status were most common, and ideal BMI and diet were less common.[278] Lastly, these results represent the youngest cohort to date where ICH is absent; our results include children that are under 12 years of age (n=117) and therefore extend beyond the age range specified for ICH in childhood and adolescence (12-19 years). Importantly, higher thresholds for older children and adolescents may incorrectly classify younger children as being in better cardiovascular health, which would likely bias results towards increased ICH.

There are a number of limitations to this study. First, certain variables that impact adiposity, such as genetic predisposition, were not assessed. Study exclusion criteria, however, attempted to identify the most common genetic conditions linked to adiposity or mediation known to impact adiposity status. Second, selection bias remains a possibility; for example, perhaps the participants with severe obesity have a different risk factor

profile than the target population of children and adolescents with severe obesity. Nonetheless, the robust sample of youth with severe obesity and concordance of baseline characteristics (Table 4.1) with known risk factors profiles[84] give confidence to minimal selection bias. Third, it can be challenging to apply criteria from newly developed constructs (i.e. ICH) to more commonly used assessment tools for cohort studies or clinical trials. For example, the ICH guidelines consider dietary intake per day or within the previous week, whereas the YAQ food frequency questionnaire asks about dietary patterns in the previous week and month. Strict adherence to AHA ICH criteria in particular was not feasible for all criteria (i.e. dietary components or physical activity); reasonable approximations were made. Fifth, the use of z-scores comes with mixed implications.[279–281] Z-scores afford the use of a continuous scale, standardize the units of the variables of interest, allow comparison across variables, and the ability to evaluate an overall ICH summary z-score as well as compare subcomponent z-scores.[279,280] Nonetheless, by transforming raw data into z-scores, the original units and clinical meaning are absent,[279] and interpretation relies on a one standard deviation unit difference which can have limited clinical meaning particularly after multiple z-scores are combined. In addition, z-scores are known to perform poorly at extreme values of BMI in youth;[281] we did not calculate a BMI z-score in the current analysis and instead opted to stratify our results by adiposity. Furthermore, z-scores that are calculated using observed means and standard deviations from the sample at hand, as was used here for several ICH components, have limited external generalizability because they are generated from a sample and not a target population of interest. Finally, in our primary analysis, we did not include smoking status in our overall z-score because there were very few current (n=2) and former (n=5) smokers, and smoking status did not have an underlying continuous scale.

In summary, poor cardiovascular health was highly prevalent in youth, and increased with adiposity status. Overall ICH z-scores were lower across higher levels of adiposity; participants with overweight, obesity, or severe obesity had lower z-scores than their normal weight counterparts. Youth remain a rich target for primary prevention efforts targeting behavioral change. Modifying the ICH to a continuous metric could further enhance its ability to stratify cardiovascular risk; data that capture a representative sample in addition to cardiovascular outcomes are critical to assessing the utility of a generalizable continuous metric. Identifying characteristics of children and adolescents

that have poor or intermediate ICH based on adiposity status has potential implications for interventions to prevent future cardiovascular disease in youth with obesity. Implementing more aggressive primary prevention intervention efforts in youth with severe obesity should be a priority.

Table 1.1. Definitions of poor, intermediate, and ideal cardiovascular health according to AHA 2020 goals: health behaviors and risk factors for children 12-19 years of age.

Metric	Poor	Intermediate	Ideal
Smoking status	Tried > 30 d ago	…	Never tried; never smoked whole cigarette
BMI	>95th percentile	85th- 95th percentile	<85th percentile
Physical activity level	None	>0 and <60 min moderate or vigorous activity every day	≥60 min moderate or vigorous activity every day
Healthy Diet Score*	0-1 components	2-3 components	4-5 components
Total Cholesterol	≥200 mg/dL	≥170 - <200 mg/dL	<170 mg/dL
Blood Pressure	>95th percentile	90-95th percentile	<90th percentile
Fasting Blood Glucose	≥126 mg/dL	100 - 125 mg/dL	<100 mg/dL

BMI indicates body mass index.

* The Healthy Diet Score is based on adherence to the following dietary recommendations: fruits and vegetables, ≥4.5 cups per day; fish, 2 or more 3.5-oz servings per week; sodium, <1500 mg/d; sugar-sweetened beverages, ≤450 kcal (36 oz) per week; and whole grains, ≥3 servings a day scaled to a 2000-kcal/d diet. Reprinted with permission Circulation.2016;134:e236-e255© American Heart Association, Inc.

Table 4.1. Baseline characteristics by adiposity status (n=300).

	Adiposity Status		
	NW	**OW/OB**	**SO**
N	113	87	100
BMI, *kg/m^2 (SD)*	18.4 (2.4)	26.5 (3.5)	35.9 (6.1)
BMI, *percentile (SD)*[1]	73.6 (6.7)	106.0 (10.0)	140.1 (16.8)
Total tissue fat, *N (%)*	25.2 (6.1)	39.5 (7.2)	48.1 (4.9)
Visceral fat mass, *g (SD)*	74 (54.0)	412.5 (275)	1097 (585)
Demographics			
Age, *years (SD)*	12.5 (2.5)	12.5 (2.5)	13.1 (2.7)
Male, *N (%)*	68 (58)	43 (48)	38 (38)
Race, *N (%)*			
White	109 (93)	74 (83)	77 (76)
Other	8 (7)	15 (17)	24 (24)
Tanner stage, *N (%)*			
1	45 (39)	23 (27)	16 (16)
2	20 (17)	18 (21)	26 (26)
3	17 (15)	18 (21)	18 (18)
4	22 (19)	15 (17)	22 (22)
5	11 (10)	12 (14)	17 (17)
Free or reduced lunch eligibility, *N (%)*	17 (15)	16 (18)	34 (34)
Clinical variables			
Weight, *kg (SD)*	44.3 (13.1)	65.9 (16.8)	93.8 (26.5)
Height, *cm (SD)*	153.2 (15.1)	156.3 (13.7)	159.8 (12.5)
WC, *cm (SD)*	63.2 (6.9)	80.5 (9.5)	100.0 (14.6)
SBP, *mm Hg (SD)*	107 (11)	116 (11)	124 (12)
SBP ≥ 90th percentile, *N (%)*	4 (3)	18 (20)	51 (50)
DBP, *mm Hg (SD)*	54 (7)	58 (9)	61 (8)
DBP ≥ 90th percentile, *N (%)*	1 (1)	3 (3)	3 (3)
Insulin, *mU/L (SD)*	4 (3)	10 (6)	17 (11)
Glucose, *mg/dl (SD)*	78 (9)	81 (9)	80 (8)
LDL-c, *mg/dl (SD)*	81 (24)	95 (24)	97 (28)
HDL-c, *mg/dl (SD)*	57 (12)	47 (12)	41 (9)
Total cholesterol, *mg/dl (SD)*	153 (27)	165 (27)	164 (31)
TG, *mg/dl (SD)*	71 (27)	113 (56)	133 (50)

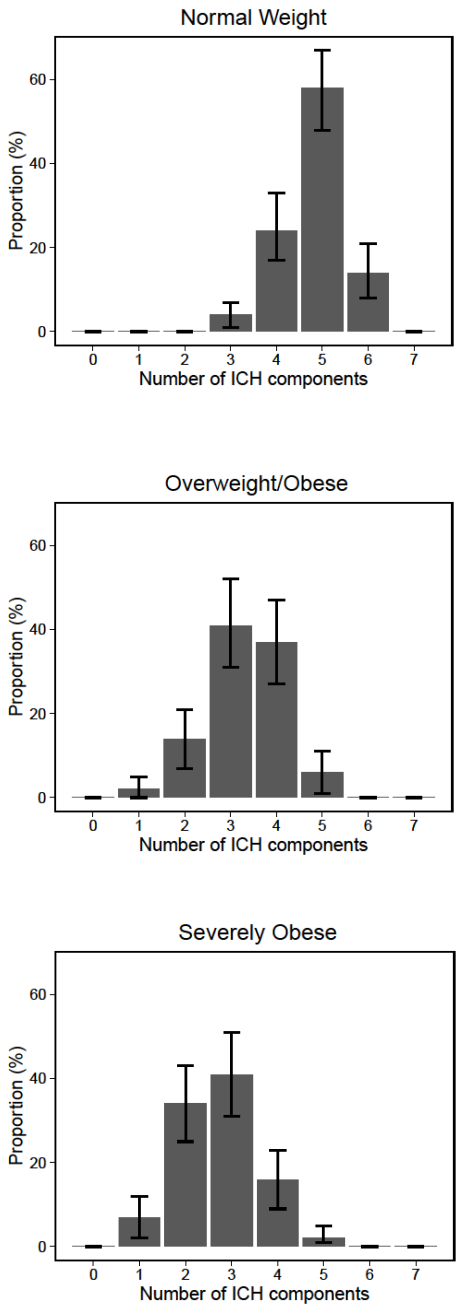
Percentages may not add up to 100% based on rounded estimates.
Abbreviations: Normal weight, NW; overweight, OW; obese, OB; severely obese, SO; waist circumference, WC; systolic blood pressure, SBP; diastolic blood pressure, DBP; low-density lipoprotein cholesterol, LDL-c; high-density lipoprotein cholesterol, HDL-c; triglycerides, TG.
[1] Age- and sex-adjusted percentiles based on CDC criteria used to account for pubertal development during adolescence.

Table 4.2. Ideal cardiovascular health (ICH) components by adiposity status (n=300).

		Adiposity Status		
	Overall, *N %*	**NW**	**OW/OB**	**SO**
N	300	113	87	100
Overall ICH prevalence, N %				
Ideal	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate	40 (13)	23 (20)	17 (19)	0 (0)
Poor	260 (87)	90 (80)	70 (81)	100 (100)
ICH Components				
Body mass index percentile, *N %*				
Ideal	113 (38)	113 (100)	0 (0)	0 (0)
Intermediate	20 (7)	0 (0)	20 (23)	0 (0)
Poor	167 (56)	0 (0)	67 (77)	100 (100)
Smoking Status, *N %*				
Ideal	298 (99)	112 (99)	87 (100)	99 (99)
Intermediate	-	-	-	-
Poor	2 (1)	1 (1)	0 (0)	1 (1)
Physical activity[2], *N %*				
Ideal	57 (19)	28 (25)	17 (19)	12 (12)
Intermediate	222 (74)	84 (74)	66 (76)	72 (72)
Poor	21 (7)	1 (1)	4 (5)	16 (16)
Healthy diet score, *N %*				
Ideal	1 (1)	0 (0)	1 (1)	0 (0)
Intermediate	62 (21)	25 (22)	23 (26)	14 (14)
Poor	237 (79)	88 (78)	63 (72)	86 (86)
Total cholesterol, *N %*				
Ideal	194 (65)	84 (74)	51 (59)	59 (59)
Intermediate	78 (26)	25 (22)	28 (32)	25 (25)
Poor	28 (9)	4 (4)	8 (9)	16 (16)
Blood pressure, *N %*				
Ideal	202 (67)	104 (92)	60 (69)	38 (38)
Intermediate	59 (20)	8 (7)	19 (22)	32 (32)
Poor	39 (13)	1 (1)	8 (9)	30 (30)
Glucose, *N %*				
Ideal	293 (98)	110 (97)	86 (99)	97 (97)
Intermediate	2 (1)	1 (1)	0 (0)	1 (1)
Poor	5 (2)	2 (2)	1 (1)	2 (2)

Percentages may not add up to 100% based on rounded estimates.
Abbreviations: normal weight, NW; overweight, OW; obese, OB; severely obese, SO.
[1] Ideal cardiovascular health defined by Lloyd-Jones *et al.* as meeting American Heart Association definitions of ideal on four health behavior metrics and three health factor metrics concurrently. Intermediate cardiovascular health defined as meeting at least one intermediate metric and no poor metrics. Poor cardiovascular health defined as having at least one poor metric.
[2] Moderate and/or vigorous physical activity.

Figure 4.1. Prevalence of number of Ideal Cardiovascular (ICH) subcomponents[1] by adiposity status (n=300).



[1]Those in the OW/OB and SO categories unable to attain 7 ICH components based on adiposity status. Error bars indicate the 95% confidence interval that would be anticipated in the population given the data.

Table 4.3. Mean z-score of Ideal Cardiovascular Health components by adiposity status (n=300).

		Adiposity Status		
	SD[1]	**NW**	**OW/OB**	**SO**
N	300	113	87	100
ICH components[2]		**ICH z-score (SD)**	**ICH z-score (SD)**	**ICH z-score (SD)**
Physical activity, *(SD)*	31	0.32 (1.0)	-0.08 (1.0)	-0.32 (0.9)
Healthy diet consumption, *(SD)*				
Fruits/vegetables	1.8	0.04 (0.9)	0.03 (0.9)	-0.07 (1.1)
Fish	0.7	0.03 (0.9)	0.16 (1.1)	-0.23 (0.9)
Whole grains	1.5	0.42 (1.0)	-0.15 (0.9)	-0.29 (0.9)
Sugar sweetened beverages	54.7	0.07 (1.0)	-0.25 (1.0)	-0.24 (1.0)
Sodium	819	0.13 (0.9)	0.07 (1.1)	-0.25 (1.1)
Overall diet summary score[3]	-	0.11 0.9)	0.09 (0.9)	-0.24 (1.1)
Total cholesterol, *(SD)*	29	0.22 (1.0)	-0.16 (0.9)	-0.14 (1.1)
Blood pressure %, *(SD)*				
SBP percentile	29	0.59 (0.9)	-0.06 (0.9)	-0.61 (0.8)
DBP percentile	22	0.29 (0.9)	-0.01 (1.0)	-0.30 (1.0)
Overall BP percentile summary score[3]	-	0.51 (0.9)	-0.04 (0.9)	-0.52 (0.9)
Glucose, *(SD)*	9	0.20 (1.0)	-0.16 (1.0)	-0.08 (0.9)
Overall ICH summary score, (SD)[3]	-	0.80 (0.9)	-0.40 (0.7)	-0.58 (0.8)

Interpretation: A z-score ≤ 0 indicates a variable is less than the mean (lower ICH); a z-score ≥ 0 indicates a variable is great than the mean (higher ICH).

Abbreviations: normal weight, NW; overweight, OW; obese, OB; severely obese, SO; standard deviation, SD; systolic blood pressure, SBP; diastolic blood pressure, DBP; blood pressure, BP; ideal cardiovascular health, ICH.

[1]Units for standard deviations: physical activity (minutes); diet: fruits/vegetables and whole grains (servings/day), fish (servings/week), sugar sweetened beverages (ounces/week), sodium (mg/day); total cholesterol and glucose (mg/dL); blood pressure (percentile). SD for absolute SBP and DBP was 13 mm Hg and 9 mm Hg, respectively.

[2]Did not include smoking status; only 2 participants were current smokers and dichotomized nature of smoking variable made z-score transformation less straight forward.

[3] Overall summary z-scores were calculated based on the average of the individual subcomponents: diet (fruits/vegetables, fish, whole grains, sugar sweetened beverages, and sodium); blood pressure percentile (systolic and diastolic blood pressure percentiles) overall ICH (physical activity, diet, SBP, total cholesterol, and blood glucose). See Methods section for a more detailed description.

Table 4.4. Mean Ideal Cardiovascular Health (ICH) diet subcomponent consumption and prevalence of ideal consumption by adiposity status (n=300).

		Adiposity Status		
	Overall	**NW**	**OW/OB**	**SO**
N	300	113	87	100
Ideal healthy diet subcomponents				
Fruits & vegetables, servings per day (SD)	3.6 (1.8)	3.6 (1.7)	3.6 (1.7)	3.4 (2.2)
Whole grains, servings per day (SD)	2.7 (1.5)	3.3 (1.5)	2.5 (1.4)	2.2 (1.3)
Fish, servings per day (SD)	0.71 (0.70)	0.73 (0.61)	0.82 (0.87)	0.55 (0.53)
SSB, ounces per week (SD)	43 (55)	47 (57)	29 (27)	56 (73)
Sodium, mg per day (SD)	2353 (819)	2466 (698)	2419 (874)	2152 (856)
Ideal healthy diet consumption[1]				
Fruits & vegetables, *N (%)*	84 (28)	28 (25)	24 (28)	30 (30)
Whole grains, *N (%)*	96 (32)	54 (48)	26 (30)	18 (18)
Fish, *N (%)*	18 (6)	5 (4)	8 (9)	2 (2)
SSB, *N (%)*	192 (64)	71 (63)	63 (72)	56 (56)
Sodium, *N (%)*	36 (12)	7 (6)	8 (9)	23 (23)

Abbreviations: Overweight, OW; obese, OB; standard deviation, SD; sugar sweetened beverages, SSB.
[1] Based on AHA guidelines, consistent with Dietary Approaches to Stop Hypertension (DASH) dietary pattern: to consume ≥4.5 cups/day of fruits and vegetables, ≥2 servings/week of fish, and ≥3 servings/d of whole grains and no more than 36 oz/week of sugar-sweetened beverages and 1500 mg/day of sodium.

Table 4.5. Differences in overall Ideal Cardiovascular Health z-score by adiposity status (n=300).

	Model 1[1]		**Model 2[2]**	
	N=300		**N= 300**	
	ICH z-score difference (95% CI)	***p*-value**	**ICH z-score difference (95% CI)**	***p*-value**
Age (per year)	-0.05 (-0.13, 0.03)	0.232	-0.04 (-0.13, 0.02)	0.346
Male (vs. Female)	0.20 (-0.09, 0.50)	0.181	0.14 (-0.14, 0.34)	0.397
Race: Black (vs. White)	-0.01 (-0.15, 0.12)	0.849	-0.08 (-0.10, 0.33)	0.761
Tanner stage	0.02 (-0.13, 0.18)	0.786	0.03 (-0.13, 0.15)	0.944
Adiposity status[3]				
Normal Weight	Referent	--	Referent	--
Overweight/obesity	-1.24 (-1.57, -0.91)	<0.0001	-1.35 (-2.3, -1.1)	<0.0001
Severe obesity	-1.41 (-1.80, -1.01)	<0.0001	-1.45 (-2.9, -0.92)	<0.0001
Free or reduced lunch eligibility	-	-	-0.10 (-0.92, 0.73)	0.812

All linear regression models examining overall cross-setional ICH z-score at mean age 12.8±2.7 years.
[1] Model 1: adjusted for age, sex, race, tanner stage, and adiposity status.
[2] Model 2: Model 1 + reduced free lunch eligibility.
[3] BMI age- and sex-adjusted percentiles based on CDC criteria used to account for pubertal development during adolescence.

Conclusion

Excess adiposity remains a serious public health threat; 33% of U.S. adolescents are classified as having overweight or obesity. Adolescent obesity is associated with a 3.5 times higher lifetime risk of cardiovascular disease (CVD) mortality, thus subclinical cardiovascular (CV) phenotypes such as carotid intima-media thickness (cIMT) have become widely accepted as relevant to earlier stages of CV disease in youth. This dissertation used two longitudinal pediatric cohort studies and one pediatric clinical trial to examine the implications of excess adiposity in youth.

In the first manuscript, we developed a risk prediction model that predicted cIMT in middle adulthood using relevant CV risk factors in adolescence. Although longitudinal associations were observed between most CVD risk factors in adolescence and increased cIMT in middle adulthood, our risk prediction model poorly predicted cIMT in middle adulthood based on discrimination and calibration metrics. Future efforts to develop a methodologically robust risk prediction model utilizing risk factors in adolescence to predict subclinical atherosclerosis in middle adulthood could benefit children and adolescents at risk for developing subclinical atherosclerosis in a number of ways. First, individuals with high-risk scores could be differentiated from those with low-risk scores without having to measure vascular pathology. Consequently, treatment optimization could be tailored for high-risk individuals. Second, risk factor treatment (behavioral or pharmacological) could be monitored over time, thereby allowing for a more personalized approach to prevention and management of atherosclerosis.

In the second manuscript, we used longitudinal data from two bi-racial cohort studies to examine the association between CV risk factors and common cIMT in two distinct periods in life: (i) between adolescence and young adulthood (mid 20's), and (ii) between young adulthood (mid 20's) and early middle age (mid 30's). Higher body mass index was associated with an increased cIMT in the younger cohort. In the older cohort, no association was present between BMI and cIMT; higher SBP was associated with increased cIMT. These results suggest that youth is a critical period for detection and intervention to preserve future cardiovascular health. Initiation of preventive strategies in adolescence and young adulthood, particularly in regard to adiposity in adolescence and blood pressure in the mid 20's, may reduce future subclinical atherosclerosis development.

The American Heart Association set 2020 Strategic Impact Goals that defined CV risk factors to be included in the concept of ideal cardiovascular health (ICH). In the final manuscript, we examined the prevalence of ICH among differing levels of adiposity in youth, including severe obesity. In addition, we generated a continuous ICH sample z-score, and examined the distribution of the ICH sample z-score stratified by adiposity groups in youth. After adjustment for demographics, children with overweight/obesity and severe obesity had lower ICH sample z-scores than those with normal weight. Pediatricians face numerous challenges when addressing weight management in a clinical setting. One of these hurdles involves addressing behavioral changes for families struggling with weight issues. On the other end of the spectrum, clinicians with expertise in weight management have limited options for clinical management of severe obesity and associated comorbidities. Although our ICH sample z-score is only applicable to our study population, conceptually it provides a platform to consider the future development of a generalizable ICH z-score created from a representative data source. Clinicians and researchers alike could potentially use a generalizable ICH z-score to: (i) provide a more comprehensive indicator of cardiovascular health than many metrics currently available (e.g. BMI), and (ii) provide a metric that may be more amenable to detect cardiovascular health improvement or deterioration as a result of behavioral change.

Taken together, these three projects provide insight into the relationship between excess adiposity and subclinical CV disease and CV health in youth. In addition, this dissertation considers metrics that have the potential to address excess adiposity prevention efforts in youth.

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