Evaluating the determinants and the effects of elevated blood pressure in a population of children and adolescents at-risk for hypertension

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Abstract

Background

Elevated casual hypertension in children can be predicted by a number of factors, including excessive BMI and poor lifestyle habits. If maintained into adolescence, hypertension can lead to increased left ventricular mass index (LVMI) and carotid intima media thickness (cIMT), and eventually cardiovascular events. However, the association of 24-hours ambulatory blood pressure (BP) on these outcomes has not been thoroughly investigated. Moreover, it is unclear if the risk factors for hypertension change throughout childhood.

Objectives

1. Determine which characteristics are associated with elevated blood pressure (BP) in childhood, and whether the same characteristics similarly impact BP throughout childhood.
2. Evaluate the extent to which elevated BP is associated with LVMI and cIMT.

Methods

Two observational cohort studies:
1. Prospective longitudinal cohort at two Quebec hospitals: visit 1: n=631, visit 2: n=564. Participants underwent a study visit, consisting of measurements of anthropometry, body composition, accelerometer data, fitness test, oral glucose tolerance test, and blood samples, as well as comprehensive lifestyle questionnaires for both patient and parents. Associations between variables and casual BP were measured to determine predictors of elevated BP.
2. Prospective cross-sectional cohort at a Montreal hospital: 134 patients were recruited in this ongoing study. 24-hour ambulatory blood pressure monitoring (ABPM) and echocardiograms were performed to ascertain BP, LVMI, and cIMT. Associations between ABPM-derived BP and LVMI and cIMT were measured.

Results

Longitudinal cohort: n=631, age=9.6 years at visit 1; n=564, age=11.7 years at visit 2; 40% of participants were overweight or obese at each visit. Adiposity and hereditary factors were associated with systolic and diastolic BP. BMIz was a significant predictor of future BP levels. Cross-sectional cohort: n=134, age=17.2 years; 36% were overweight or obese at the study visit. BMIz and lean body mass were associated with LVMI, while only BP was associated with cIMT. Other factors were independently associated with both measures, but were not significant when the models were adjusted for additional variables.

Conclusions

Hypertension is associated with several measurable variables, although children and adolescents that present with these risk factors often go unscreened and untreated. The risk factors themselves and the associated ambulatory hypertension can lead to significant end-organ damage in adolescents. Research should focus on developing and testing better measurement indices, while policy and protocol changes should make BP screening and tracking a more widely implemented practice.

Résumé

Introduction

L'hypertension artérielle élevée chez les enfants peut être prédite par de nombreux facteurs, incluant un IMC élevé et de mauvaises habitudes de vie. On ne sait pas si l'impact de ces facteurs sur la pression artérielle (PA) change pendant l'enfance. Si elle est maintenue dans l'adolescence, l'hypertension peut mener à une augmentation de l'indice de masse ventriculaire gauche (IMVG) et de l'épaisseur de l'intima média carotidienne (ÉIMc) et, éventuellement, des événements cardiovasculaires. Cependant, l'impact de la pression artérielle ambulatoire (dérivée du MAPA) sur ces résultats reste inconnu.

Objectifs

1. Déterminer quelles caractéristiques sont associées avec une PA élevée pendant l'enfance et si ces caractéristiques spécifiques restent influentes au cours de l'enfance.
2. Évaluer dans quelles mesures une PA élevée est associée avec l'IMVG et l'ÉIMc.

Méthodes

Deux études d'observation de cohortes :

1. Une Étude longitudinale conduite dans deux hôpitaux du Québec: visite 1: n = 631, visite 2: n = 564. Les participants ont fait une visite d'étude pendant laquelle la mesure d'anthropométrie et l'indice de masse corporelle ont été collectés. un test de condition physique, un test oral de tolérance au glucose, des échantillons de sang ont été administrés aux patients. Des questionnaires concernant les habitudes de vie ont été remplits par les patients et leurs parents. Les données fournies par l'accéléromètre ont aussi été regardées.
2. Une étude prospective transversale à l'hôpital Sainte-Justine à Montréal: 134 patients ont été recrutés pour cette étude en cours. Étude transversale: Mesures Automatiques de la Pression Artérielle (MAPA) à domicile pendant 24 heures et un échocardiogramme ont été effectués pour déterminer l'hypertension, l'IMV et l'ÉIMc. Dans l'étude longitudinale, les associations entre les différentes variables et la PA ont été mesurées pour déterminer les prédicteurs de la PA élevée. Dans l'étude transversale, les associations ont été mesurées entre la BP du MAPA et l'IMVG et l'ÉIMc.

Résultats

Cohorte longitudinale: n = 631, âge = 9,6 ans à la visite 1. N = 564, âge = 11,7 ans à la 2e visite. 40% des participants étaient en surpoids ou obèses à chaque visite. L'adiposité et les facteurs héréditaires étaient associés avec la BP systolique et diastolique, et l'IMC était un prédicteur significatif des niveaux de PA futurs.

Cohorte transversale: n = 134, âge = 17,2 ans. 36% étaient en surpoids ou obèses lors de la visite d'étude. L'IMC et la masse corporelle maigre ont été associés avec le IMVG, et seulement la PA était associée avec l'ÉIMc. Les autres facteurs ont été indépendamment associés avec les deux mesures cardiaques, mais n'étaient pas significatifs lorsque des variables supplémentaires ont été ajoutées pour ajuster le modèle.

Conclusions

L'hypertension est associée avec plusieurs variables qu'il est possible de mesurer, mais les enfants et les adolescents qui présentent ces facteurs de risque ne sont pas souvent suivis par leur médecin. Les facteurs de risque eux-mêmes ainsi que l'hypertension ambulatoire associée peuvent causer des dégâts significatifs au niveau des organes chez les adolescents. La recherche devrait être concentrée sur le développement et sur les essais visant à trouver de meilleurs mesures. De plus, les politiques et les protocoles devraient être modifiés afin que le dépistage de la PA soit implanté comme pratique.

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Preface & contribution of authors

This thesis is original work by the author, Joseph Windheim.

The two original manuscripts were conceptualized and designed by Mr. Windheim and Dr. Michael Zappitelli.

Mr. Windheim carried out part of the patient recruitment, study visits, data entry, and statistical analyses, and drafted the initial manuscripts. Dr. Zappitelli reviewed and edited all analyses and manuscript work that was done.

Drs. Melanie Henderson and Zappitelli are the principal investigators for the QUALITY and QUALITY-BP studies, respectively; they participated in conceptualizing and designing the study and the database used for the study, and coordinated and supervised data collection.

Ms. Catherine Pelletier and Ms. Ginette Lagacé helped to recruit patients, perform the study visits for the QUALITY and QUALITY-BP studies, enter collected data, and conduct the blood and urine sample analyses. I also acknowledge Soren Harnois-Leblanc, Julie Lavoie, Maryse Thibeault, Myriam Leclerc, and the rest of the CHU Sainte-Justine team who were instrumental in the recruitment of patients, the sample analyses, and the data entry.

Mr. Windheim, Mr. Mike Pizzi, and Dr. Zappitelli together designed the database used for the QUALITY-BP study.

The review manuscript was written by Mr. Windheim, and edited by Dr. Zappitelli.

Data analysis was performed using STATA® version 12 (College Station, TX, USA) by the author, with assistance from Dr. Zappitelli.

Abbreviations

English

ABPM	Ambulatory Blood Pressure Monitor
ACE	Angiotensin Converting Enzyme
ADH	Anti-Diuretic Hormone
AMBGC	Annual McGill Biomedical Graduate Conference
ANF	Atrial Natriuretic Factor
Ang	Angiotensin
ANS	Autonomic Nervous System
ASE	American Society of Echocardiography
AT1(2)R	Angiotensin II Receptor Type 1 (Type 2)
BMI (z)	Body Mass Index (z-score)
BMIcv	Body Mass Index cardiovascular threshold
BP (z)	Blood Pressure (z-score)
BSA	Body Surface Area
CDC	Centers for Disease Control
CF%	Central Fat Percentage
cIMT	carotid Intima Media Thickness
CNS	Central Nervous System
CRP	C-Reactive Protein
CSN	Canadian Society of Nephrology
DBP (z)	Diastolic Blood Pressure (z-score)
DEXA	Dual-Energy X-ray Absorptiometry
ECF	Extracellular Fluid
ECG	Electrocardiography/electrocardiogram
FFQ	Food Frequency Questionnaire
GFR	Glomerular Filtration Rate
HC	Hip Circumference
HDL-C	High Density Lipoprotein - Cholesterol
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HR	Heart Rate
HTN	Hypertension
IQR	Interquartile Range
LBM	Lean Body Mass
LDL-C	Low Density Lipoprotein - Cholesterol
LICO	Low Income Cut Off
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Index
MAP	Mean Arterial Pressure
MAPK	Mitogen-Activated Protein Kinase
Matsuda ISI	Matsuda Insulin Sensitivity Index
MCH	Montreal Children's Hospital
MRI	Magnetic Resonance Imaging

MSNA	Muscle Sympathetic Nervous Activity
MVPA	Moderate-and-Vigorous Physical Activity
NHBPEP	National High Blood Pressure Education Program
NSAID	Nonsteroidal Anti-Inflammatory Drug
OGTT	Oral Glucose Tolerance Test
OSA	Obstructive Sleep Apnea
QUALITY	Quebec Adiposity and Lifestyle Investigation and Youth
RAAS	Renin-Angiotensin-Aldosterone System
RWT	Relative Wall Thickness
SA node	Sinoatrial node
SBP (z)	Systolic Blood Pressure (z-score)
SCr	Serum Creatinine
SD	Standard Deviation
SSB	Sugar-Sweetened Beverage
TDI	Tissue Doppler Imaging
V1 / V2	Visit 1 / Visit 2
VO2 max	Peak Oxygen Uptake
WC	Waist Circumference
WCH	White Coat Hypertension
WHO	World Health Organization

French

CHU	Centre Hospitalier Universitaire
ÉIMc	Épaisseur de l'Intima Média carotidienne
IMC	Indice de Masse Corporelle
IMVG	Indice de Masse Ventriculaire Gauche
MAPA	Mésure Automatique de la Pression Artérielle
PA	Pression Artérielle

Echocardiography

AR	Aorta Root Diameter
E/e' lat	Early Mitral Inflow Velocity/Mitral Annular Early Diastolic Velocity
EF	Ejection Fraction
IVS (d)	Interventricular Septum (diastolic)
LA	Left Atrium Diameter
LVID (d)	Left Ventricular Internal Diameter (diastolic)
LVPW (d)	Left Ventricular Posterior Wall (diastolic)
MV E/A	Mitral Valve E Peak/Mitral Valve A Peak

Chapter 1: Overall introduction and aims

1.1 Overview

Hypertension in youth is universally perceived as a major health concern, and it has been and continues to be the focus of numerous large-scale studies and government initiatives. If hypertension persists throughout the patient's youth, it can cause significant and irreversible damage to the heart and vascular system, kidneys, brain, and eyes, and can heighten the risk for myocardial infarction and stroke. Hypertension in children is defined using normative curves, such that patients with BP values exceeding the 95th percentile of BP are hypertensive, irrespective of the fact that lower levels of BP may also cause pathologies.

While there are several contributors to elevated BP, the majority of hypertension cases are classified as "essential", meaning that they have no identifiable cause. Several factors, such as adiposity and high salt consumption, are known to contribute to elevated BP. Yet few studies have investigated whether there exist other meaningful factors in children genetically predisposed to developing hypertension. It is also unclear if, and to what extent, the factors' influences change as the children get older. This information is essential in order to properly identify the major risk factors for hypertension at each age, and administer appropriate treatment.

When a child is diagnosed with essential hypertension, the physician first recommends lifestyle changes, such as modifications to the child's diet and exercise regimen. Failing that, the physician may prescribe anti-hypertensive medications. However, while screening for hypertension is practiced in Canada, few children with an abnormal BP measurement have a follow-up measurements performed at subsequent appointments [1]. Furthermore, some organizations do not recommend screening, arguing that the current data does not indicate that the potential benefits outweigh the harms. Studies evaluating the link between BP and end-organ damage in children are needed to establish a conclusion and properly direct clinical care. In addition, large-scale longitudinal studies in children are needed to determine health risks at each BP level. This is necessary for the development of outcome-based hypertension thresholds.

1.2 Thesis hypotheses and aims

The hypotheses of my thesis are as follows:

1. Elevated BP is associated with a number of physical, lifestyle, and hereditary factors in children, particularly adiposity and adiposity-related measures.

2. Throughout childhood, adiposity remains a significant predictor of elevated BP.
3. Elevated BP and adiposity are associated with increased LVMI and cIMT in adolescents.

To address these hypotheses, my thesis has the following aims:

1. Determine the factors associated with elevated BP in early and late childhood.
2. Determine if BMI z-score is a significant predictor of future BP levels.
3. Evaluate the individual relationships between LVMI/cIMT and 24-hour ambulatory BP monitoring measures (daytime, nighttime, and 24-hour BP, and nighttime dipping) and adiposity measures (BMI z-score and LBM) in adolescents.

1.3 Outline of thesis

Chapter 2 is a review of the literature surrounding BP and hypertension in children. It covers the definition of hypertension, the methods and devices used to measure BP, the forms of end-organ damage resulting from hypertension, the normal mechanisms of BP regulation, and the most important factors known to be associated hypertension in youth. Chapter 3 is a scoping review on the relationship between BP and LVM in children and adolescents; we believed this section to be necessary, as most studies focus on the impact of adiposity on changes in the heart's geometry, and neglect discussing BP as an independent contributor. The subsequent two chapters consist of the experimental portion of my thesis work, using data from a longitudinal study of children at risk for obesity (the QUALITY study and the QUALITY-BP study). Chapter 4 is a manuscript investigating the longitudinal associations between casual BPz (BP z-score) and several factors collected from children in the QUALITY study. I determined if associations changed as the children aged, and if BMIz predicts future BPz. Chapter 5 is a manuscript evaluating associations between BP and cardiac damage measures (LVMI, cIMT). Chapter 6 summarizes my findings and outlines future research directions on hypertension and end-organ damage in youth.

Chapter 2: Background and literature review on BP and cardiovascular health in youth

2.1 Blood pressure in youth

2.1.1 Overview

Blood pressure (BP) results from the interplay of several factors, most importantly cardiac output, vascular resistance, and kidney activity. BP is tightly regulated in children and adults to

ensure adequate perfusion to the vital organs and extremities. It is controlled by mechanisms at the systemic level, by the autonomic nervous system (ANS) and the endocrine system, and at the local level, by paracrine secretions and smooth muscle contractions. These systems work in parallel, often antagonistically and redundantly, allowing for precise adjustments and rapid adaptability in the face of changing circumstances. Elevated BP and hypertension in children and adolescents may cause significant damage to the heart and kidneys, and may persist and worsen through adulthood, significantly increasing their risk for adverse cardiac events [2]. In fact, hypertension is the leading cause of premature death and disability among adults, in both well-developed and less-developed countries [3], and is associated with 10% of healthcare expenditures, amounting to an estimated total of $370 billion USD worldwide [4]. The mechanisms of BP regulation and the outcomes of elevated BP will be discussed in more detail in the ensuing sections.

2.1.2 Hypertension and associated pathologies

The average systolic and diastolic BP (SBP and DBP, respectively) in newborns is 64 mmHg and 41 mmHg, respectively. As the child develops, physiological and hormonal influences change and the BP increases. The normal BP range rises steadily during childhood and adolescence, until the age of 18, at which time normal resting SBP/DBP is defined as 120/80 mmHg. This remains the standard throughout adulthood. Hypertension in adults is defined as casual SBP and/or DBP exceeding 140/90 mmHg. These elevated levels of BP are strongly associated with cardiovascular damage [5]; the Framingham Study found that adults with hypertension were 3 times as likely to develop a stroke and or cardiac failure than normotensive adults [6]. Hypertension is also the most common risk factor for congestive heart failure [7].

Adult hypertension is believed to have its origin in youth [3]; the large-scale Bogalusa Heart Study found that nearly 50% of hypertensive adult participants had suffered from elevated childhood BP [8]. Studies have demonstrated that high BP contributes to vascular abnormalities, including chronic kidney disease, cardiovascular disease and left ventricular hypertrophy (LVH), which persist throughout the patient's lifetime [9].

In some instances, for example when normotensive patients are treated in a clinical setting, they may experience a transitory increase in casual BP values within the hypertensive range. This phenomenon may be the result of anxiety caused by being in hospital or because the

patient is intimidated by the attending medical professionals; therefore, the condition is often referred to as "white-coat hypertension" (WCH). Performance of 24-hour ambulatory blood pressure monitoring (ABPM) performed in the patient's usual setting (e.g., home) can diagnose WCH, by demonstrating that day and night BP values are in fact normal outside of the clinical setting (i.e., their "ambulatory BP" is normal). The ABPM and its advantages and limitations are discussed in depth in Chapter 2.2.2.

In children and adolescents, reported rates of WCH vary widely from 1% to 88% [10, 11]; however, research often demonstrates a higher prevalence of WCH among obese children [12]. This has led to further evaluation to consider the importance of WCH. Children with WCH have been found to have left ventricular mass index (LVMI) values that lie between those of normotensive and sustained hypertensive children [13, 14], and preliminary evidence suggests that those with WCH are at increased risk to progress to sustained hypertension [13]. This supports the theory that WCH is an intermediate state that may develop into persistent hypertension.

In contrast, if a patient has normal casual BP (BP measured in office) but has ambulatory hypertension (on ABPM), they are said to have "masked hypertension". Lurbe et al. found that masked hypertension occurred in approximately 10% of children and adolescents, and that its presence predisposed patients to develop sustained hypertension and left ventricular hypertrophy (LVH), an accepted measure of cardiovascular risk in youth [10].

Normally, BP drops during sleep because of the absence of daytime stressors and because of diurnal changes in the endocrine and autonomic nervous systems. Blunted nighttime BP dipping (or lack of nighttime dipping) is considered a third form of hypertension. What constitutes a normal magnitude of BP dipping in children is not clear, so the adult definition for non-dipping (<10% drop in SBP and DBP at night) is commonly used [15]. Using this definition, up to 30% of children are "non-dippers" [15]. In children with hypertension, non-dipping status is associated with elevated LVMI [16], and in normotensive adults, it contributes to higher LVMI and cardiac remodeling [17]. Non-dipping has not been shown to contribute to renal damage in adults [17].

2.2 BP-measurement methods

2.2.1 Casual BP measurement techniques

Two methods of measuring casual BP are currently practiced. The auscultatory method is administered by a healthcare professional and requires only a BP cuff and a stethoscope. By listening for the appearance and subsequent disappearance of Korotkoff sounds, caused by turbulent blood flow through a semi-occluded blood vessel, the administrator can determine the patient's SBP and DBP.

The other method is oscillometry, which is performed automatically by an electronic machine. In contrast to the previous method, the oscillometric device alters the cuff pressure, and records the minute artery wall oscillations. The collected data is then fed into an algorithm, and the patient's SBP and DBP are calculated.

The oscillometric device is more widely used in clinical settings, for both patient care and clinical research, for a number of reasons: first, the automated machine allows anyone to use it, without the need for trained healthcare professionals. Second, in theory, the device will have a lower inter-measure variability, and less bias towards previously collected measures. Third, the integrity of the oscillometric data collection is not influenced by outside noises, present in busy clinical or intensive care centers [18]. The main drawbacks with respect to the oscillometric method are that the machines are expensive and the accuracy of the various oscillometric devices have not all been validated in children [18].

In general, when the two methods are compared using the same patient population, they yield significantly different results [19]. Because each oscillometric device model is different, it is difficult to assess the objective accuracy of the oscillometry method as a whole, let alone to compare it to auscultation. However, one study that compared the two methods to direct radial artery BP measurement in children found that the oscillatory device that was used gave similar results, while the auscultatory measurements were not nearly as satisfactory [20].

The National High Blood Pressure Education Program in the United States recommends auscultation as the preferred method of BP measurement in children and adolescents [21]. The European Society of Hypertension also recommends auscultation over oscillometry, citing the latter method's heterogeneity and lack of thorough testing [22]. The Canadian guidelines are less definitive, allowing for the use of either method, but with auscultation to be used to validate abnormal oscillometric values [23].

2.2.2 Ambulatory BP monitoring

A more recent development has revolutionized the field of BP measurement. In 1962, the first semiautomatic ambulatory blood pressure monitor (ABPM) was put to use by a group at the University of California, San Francisco [24]. The device consisted of an arm cuff worn by the patient and a portable BP recorder Worn over the course of several hours as the patient went about his daily life, the cuff inflated at regular intervals, and the BP was determined and stored in the recorder for later analysis. Since then the device has been modernized[1], but the fundamental idea, and its advantages over the casual methods have remained the same: while previously, BP was only measured in the hospital, a setting which can artificially raise or lower the patient's BP (white-coat hypertension and masked hypertension, respectively), the ABPM takes multiple recordings throughout the patient's day generating a true reflection of his BP. The ABPM also collects otherwise inaccessible information, including nighttime BP and BP load (the percentage of BP readings > 95th percentile).

2.2.3 ABPM strengths and limitations

Due to its numerous and frequent recordings (often >30), ABPM-measured BP allows for the detection of smaller changes in BP. Casual BP, on the other hand, relies on a minimum of only 3 measures to diagnose hypertension, and does not allow for stratification of high BP into specific abnormalities.

As mentioned, the ABPM also records BP load (percentage of values that are above threshold values of hypertension). In adults, BP load has been found to be more predictive of end-organ damage than mean BP [25]. High BP load in pre-hypertensive and hypertensive children accelerates renal damage, although it does not directly contribute to LVH [26, 27]. The thresholds and loads for each type of hypertension are listed in Table 1.

[1] When first devised, the portable BP recorder included a microphone, to measure the SBP and DBP using the Korotkoff sounds. Today, the device is computerized and calculates BP using an oscillometric algorithm.

Table 1: American Heart Association staging of casual and ambulatory BP in children

Classification	Casual BP	Mean Ambulatory SBP and/or DBP (day, night, or 24hr)	SBP and/or DBP load (%) (day, night, or 24hr)
Normal BP	<90th %tile	<95th %tile	<25
White coat HTN	≥95th %tile	<95th %tile	<25
Prehypertension	≥90th %tile or >120/80mmHg	<95th %tile	≥25
Masked hypertension	<95th %tile	>95th %tile	≥25
Ambulatory HTN	>95th %tile	>95th %tile	≥25-50
Severe ambulatory HTN	>95th %tile	>95th %tile	>50
Other:			
Non-BP dipping	([mean daytime BP] – [mean nighttime BP])/mean daytime BP x 100 <10%		

Figure 2. *The BP percentile thresholds and loads for each form of hypertension. Casual BP values based on National High BP Education Program Task Force normative data [21]. ABP and BP load values based on the American Heart Associations normative data [28].*

ABPM data is evaluated in three ways: daytime values alone, nighttime values alone, and the cumulative 24-hour values. Because BP normally dips during the night, the hypertension thresholds are different for each of the three periods of time examined. These thresholds were developed using the data from a large-scale study of normotensive and hypertensive adults [29]: they define hypertension as >135/85 mmHg for 24-hour ABPM, >140/90 mmHg for daytime ABPM, and >125/75 mmHg for nighttime ABPM. In children, the hypertension threshold is the 95th percentile for the respective period of time being considered. Because of the mounting evidence that BP load is significant in influencing end-organ damage, it is now being considered in conjunction with mean BP to determine the need for further tests or treatment.

Increasingly, ABPM is being used in clinical and research settings. The American Heart Association recommends the use of ABPM in the diagnosis and confirmation of hypertension in children, and for the measurement of BP variability [30].

In our own research studies, we have observed the few, but important, limitations of ABPM, particularly in its use in pediatric populations. Often, the cuff is uncomfortable, so that when inflating, the child has a tendency to move around. Excessive motion may invalidate the ABPM's reading, and if there are too few valid readings, the patient data may not be eligible for inclusion in the analyses (in order to be used, ≥75% of the total readings must be valid). The

discomfort caused by the cuff also affects the patient's ability to sleep, reducing the ABPM's potential to capture nighttime readings. Finally, it is at the discretion of the patient what medications he takes, what caffeine- and sugar-rich foods he consumes, and what physically or emotional activities he engages in; all of these factors can affect the BP readings, and while the patient is encouraged to fill out a form indicating whether these factors were present, often, they are not taken into consideration at the time of analysis.

2.2.4 Associations between ABPM values and left ventricular mass (LVM) & carotid intima media thickness (cIMT)

ABPM measures as a whole are more strongly associated with LVM and cIMT (two accepted measures of cardiovascular health in youth) than is casual BP [31, 32]. Awake BP variability, a measure obtainable only using ABPM, is associated with albuminuria, increased LVMI, and thickened cIMT [33, 34].

Nocturnal BP is a significant risk factor for cardiovascular-related mortality and morbidity, in both hypertensive and general adult populations. Elevated nocturnal BP – in other words, an inadequate reduction of nocturnal BP (non-dipping) – is associated with central aortic stiffness in patients with hypertension [35]. Microalbuminuria (a measure of renal disease) is also more common in non-dippers [36]. Alternatively, "extreme dipping", characterized by a decrease in nocturnal BP by >20%, is associated with nervous tissue damage and myocardial ischemia [36].

2.3 Outcomes of hypertension

2.3.1 Cardiac damage

In children and adolescents, LVH is the most common form of end-organ damage resulting from elevated BP, occurring in more than 30% of children with hypertension [27]. However, the role of BP in the manifestation of LVH is unclear, as are the mediating effects of factors such as adiposity, race, and genetics. This topic is explored in depth in Chapter 3.

Elevated BP can also result in an increased carotid intima medial thickness (cIMT) in youth [37]. Stabouli et al. have determined that obese children are more at risk for developing increased cIMT in childhood [12]. [12]. In normal children, cIMT is around 400 μm, and at cIMT levels over 900 μm, children have increased risk of future atherosclerosis [38, 39].

However, studies have found that childhood obesity is not significantly associated with cIMT in adulthood [40].

In adults, cIMT has emerged as a marker of early atherosclerosis and a harbinger of serious cardiovascular pathologies. Although elevated cIMT is predictive of cardiovascular events, accounting for cIMT did not improve risk stratification in individuals [41, 42].

2.3.2 Renal damage

Kidney function is most accurately measured by determining the glomerular filtration rate (GFR). The gold standard method of measuring a patient's GFR is to inject him/her with an inert compound that is neither secreted nor reabsorbed by the kidney or other organ. By measuring the concentrations of said compound in the blood and the urine, the GFR (renal clearance of the substance) can be calculated. Because gold standard GFR measurement is cumbersome and time consuming, a surrogate marker of GFR is most commonly used: the measurement of serum creatinine (SCr) concentrations reflects GFR, since it is filtered by the kidney and reflects renal clearance. However, SCr concentrations may be influenced by several factors, such as age, muscle mass, and diet, independent of renal function [43]. More recently, Cystatin C is gaining favor as an endogenous measure of GFR. Cystatin C is a cysteine proteinase inhibitor that is synthesized by all nucleated cells. Cystatin C is cleared from the body by glomerular filtration, but is not secreted by the kidney. A recent meta-analysis showed that Cystatin C is a superior method of estimating of GFR, as compared to SCr [43].

2.4 BP regulation

Before discussing the lifestyle and environmental factors that contribute to hypertension, it is important to understand how BP is normally regulated. This section is a brief overview of the central, renal and hormonal physiology of BP regulation.

2.4.1 Autonomic nervous system (ANS)-mediated BP regulation and associated pathologies

Although the kidneys are considered the primary regulators of BP, the ANS – responsible for causing "neurogenic" hypertension – plays a vital part in the maintenance of normal BP. Within the ANS, the sympathetic and parasympathetic branches have roles in both acute and chronic control of circulation; the ANS can quickly adjust BP according to behavioural, emotional, and physiological stresses, and is responsible for keeping BP at a given set point in the long-run [44].

The parasympathetic arm innervates the heart via the vagal nerve, while the sympathetic arm is more broadly regulatory, innervating the heart, blood vessels, kidneys, and adrenal medulla [44].

Baroreceptors are mechanoreceptors that detect the stretching or collapsing of arterial walls indicative of changes in the arterial BP. In response, in what is known as the baroreflex, baroreceptors transmit afferent signals to the brain in order to return the mean arterial pressure (MAP) to normal levels. Baroreceptors exist in two variants: high-pressure arterial baroreceptors and low-pressure baroreceptors, which differ based on their response stimuli. Low-pressure baroreceptors, or volume receptors, are located in large systemic and pulmonary veins and the chambers of the heart, most importantly the right and left atria, and are triggered in instances of inadequate blood volume or blood pressure. Of note, in response to increased central venous pressure, the volume receptors in the left atrium generate a reflex tachycardia. In contrast, high-pressure arterial baroreceptors, as their name suggests, are activated when BP exceeds a defined threshold. The most influential arterial baroreceptors are located in the carotid sinus and aortic arch, and only this type of baroreceptors can rapidly regulate MAP [45].

Changes in BP trigger a cascade of events. If, for instance, there is a substantial decrease in atrial filling, volume baroreceptors will transmit signals to the brain. As a result, venous smooth muscle tone will increase and antidiuretic hormone (ADH) will be released from the neurohypophysis [46]. Because of this dual pathway of regulation, it has been suggested that sympathetic efferents are responsible for both short-term (changes in resistance) and long-term (changes in blood volume) BP alterations.

Heart rate (HR) is controlled by the sinoatrial (SA) node, located in the right atrium. Traditional knowledge has held that changes in HR are the result of reciprocal changes in both sympathetic and parasympathetic tone [47]. However, this has been called into question by early experiments which have found that sympathetic and parasympathetic systems are independently responsible for BP elevations and reductions, respectively [48]. Others have shown that the sympathetic and parasympathetic systems can interact indirectly, with vagal (parasympathetic) nerve activity opposing heightened sympathetic tone [49].

For long-term regulation, the function of the ANS varies by gender and age. There is also inter-individual variation. Baseline muscle sympathetic nerve activity (MSNA), the primary index of sympathetic activity, can differ between normotensive individuals by as much as 5- to 10-fold [50]. In adults, there may also be gender variability. In men, MSNA is directly

proportional to total peripheral resistance and inversely proportional to cardiac output and adrenergic sensitivity, while in women, those relationships have not been proven. In older men and women, the association between MSNA level and BP is stronger, suggesting that reproductive hormones may impact on the relationship. This may explain the increased incidence of hypertension in post-menopausal women [50]. In addition, there is mounting evidence that the sympathetic system exerts its long-term influence via renal sympathetic innervation and controls the release of adrenergic hormones that regulate BP in the long-term [51].

Baseline activity of barosensitive nerves is thought by some to be the most determinative factor for controlling long-term BP levels, and is synchronized with heart rate and respiration [44]. While minute-to-minute, BP varies to accommodate stress and behaviours, under normal conditions, long-term stability is maintained. Many studies have reported that baroreceptors are involved in stabilizing daily BP; in one elucidatory experiment, sinoaortic baroreceptor-denervated dogs exhibited marked BP lability over a 96-minute period [52]. However, the importance of baroreceptors in signalling long-term hypertension has been repudiated for three reasons: first, the ability of the nervous system to maintain regular BP is limited, and it alone cannot possibly be responsible for maintaining BP within such a limited range. Second, arterial baroreceptors undergo rapid adaptation, so it is unlikely that they are involved in long-term regulation [45]. Third, destruction of the baroreceptors does not greatly affect MAP levels [53].

Increased sympathetic tone, responsible for the fight-or-flight response, results in elevated cardiac output and HR. Early hypertension is characterized by an increase in cardiac output but normal peripheral resistance, termed the “hyperkinetic state” [54]. It is possible that this dissociation is the result of sympathetic hyperactivation, which increases both cardiac output and ß-adrenergic sensitivity, blocking the normal compensatory vasodilatory response in blood vessels [50]. This theory was supported by the presence of increased levels of catecholamines in borderline hypertensive patients, suggesting rapid and incessant firing of the nervous system [54]. Denervation of sympathetic afferents to the heart abolishes this state [55]. These patients have also been shown to have decreased parasympathetic activity. Because both branches of the ANS are deregulated, this suggests that the aetiology of the hypertension lies in the CNS [55].

Hypertension maintains itself: exposure to elevated sympathetic activation for long periods of time decreases β-adrenergic sensitivity in the heart, and prolonged elevated BP causes hypertrophy of the vascular wall. Under these circumstances, at rest, the blood vessel lumen is

decreased, and during vasoconstriction, it decreases to an even greater degree. In addition, vascular responsiveness to α- and β-androgens may be elevated [55]. As hypertension advances, sympathetic hyperactivity is less evident, i.e., as the vascular wall thickens, it takes less sympathetic activity to achieve the same heightened BP [55]. It is postulated that this is due to increased sensitivity and responsiveness of the target organs to sympathetic drive [55].

2.4.2 Kidney-mediated BP regulation, the renin-angiotensin-aldosterone system (RAAS), and associated pathologies

The kidneys are the most important organ system for the detection and regulation of BP, through fluid homeostasis. The dominant class of sympathetic efferents – barosensitive nerves – controls renin secretion, tubular sodium absorption, and renal blood flow [44]. In 1972, Guyton and colleagues proposed the acute pressure-natriuresis relationship, by which any sodium imbalance is counterbalanced by a cascade of events that maintains the BP [56]. For instance, an increase in sodium absorption increases total extracellular fluid (ECF) volume, increasing cardiac output and BP. Over a number of hours or days, the elevated BP increases salt output, returning the ECF to its normal level. The pressure-natriuresis curve is heavily modulated by neurohormonal factors, increasing BP's resistance to change [56]. Guyton's model was revolutionary as it challenged the long-held idea that BP is controlled exclusively by cardiac output and vascular resistance.

Hormonal control of BP is localized mainly at the pituitary gland and the adrenal glands, which sit atop the kidneys. The renin-angiotensin-aldosterone system (RAAS) is responsible for systemic control of fluid and electrolyte balance, effectively maintaining BP. Recent studies have also elucidated the existence of local tissue RAAS.

There is substantial evidence that RAAS imbalances can affect BP, and that disruption of the RAAS axis is central to many, though not all, forms of hypertension [57]. In rats, spontaneous hypertension was cured after infusion of blockers of angiotensin II (Ang II), the main effector hormone of the RAAS [57]. Furthermore, when areas of the brain containing Ang II receptors were lesioned, hypertension was attenuated [57]. Trials on humans, in which patients with LVH were treated with Ang II receptor antagonists have yielded favorable results [58].

2.4.3 Angiotension II and associated pathologies

The juxtaglomerular (JG) cells of the renal afferent arterioles release renin, an aspartyl protease. In addition to the normal promoter region, the renin gene has a regulatory region, with the

potential to either inhibit or enhance its transcription. It has been proposed that vitamin D3 may play a role in the down-regulation of renin transcription, as the vitamin D3 receptor is homologous to the retinoic acid receptor, which has been shown to reduce RAAS activity, resulting in lower BP [59, 60]. The association between vitamin D3 deficiency and hypertension is discussed briefly in Chapter 2.5.1.

Renin is encoded by one gene, and renin mRNA is translated into the intermediate polypeptide, preprorenin. In the JG cell, the proprotein is cleaved and the active renin is packaged into secretory granules. While active renin is released only in response to a stimulus, prorenin is released continuously. Prorenin can itself bind to the (pro)renin receptor [61] or can be converted to active renin by extracellular enzymes [62]. Angiotensin II can also be produced directly from the angiotensinogen precursor by a number of other enzymes, including tissue plasminogen activator, cathepsin G, and tonin [58].

Many factors influence renin release, one of which is the tubuloglomerular feedback loop, which autoregulates renal blood flow. Tubular chloride concentration is an indication of excessive GFR. Chloride delivered to the macula densa interacts with the adjacent JG cells, stimulating the release of renin. Renin then induces vasoconstriction in the renal artery, reducing GFR, and bringing the sodium chloride concentration back to the set-point [59]. The sympathetic nervous system can also cause renin release via ß-adrenoreceptor-mediated cAMP production.

In the RAAS pathway, renin cleaves a short segment off of angiotensinogen, a plasma protein, to generate angiotensin I (Ang I). While most circulating angiotensinogen is produced in the liver, it is also released by the heart, kidneys, and adipose tissue [62]. Angiotensin converting enzyme (ACE) and ACE2, present in the lungs and kidneys respectively, then hydrolyze Ang I into the biologically active Ang II [63]. The effects of Ang II are immediate. Besides its effects on the heart and kidney via aldosterone release and anti-natriuresis, Ang II acts on the sympathetic nervous system, enhancing cerebral blood flow. With chronic exposure, Ang II takes on cytokine-like properties, increasing cell growth and migration, ECM deposition, and vascular remodelling [64].

Ang II has two receptors, angiotensin II type 1 receptor (AT1R) and AT2R, which have heterogeneous distribution throughout the body. AT1R is more widely expressed and is believed to be the primary mediator of Ang II's BP regulatory effects [58, 65]. AT1R is found in the vascular system, and when activated, causes vasoconstriction; in the adrenal cortex, it prompts

aldosterone release and sodium absorption; in the brain, it increases BP through the sympathetic nervous system; in the kidney, it leads to renal vasoconstriction and sodium reabsorption [65]. AT1R mediates BP regulation by increasing proliferation of vascular smooth muscle cells, coronary endothelial cells, and cardiomyocytes, implicating it in various pathologies such as left ventricular hypertrophy (LVH) [58, 66]. Activated AT1R also leads to activation of protein kinase C (PKC). PKC phosphorylates tyrosine kinase and mitogen-activated protein kinase (MAPK) and induces the release of intracellular Ca2+, increasing cardiac contraction.

Knockout experiments have shown that AT2R may oppose the effects of AT1R [58]. AT2R is present at much higher levels throughout fetal development than it is during adulthood. It is upregulated in heart failure, and skin and nervous system lesions, and appears to be involved in the control of cell proliferation and differentiation [58]. AT2R may also contribute to heart disease, given that it is found in much higher density in diseased tissue than is AT1R [66].

2.4.4 Cardiac control of BP, atrial natriuretic factor (ANF), and associated pathologies

The heart itself is an endocrine gland, producing a polypeptide hormone that is involved in the modulation of BP: atrial natriuretic factor (ANF). Secreted by atrial muscle cells, ANF interacts with the other BP-regulation mechanisms [67, 68].

ANF is released in times of elevated cardiac filling, and is important in counteracting states of volume expansion, congestive heart failure, renal failure, and hypertension [69, 70]. ANF is a diuretic, enhancing the kidney's sodium excretion and reducing arterial pressure [71]. ANF receptors have been identified in a number of tissues where the peptide exerts its effect: smooth muscle cells of the vasculature, mesangial cells of the renal glomerulus, renal papilla cells, adrenal cells, and a number of regions of the brain, including the pituitary gland [70].

In the kidneys, ANF stimulates an increase in GFR, by increasing hydrostatic pressure and the filtration coefficient. The former effect is thought to be via increased efferent arteriole resistance and decreased afferent arteriole resistance; the latter effect is poorly understood [70]. ANF also increases blood flow through the inner medulla and papilla, decreasing hypertonicity in the medullary interstitium, effectively leading to increased water excretion [70]. It is unclear whether ANF has a role in directly altering Na absorption in the tubular epithelial cells.

ANF also has vasodilatory effects, by causing (a) vasorelaxation in veins, decreasing venous return and (b) fluid outflow from blood vessels [70]. In the pituitary gland, ANF blocks

the release of aldosterone, renin, vasopressin, and other hormones. In addition ANF has also been shown to modulate the firing rates of neurons in the CNS, reducing BP [70, 72].

2.5 Factors that contribute to hypertension and LVH

Various lifestyle, genetic, and physical factors strongly contribute to the development of hypertension and LVH in youth, by interfering with the regulatory mechanisms, either directly or indirectly. Several of the most important contributing factors are discussed below.

2.5.1 Diet

The most accurate method of assessing dietary sodium intake is to conduct a 24-hour urine collection. In children and adolescents, sodium intake is significantly associated with elevated SBP. The relationship remains significant even after controlling for confounding factors [73]. A meta-analysis of 11 pediatric studies showed that for each reduction of 1g of sodium per day, SBP drops by 0.4 mm Hg, a modest but important decrease [74].

Salt intake is also related to soft drink consumption in youth. In children, sodium consumption is a major determinant of fluid consumption, and soft drinks are a significant source of both fluids and calories [75, 76]. According to one study, children who cut their salt intake in half, to recommended levels, had significantly reduced total fluid and soft drink consumption [75]. It has been found that while salt reduction is highly beneficial, calorie restriction alone has no independent effect on BP in adults [77]. This may suggest that deleterious effects of soft drinks are largely contributed by increased salt intake. Interestingly, the sensitivity of BP to sodium is modulated by weight loss, with obese individuals being at the greatest sensitivity [78].

Most studies have found a significant and independent association between LVM and urinary sodium excretion, in hypertensive patients [79]. This is expected, as sodium not only increases water retention, but it also produces an enhanced pressure response to norepinephrine and Ang II, increasing sympathetic tone [79]. However, previous studies on this topic have been inconsistent, possibly due to differences in study designs. For example, in the Framingham Heart Study, the association between LVM and urine sodium to creatinine concentration was not found to be significant [80]. The U.S., Canadian, and European Societies of Hypertension recommend limiting sodium intake as a lifestyle change to combat widespread hypertension [81-83].

Vitamin D deficiency is a common finding in patients with essential hypertension. In adults, serum vitamin D levels, were inversely related to LVM [84]. One explanation is that

vitamin D suppresses the renin-angiotensin-aldosterone pathway and is integral to the inhibition of various pro-hypertensive pathways in cardiomyocytes; when vitamin D is not present, cardiomyocytes grow uncontrollably [84]. However, in youth, activated vitamin D levels did not differ between severities of hypertension, LVMI, or cIMT [85]. This may be because another pathway was responsible, or that vitamin D is not associated with those abnormalities. It is also unclear whether the predictive properties of vitamin D were different in adults and youth because of differences in the populations, or if the organ damage measures were defined differently.

A clinical trial in normotensive adults found that a diet rich in fruits, vegetables, low-fat dairy products, and low in saturated and total fat, significantly reduced SBP and DBP [86].

2.5.2 Genetics

Familial aggregation of blood pressure between parents and children, and between siblings, has been shown in many studies [87-89]. A genetic basis for these associations has been proposed, as genetic loci for hypertension in youth have been identified [90, 91]. An Italian study found that allelic variants of RAAS genes carry a small risk of developing hypertension [92]. Early hypertension screening in genetically at-risk youth may be a viable option. Combinations of genetic and environmental factors likely impact hypertension risk; in fact, familial obesity, an important risk factor for hypertension, may play the most important role [93].

2.5.3 Medications and recreational drugs

Many medications raise BP. A literature review found that most oral NSAIDs, in particular ibuprofen, raised SBP and DBP in as little as four weeks of treatment. The mechanism of action was speculated to be NSAIDs' COX-2 inhibition, which stops the production of vasodilatory prostaglandins [94]. Certain cancer drugs have also been found to cause hypertension, primarily because they are often accompanied by the use of angiogenesis inhibitors [95].

Cocaine is a dangerous and addictive drug [96]. It is also a potent vasoconstrictor, and stimulates the sympathetic nervous system and adrenal glands [97]. Within minutes of intranasal administration, BP and heart rate increase drastically [96]. This can lead to cerebral hemorrhage, seizures, intestinal ischemia, myocardial infarction, and other fatal effects. In pregnant women, cocaine use can result in placental vasoconstriction, increasing the rate of spontaneous abortion and putting the fetus at greater risk for congenital malformations [96].

Acutely, smoked marijuana stimulates the cardiovascular system, raising heart rate by 20% to 100%, for up to 3 hours after inhalation [98, 99]. This increase, as well as an increase in cardiac output, is mediated by increased vagal and sympathetic activities [100]. However, after several days of frequent exposure, the user develops temporary tolerance to increased heart rate and BP [99]. While acute cardiovascular events following marijuana exposure have occurred, and myocardial infarctions are 4.8 times more likely immediately following use [101], marijuana is not considered to be a serious health concern for young, healthy people [99].

There are several ways to consume tobacco: smokeless tobacco (i.e. chewing tobacco or snuff) which is ingested orally or nasally; cigars and pipe tobacco, which are smoked and are mostly raw tobacco; and cigarettes, which are also smoked and contain more than 4000 added chemicals besides tobacco. In evaluating cardiovascular effects of tobacco, these distinctions are unimportant as nicotine blood levels resulting from the use of either form are similar [102], and nicotine is the principal cardioactive compound in tobacco [102, 103]. There is little consensus as to whether tobacco or cigarette users have higher or lower BP than non-smokers [102], but tobacco ingestion has negative impacts on other many organ systems [104].

Maternal smoking during pregnancy is strongly associated with low infant birth weight [105-107]. Some believe that this relationship is a result of shared lifestyle factors by the mother and child, and has little to do with maternal smoking [108], although this view is unpopular among scientists. A genotyping study found that the adverse effects of maternal smoking were modified depending on the maternal CYP1A1 and GSTT1 genotypes, both of which encode enzyme activity [107].

Endogenous opiate peptides inhibit sympathetic activity by acting on brain areas involved in BP control [109]. Abrupt cessation of opiate use can cause hypertension [110].

Two small studies, involving fewer than 10 participants, evaluated the physiological reactions following oral administration of LSD-25 or psilocybin. Both drugs resulted in a transient increase in BP [98, 111]. Methamphetamines and amphetamines increase BP by raising the concentrations of catecholamines in the central and peripheral nervous systems [112]. Hypertension is both an acute and, with prolonged use, a chronic effect of methamphetamine

use, and is commonly observed among methamphetamine users admitted to emergency rooms [112]. Its use is also associated with a host of other cardiovascular problems [112].

Alcohol is a common environmental factor linked to hypertension. Many large-scale studies have found that in adult men and women, alcohol consumption greater than 2 drinks per day was associated with higher SBP and DBP [113, 114]. Interestingly, participants who had engaged in heavy drinking (≥3 drinks/day) in the remote past had lower BP. In adolescents, alcohol intake was positively, though weakly, associated with BP levels [115]. The mechanisms of alcohol's influence on BP are unknown, but evidence suggest that it may be via sympathoactivation [114] or because of its association with cigarette smoking, coffee consumption, and other lifestyle and physiological factors that increase BP [113, 114]. High alcohol consumption has also been shown to be independently associated with LVH [114].

Coffee, the most commonly used stimulant, is associated with higher BP [116]. The pressor effects were found to be short-term, as in participants of studies lasting more than 2 weeks, the cardiovascular system appeared to adapt to the effects of coffee [116]. Plasma caffeine levels peak 60 minutes following oral coffee consumption, which coincides with the peak BP [117]. Caffeine exerts its effects through increase in excretion of catecholamines and an increase in renin activity [117]. Although caffeine does not raise BP to concerning levels in normotensive people, the effects of caffeine may be problematic in hypertensive people. Other beverages and foods, such as energy drinks, teas, and chocolate, also contain caffeine, so their consumption and may exert similar effects.

Table 2 lists the prevalence of common drug use among American adolescents, and the effects that the drugs have on BP.

Table 2: The prevalence of drug use among American adults and adolescents, and their effects on BP.

Drug	**Prevalence of use in U.S. aged 12+ (%, 2015)***	**Prevalence of use in U.S. aged 12-17 (%, 2015)***	**Effect on BP**
Cocaine	1.9	0.6	Significant transient increase in BP

Marijuana	13.5	12.6	Significant increase in BP, maintained for > 3hrs
Cigarettes	23.1	8.1	Unresolved
Smokeless Tobacco & Cigars	13.8	8.6	
Heroin	0.3	0.1	Transient decrease in BP
Hallucinogens	1.8	2.1	Transient increase in BP
Methamphetamines/ Amphetamines	0.6	0.2	Acute and chronic elevation of BP
Binge Alcohol Use†	24.9	5.8	Small increase in BP

Data taken from the United States Substance Abuse and Mental Health Services Administration (SAMHSA): https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.pdf and the Federal Drug Administration (FDA): https://www.fda.gov/downloads/aboutfda/centersoffices/officeoffoods/cfsan/cfsanfoiaelectronicreadingroom/ucm333191.pdf
For Canadian figures, see: http://www.hc-sc.gc.ca/hc-ps/drugs-drogues/stat/_2011/summary-sommaire-eng.php
* Prevalence for drug use in past year
† Prevalence for past month only

2.5.4 Social stress and physical activity

Psychological stress leads to acute rise in BP [118], and in general, girls have more emotional stress than boys [119]. There is also a relationship between large acute BP responses to emotional stimuli, and long-term development of hypertension [120, 121]. Furthermore, in adults, stress type influences the magnitude of BP change; SBP is more elevated and the elevation is sustained for longer, when people are allowed partial or full control of their stressful situation, as opposed to when they are not [122]. High work stress is associated with higher SBP levels [123]. This is more pronounced when there is an imbalance between a person's work effort and his perceived reward [123]. A postulated mechanism is that mental stress enhances sympathetic activity, which acts to retain sodium and increase blood volume [124], and decreases vagal tone [123]. Sympathetic activity is further enhanced in people with known risks for hypertension [121, 124].

Besides its role in reducing adiposity and related factors, there is little evidence to suggest that physical activity independently reduces BP in school-aged children [125]. However, many studies have reported that young adults engaging in vigorous physical activity are

protected against future hypertension [126]. The effect is greater for adolescents engaging in team sports, who receive social support and adulation from their peers [119, 127]. Frequent physical activity is strongly associated with psychological well-being in youth, although the many potential confounding factors make establishing a causative link tenuous [127].

2.5.5 Sleep and obstructive sleep apnea

Sleep disruption has been shown to increase BP in normotensive and hypertensive adults [128], independent of adiposity [129]. A study found that adolescents with poor sleep quality were 3.5 times more likely to be pre-hypertensive or hypertensive. Moreover, those with short sleep duration were 2.5 times more likely [129]. In addition, long-term administration of melatonin has been shown to reduce BP [128]. The cause of these associations is unknown.

Obstructive sleep apnea (OSA) occurs when the upper respiratory pathway is partially or fully obstructed, causing sleep disruptions. Many studies have shown an association between obesity and OSA [130]. In children, OSA and sleep-disordered breathing increase BP and BP variability and reduce nocturnal BP dipping [131-133]. On arousal, normotensive people experience a morning BP surge, mediated mostly by the sympathetic system. OSA, characterized by irregular wakefulness, could thus cause hypertension by sympathetic hyperactivation [133]. Increased sleep debt is also associated with elevated sympathetic tone and cortisol release [134].

OSA is associated with cardiac remodeling and an 11-fold increase in the risk for LVH [135]. The resulting LVH is not necessarily a result of the elevated BP; studies have shown that episodic hypoxia and certain humoral factors can each independently lead to LVH [135].

2.5.6 Adiposity

Adiposity is one of the most important risk factors for elevated BP and hypertension in youth [136]. Several methods are used to assess adiposity in children. BMI is most common because its calculation requires only the patient's height and weight. However, BMI percentiles, the measure used to designate obesity, may be insufficient and insensitive in predicting end-organ damage [137]. An alternative is BMIcv: sex-, age-, and race-specific thresholds for cardiovascular complications. Although BMIcv represents relatively low BMI percentiles, it was found to be more sensitive for predicting LVH than the more traditional 85th or 95th percentile-based BMI definitions [137].

Other adiposity measures in children are visceral fat and waist circumference, both were found to be more powerful predictors of LVH than BMI [138]. Daniels et al. found that lean body mass was a much stronger predictor of LVM than was crude fat mass, although the two were strongly correlated [139]. Fat mass percentage and abdominal fat mass were associated with cardiovascular risk factors, independent of BMI, with visceral abdominal and subcutaneous abdominal fat mass having similar strengths of association, and with fat mass percentage being most strongly linked to LVM [140]. Adolescents with high central adiposity have a higher prevalence of hypertension [141]. Adiposity measures worsened from the normotensive to pre-hypertensive to hypertensive states [142]. Maximova et al. showed that several common adiposity indicators, including BMI, waist circumference, and fat mass percentage are strongly correlated [143]. They recommend using BMI to measure adiposity, because of its simple calculation and wide applicability.

Adolescent obesity is associated with casual and ABPM-measured BP. [144]. Obese children have a 3.5-fold increased likelihood of having hypertension than non-obese children [145]. Frighteningly, 80% of obese children will go on to become obese adults [145].

Sorof et al. found that as BMI percentile increased, so did the proportion of children with hypertension [146]. Mechanisms are unclear, but adiposity is thought to disturb autonomic BP regulation [136, 146]. Obesity is determined by a combination of environmental and genetic factors, ultimately resulting in increased sympathetic activity, increased sodium retention and elevated plasma renin and aldosterone [145]. Adiposity is often the most modifiable risk factor and represents a viable target for combatting high BP. A study found that adolescents in the 75th percentile of weight, who went through a rigorous 20-week weight loss program had lower post-program SBP and DBP [147]. The weight loss program also influenced the degree to which BP dropped, with a combination of exercise and caloric restriction producing the best results [147].

2.5.7 Premature birth

A systematic review showed that low birth weight is associated with higher BP, which may be related to lower endowed renal mass [148].

2.6 Current interventions to treat hypertension

A review of over 100 trials designed to reduce BP in adults revealed that the most successful strategies were (i) controlled diet (ii) regular exercise (iii) restricted salt and (iv) restricted alcohol. Other therapies and obscure vitamin supplement-based methods were unsuccessful [149]. If, once lifestyle modifications are implemented, BP is not sufficiently lowered, or if the patient has additional complicating factors, pharmacotherapy can be initiated. Guidelines propose that ACE inhibitors, angiotensin receptor blockers, calcium channel antagonists, β-receptor antagonists, and diuretics are recognized BP-lowering medications [150]. The specific medication used depends on the severity of hypertension in the patient, the patient's age and physical condition, and the practices at the specific hospital where the patient is being treated.

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Chapter 3: The associations between LVMI and adiposity, blood pressure, and other physical and lifestyle factors: a scoping review

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3.1 Foreword to this manuscript chapter

Since one of the foci of my thesis is to examine the association between BP and LVM in youth, I performed a detailed literature review on this topic. In reviewing the literature, I identified that few knowledge synthesis reviews have addressed the issues surrounding how to measure or estimate LVM in children and the strong interplay between adiposity, BP and LVM. This scoping review (previously referred to as a "narrative" review) provides an overview of how to express LVM in youth and associated controversies, in addition to factors affecting the BP-LVM relation, including adiposity. Some of the information presented in this manuscript may be somewhat redundant with information provided in the thesis background above. Dr. Bethany Foster is a colleague of Dr. Zappitelli's at the MCH who has expertise on estimating LVM in children and Dr. Jean-Luc Bigras is a cardiologist at Hôpital Sainte-Justine. The draft of this manuscript will be reviewed by all authors and submitted to Pediatric Cardiology (thus, a reading audience comprised mostly of pediatric cardiologists or individuals performing research on hypertension or caring for children with hypertension). Upon contacting the editor of this journal, they confirmed interest in considering a review article on this topic for publication.

3.2 Manuscript 1

Abstract

Excessively elevated left ventricular mass (LVM), called left ventricular hypertrophy (LVH) is one of the most common cardiac abnormalities in youth, and is known to be associated with increased adiposity. However, the relationship between adiposity and LVM is complex, with many factors having roles in the development of cardiac rearrangement. In addition, adiposity is associated with elevated blood pressure (BP), although few studies have focused on the direct

relationship between BP and LVM. Those studies that have, have yielded ambiguous results. Moreover, it is difficult to make broad statements regarding LVH, because LVH is a heterogeneous disorder, with different forms arising under different conditions; in turn, each form is associated with a different risk profile. The aims of this review are to discuss what is known about the BP-LVM relationship, the methods to measure LVM and BP and to scale LVM, and the adiposity-LVM relationship and its mediating factors. Current treatment recommendations are to first address obvious lifestyle factors that may be contributing to the hypertension or LVH, and failing sufficient improvement, to begin drug treatment. Future research should resolve two problems; firstly, researchers must determine at what levels of BP, children begin to show signs of end-organ damage. Secondly, BP screening in children should be closely evaluated to determine if it is sufficiently efficacious and efficient for widespread implementation.

Introduction

Increased left ventricular mass (LVM) is known to be associated with increased adiposity [1-3]; increased adiposity is in turn associated with elevated blood pressure (BP) [4, 5]. Yet, there are few studies that evaluate the independent relationship between BP and LVM. Furthermore, information regarding confounding factors that contribute to high LVM among obese individuals is sparse. This review reviews data regarding the link between BP and LVM. Several factors affecting this relation will be addressed separately, with a discussion on whether they are associated with elevated LVM in normal or obese youth.

The relationship between BP and LVM

Hypertension in children is defined as systolic blood pressure (SBP) or diastolic blood pressure (DBP) greater than or equal to the 95th percentile, as per age, sex and height-specific normative values [6]. In 2010, the prevalence of hypertension in youth as per the definition above, was estimated at 3-4% in the United States, and 1% in Canada [7, 8]; in obese children, the prevalence was considerably higher, at 15-30% [9, 10]. Obese children also have a 3-fold greater risk of developing hypertension than normotensive children [5]. With the increase in childhood obesity and diabetes [11], and the drop in quality of diet [12], there is concern that hypertension incidence in children may also rise.

In youth, an increase in BP levels is detrimental to cardiac health. Elevated BP is associated with cardiac abnormalities, including left ventricular hypertrophy (LVH). LVH is an increase in left ventricular mass (LVM) and a distortion of normal left ventricular geometry [13]. However, the relationship between BP and LVM in children has yet to be fully characterized and there is still controversy as to the magnitude with which BP is associated with LVM and how other factors play a role in that relationship. Although elevated SBP and DBP have been shown to be associated with higher LVM in children and adolescents, SBP has been shown to be more strongly correlated with LVM [14]. Going even further, McNiece et al. found that SBP, but not DBP, was significantly associated with LVM in children [15]. Although many studies support that elevated BP is associated with elevated LVM, there is substantial evidence that BP may not be the only causative factor and that the BP-LVM relation may be significantly modified by other factors. For example, several studies have shown that the effects of elevated BP on LV geometry are amplified in patients among some racial minority groups [16, 17] and among patients with certain comorbidities, such as chronic kidney disease [18]. It is unclear whether gender differences have a modulatory role in the effect of hypertension on LVH [17, 19, 20].

LVH is a progressive disorder, fueled by years of cardiac stress and subclinical changes in cardiac structure. Beginning in childhood, excess adiposity and other risk factors lead to cumulative increases in LVM throughout adolescence and into adulthood [21, 22]. Adult LVH has been shown to increase the risk for cardiac events [23, 24], with the rate of cardiovascular disease nearly double in those with LVH [23]. It has been proposed that LVH is the result of the following pathway: as arterial BP rises in the patient, so does the afterload against which the left heart must pump. Therefore, the left ventricle must do more work in order to maintain cardiac output, thereby causing the cardiac tissue to hypertrophy [25]. Yet, in obese children and adolescents, pathological changes to the left ventricle may precede the presence of hypertension [26, 27]. Similarly, independent studies, which have included patients of several ethnicities, found that at the time of hypertension diagnosis, up to 41% of children or adolescents, some of whom were obese, presented with LVH [15, 28, 29]. Even among children with mild, untreated hypertension, 34% were found to have LVH [30]. The Strong Heart Study has indicated that LVM values can be used to predict future hypertension development in adults with normal BP levels [31, 32]; for each standard deviation increase in LVMI, the probability of incident hypertension increases by 36% [31].

The prevalence of hypertension in adulthood puts a heavy burden on the United States' healthcare system. With 80 million U.S. adults – 1 in 3 people – suffering from hypertension, the direct and indirect costs amount to more than $46 billion [33]. In light of these findings, and considering the effects on both the individual and society, BP screening in children and monitoring LVM in children with high BP, is imperative, especially in patients with increased cardiovascular risk.

Defining LVMI: issues of scaling to body size

Because LVM is strongly related to body size, LVM is often standardized (or divided) by body surface area or by height, resulting in left ventricular mass index (LVMI). The goal of standardization is to be able to interpret and express LVM across the size spectrum throughout childhood. The National High Blood Pressure Education Program Working Group recommends using height raised to an allometric power (usually $m^{2.7}$) to index LVM [6], a method developed by de Simone et al. and most commonly used in clinical and research practice [34]. In the past two decades, there has been renewed debate regarding which scaling factor should be used for LVM. Using the method outlined by de Simone et al., LVH is defined as LVMI > 51 $g/m^{2.7}$ in adults, and > 38.5 $g/m^{2.7}$ in children. The proposed cut-off value (38.5 $g/m^{2.7}$) is the same for both sexes, and across all ages, two factors which may influence LVM [35], particularly throughout puberty [36]. Failure to account for these potentially confounding factors results in a simple, though intrinsically inaccurate, method for expressing LVM. Misclassifying patients as having LVH when they do not, may have negative patient and system effects including inappropriate diagnosis and anxiety-provoking unneeded investigations, as well as impact on increased health care costs for investigations. Misclassifying patients as not having LVH when they in fact do, has obvious negative effects related to inappropriate lack of follow-up, diagnosis and treatment.

Percentile normative curves for LVMI ($height^{2.7}$) have been developed, using retrospective data from more than 2,000 non-obese children who ranged in age from 0-18 years old [37]. All of the children were determined by echocardiography to have normal cardiac anatomy and the percentiles were created separately for boys and girls. LVH in children is here defined as LVMI greater than or equal to the 95^{th} or 99^{th} age- and gender-specific percentiles. This definition, therefore, takes into account the changing physiology and anatomy of the heart

as the patient progresses through childhood and the anatomical differences that exist between genders, and may have prognostic value for cardiovascular disease [38].

The use of LVMI percentiles has value, however, scaling LVM by height has been shown to lack robustness. It was acknowledged from the time of its conception that scaling by height does not fully account for differences in adiposity across individuals [34]. The resulting LVMI scaled by height increases with decreasing body size (i.e., height) [39], demonstrating that its intended normalization effect is lacking (i.e., scaling by height does not remove the variation in LVM due to body size). In fact, scaling by height resulted in LVH misclassification in nearly 20% of at-risk children and a false-positive rate of 21% [40].

Despite the issues associated with scaling LVM to height described above, height has proven to be a more accurate scaling method in predicting cardiovascular disease than body surface area is; body surface area fails to account for variability in height [41]. Lean body mass (LBM) has been proposed as a preferred alternative to scale LVM, because it preserves the ability to assess the effect of adiposity on LVM [42]. LBM has been shown to scale better with LVM than height (e.g., scaling LVM to height is invalid in children shorter than 140cm) or body surface area (BSA) (which is too heavily dependent on weight) [40]. While LBM also increases with increasing height and weight, LVM scaled to LBM is more robust across the height and weight spectra [43, 44]. However, gold standard LBM measurement requires the use of bioelectric impedance analysis or duel-energy X-ray absorption technique, neither of which is commonly used in-clinic. In response to this obstacle, Foster et al. developed a predictive equation for LBM, using measures of height, weight, BMI, age, and population ancestry [45]. The latter variable, essentially determining if the patient has African-American ancestry, is important to account for the differences in body composition that exist between races, although its inclusion in the equation is not necessary. While Foster's equation predicted LBM very well when compared to duel-energy X-ray absorption data ($r = .97$, mean difference = 0.6%), and was validated in obese South Carolinian children and adolescents [46], it is unknown how the equation would fare in extreme or disease populations, such as for athletes and chronically ill patients [42]. Foster also created reference centiles for LVM scaled to LBM, where the LBM values used were calculated using Foster's predictive equations. The centiles are stratified by sex, and have been demonstrated to adequately account for broad differences of height and weight [40]. Although the formula-based LBM estimation used to create the centiles is limited in

some regards (versus using gold standard LBM measurement), this method eliminates sex differences and is a more valid normalizer of cardiac geometry than the other commonly used techniques [42]. Until more data is available that compare the various methods and determine which best normalizes LVM in youth, many authors maintain that height$^{2.7}$, should continue to be used to scale LVM given its wide use, ease and existing normative values.

Classifying LVH

Left ventricular hypertrophy (LVH) is traditionally subdivided into three pathologies: eccentric hypertrophy, concentric remodelling, and concentric hypertrophy. This classification has evolved based on the differential response of the cardiac muscles to different types of stress the heart undergoes in conditions like hypertension. Eccentric hypertrophy, the cardiac response to volume overload, is characterized by elevated LVMI but normal relative wall thickness (RWT)[2]. Concentric remodelling, the cardiac response to chronic *pressure or volume* overload, is normal LVMI with elevated RWT [47]. When both LVMI and RWT are elevated the patient is said to suffer from concentric hypertrophy (Figure 1). Among the LVH subtypes, concentric hypertrophy carries the greatest risk for cardiovascular events in adults [48].

[2] RWT is defined as (2 x posterior wall thickness) / (left ventricular diastolic diameter)

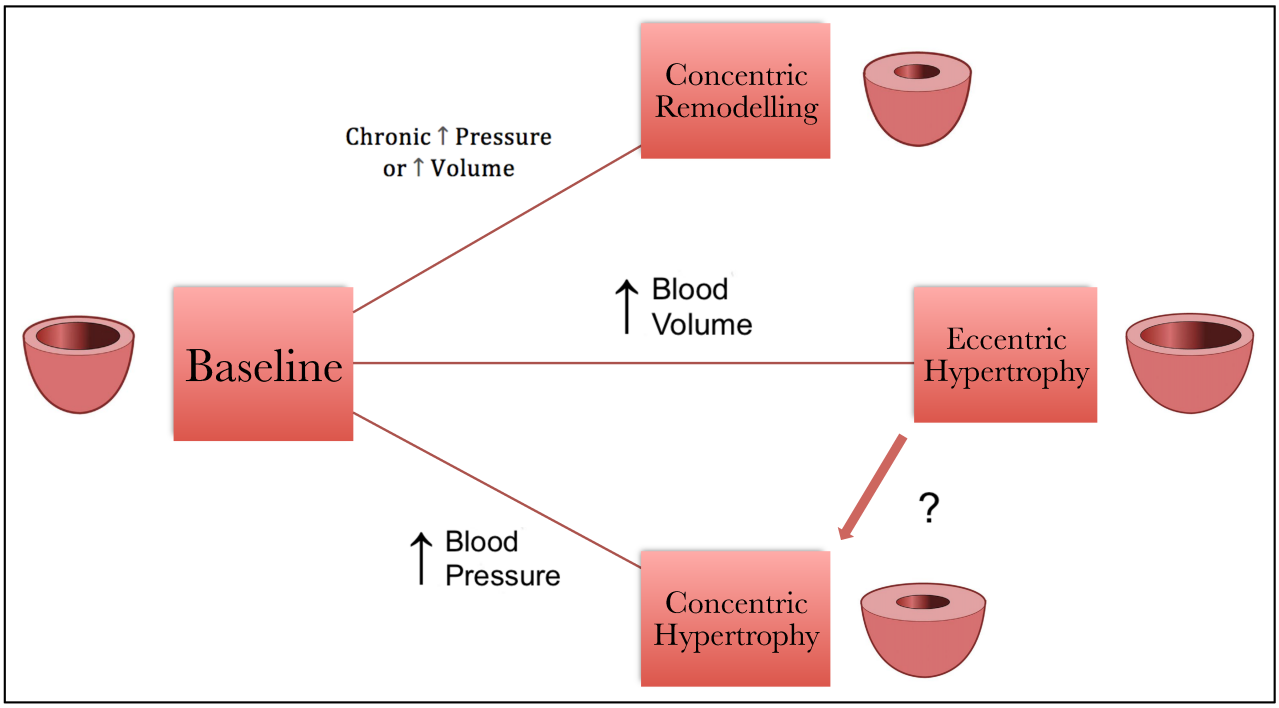

Figure 1. Flowchart showing the progression of a normal heart to the different forms of LVH under different conditions. Some believe that eccentric hypertrophy is the precursor to concentric hypertrophy. Graphics of the heart taken from Rodriguez et al., 2010 [49].

Concentric hypertrophy is more strongly associated with elevated SBP in children and adolescents, than is eccentric hypertrophy [50-52]. Numerous studies have also demonstrated associations of varying strengths between all three forms of LVH and various cardiovascular risk factors other than hypertension (including obesity [51-53], fasting glucose [52], and insulin resistance measures [51, 52]). There is uncertainty as to why the different forms of hypertrophy are associated with different factors and are associated with different risks. This may be because the two hypertrophic states, eccentric and concentric hypertrophy, could represent two points in the evolution of a single pathology. In a study on children with persistently elevated BP, Daniels et al. found that children with concentric hypertrophy had a longer history of hypertension than those with eccentric hypertrophy [44]. This supports the hypothesis that eccentric hypertrophy precedes the development of concentric hypertrophy.

It is important that the development of LVH in children and adolescents is clearly defined; by understanding the progression of the pathology, physicians will be better equipped to treat it.

While many studies indicate that elevated SBP is most strongly linked with the concentric hypertrophic condition, there is a need for further research in young cohorts, to elucidate the extent to which these entities are more or less associated with cardiovascular outcomes in youth and which require immediate intervention. For the sake of simplicity and brevity, the remainder of the review will not differentiate between the types of LVH.

Measurement of heart geometry

There is little consensus regarding the ideal method with which to detect target organ damage in children. Consideration must be given to the sensitivity and specificity, cost, and availability of each method. Cardiac MRI is generally considered to be the gold standard in detecting cardiac abnormalities, and is the benchmark against which other measures are compared [54]. However, it is a relatively expensive and laborious test to perform, and therefore is less frequently used compared to echocardiograms. The two methods most frequently compared for clinical use are echocardiography and electrocardiography (ECG). Echocardiograms use ultrasound waves used to compile images of the patient's heart, allowing for easy diagnoses and more precise characterization of cardiac pathologies such as LVH. ECGs measure the electrical activity of the patient's heart, from which pathologies can be indirectly inferred. Using one of several sets of criteria [55], LVH can be determined with varying degrees of confidence, however the extent of damage present cannot be assessed. It is generally accepted that echocardiograms provide a more reliable diagnosis of LVH than do ECGs [56, 57]. See Table 1 below for a summary of the different types of echocardiograms.

Table 1: Comparing the strengths and weaknesses of M-Mode, 2D, 3D, and TDI echocardiography

Echocardiogram Type	**"Motion-Mode" (M-Mode)**	**2-Dimensional (2D)**	**3-Dimensional (3D)**	**Tissue Doppler Imaging (TDI)**
Method	Captures motion of heart	Captures 2D image of heart	Captures 3D image of heart	Captures blood flow through heart's chambers and valves
Information Gathered	Measures size of heart structures, thickness of heart walls, and ventricular function	Measures real-time motion of heart and allows for observation of heart	Similar to 2D echocardiogram, but enhanced imaging of heart	Measures cardiac output and detects heart wall/valve abnormalities

Pros & Cons	Based on prefixed LV geometry [58]	Most commonly used echo modality; based on prefixed LV geometry [58]; results in foreshortened view of the LV [59]	Not dependent on geometric modeling, so more specific; more accurate measurement of chamber volumes [59]	Can differentiate between different types of LVH (physiological vs. pathological) [60]
Ability to Diagnose LVH	Overestimated magnitude of LVM [58]	Underestimated magnitude of LVH [58]	Very consistent with MRI results [58]	Very consistent with MRI results [61]

Studies have found that among the various types of echocardiography, M-mode echo overestimates the presence of LVM, 2D echo underestimates it, and 3D echo shows the best agreement with MRI [58]. Tissue Doppler Imaging was found to correlate very well with results from MRI exams [61].

Despite the wide availability of echocardiography, and its utility in detecting and diagnosing cardiac disease, certain cohorts most at-risk, such as those with hypertension, anemia, or kidney disease do not receive echocardiograms. One study found that only 1 in 4 adolescents with hypertension had received an echocardiogram [62]. Even more surprising, in children with end-stage renal disease, although 92% of the patients had cardiovascular risk factors, only 35% received an echocardiogram [63]. In the end-stage renal disease patients, chest x-rays, which possess poor diagnostic ability, were used almost twice as frequently as echocardiograms to diagnose cardiac disease [63]. Hypertensive patients should undergo periodic testing, but more importantly, patients with serious comorbidities should undergo echocardiograms regularly to monitor BP progression.

Factors impacting the BP-LVM relation

Adiposity

Numerous studies have shown that obesity is an independent factor, and is one of the most significant contributors, to the development of high LVM in children and adolescents [1, 52, 54]. Obese children are at much higher risk of suffering from both forms of LVH; a meta-analysis of 15 adult studies found that with increasing adiposity, cardiovascular risk increases not linearly, but exponentially, with an overall odds ratio of 4.2 for LVH in obese *vs.* non-obese individuals

[64]. When obesity is accompanied by hypertension, there is a synergistic effect that further accelerates the cardiac remodelling process [16, 26, 47, 52].

A number of factors contribute to the development of LVH in obese children and adolescents. Firstly, and perhaps most importantly, with increasing adipose tissue, the body demands greater metabolic resources, requiring a larger cardiac workload. This results in cardiac remodelling [52] – the hypertrophy of cardiomyocytes – which begins as an adaptive response, but quickly progresses into a pathological condition. Compounding this problem, is data showing that the majority of adolescents consume sugar-sweetened beverages (SSBs) [65] and sodium [66] in quantities that far surpass health recommendations. Elevated SSB consumption has been shown to increase adiposity, and heighten the risk for metabolic syndrome and diabetes [67] [68]. Meanwhile, elevated sodium intake in youth has been shown to increase fluid retention, blood volume and afterload. Each of these effects contributes to increasing the strain on the heart [69]. Neurohormonal factors can also induce myocardial hypertrophy. Obese individuals are more at risk for developing insulin resistance, which is itself an independent predictor of increased LVM [70]. Adipocytes produce leptin, which has been shown to increase the size of pediatric myocytes in vitro, and induce expression of cardiac hypertrophy markers [71]. Excess adipose tissue also activates the renin-angiotensin-aldosterone system (RAAS), inducing myocardial growth and fibrosis [72]. Finally, obese children more commonly have sleep apnea and insulin resistance, both of which can result in hyperstimulation of the sympathetic nervous system, and ultimately lead to cardiac hypertrophy [47].

Several methods are used to assess adiposity in children. BMI is most common because its calculation requires only the patient's height and weight. With the availability of population-based normative percentile values, patients can be grouped into categories depending on where they fall in the distribution. In children, overweight is defined as BMI at or above the 85th percentile, and obesity is defined as BMI at or above the 95th percentile. Others ways of measuring adiposity include abdominal fat [73], waist circumference [74], waist-to-height ratio [74] and the recently introduced BMIcv (BMI cardiovascular risk thresholds) [75], all of which are associated with cardiovascular risk. There does not seem to be a substantial difference in the association between adiposity and BP using different adiposity measures type (e.g., BMI, waist circumference), but the extent to which different ways of expressing adiposity impacts on its association with LVM is unclear.

Pediatric data have shown that when adiposity is decreased (i.e., with weight loss), even with no change in BP, LVM is significantly reduced [76]. The reduction is even more striking in obese adolescents, in which LVH prevalence was reduced from 28% to 3% in the setting of weight loss [77]. Similarly, after 6 months of anti-hypertensive treatment and decreases in BP levels, patients showed marked reduction in LVM, even with no accompanying change in BMI [78]. However, the relationship between BP and LVM is still controversial, and some studies have found that BP is only weakly associated with LVM [27, 79].

Evidently, there is a need for more research to determine if BP is a significant, independent contributor to increased LVM, or if it is only important when paired with obesity. It is clear that adiposity is a major contributor to cardiac geometrical rearrangements, but there may be other factors that augment the likelihood of developing LVH.

Prematurity & age

In contrast to some previous studies, certain groups have recently found a significant association between premature birth and LVM in adolescence, independent of adolescent weight and BP status [54, 80]. In many premature individuals, the increase in LVM is significant, translating to a 50% increase in the risk for cardiovascular events in adulthood [80]. The pathology is postulated to be because preterm infants have fewer cardiomyocytes than normal, and under increased duress, they become hypertrophied.

Age is a factor whose significance to the development of LVM in children cannot be easily measured because it is so closely correlated with other variables; these include height, weight, LBM, and BP, all of which are mediated through hormonal pathways that are most active throughout puberty.

Familial

While it is widely acknowledged that BP is influenced by hereditary components, genetic influence on LVM is unclear [81]. Normotensive offspring of hypertensive parents have greater LVM than normal, suggesting a genetic component [82]. At least one genetic polymorphism is indirectly associated with increased LVM in the context of obesity: in obese individuals, a mutation in the adiponectin gene, which encodes a protein secreted from adipose tissue to regulate glucose metabolism, is associated with elevated LVM [83].

In otherwise-healthy people, mutations in angiotensin-converting enzyme (ACE) that increase ACE activity, have been found to contribute to LVH [84]. However, these results are not universally accepted, with some studies concluding that there is no association [85, 86]; one such study noted that genetic studies are susceptible to several difficulties that make consistent replication difficult. The include inadequate control and experimental group sizes [85].

Race

Black children with hypertension are at a much higher risk of developing LVH, up to a six-fold increase over white children [87, 88]. This could be, in part, because black children are born with a greater LVM than their white counterparts [89]. However, in general, normotensive black adolescents have lower BP than white adolescents [90], so the rise in LVM may be entirely due to non-BP factors.

The observed racial difference may be because black boys have increased levels of aldosterone, compared to their white counterparts [91]. Elevated aldosterone levels increased fluid retention and LVM in black boys, but had little effect in white boys. Therefore, it may make sense for the treatment of hypertension in black adolescents to focus on reducing the activity of plasma aldosterone, via an aldosterone blocker or an Angiotensin II receptor blocker. Another study found that after adjusting for important confounders, such as obesity, black status was not independently associated with LVH [92].

A similar increase in LVH prevalence was found in Hispanic children [17], although this may also be attributed to the increased obesity and decreased diet quality among this population. The association of obesity and LVM has not been studied in depth in other minority groups.

Nutrition (vitamin D & sodium)

Vitamin D deficiency is a common finding in patients with essential hypertension, and there is evidence that vitamin D acts to suppress the RAAS and inhibit cardiomyocyte hypertrophy [93]. In adults, serum vitamin D levels were inversely related to LVM [94]. Similarly in children with chronic kidney disease, who are at risk for increased LVM, lower vitamin D levels are associated with increased LVM [95].

Studies have found a significant association between LVM and urinary sodium excretion in hypertensive patients, and in some cases sodium was an independent factor for LVM [96].

Sodium not only increases water retention; it also produces an enhanced pressure response to norepinephrine and angiotensin II, increasing sympathetic tone [96]. However, previous studies on this topic have produced inconsistent results. Often, the observed association between sodium and LVM may be attributable to the presence of other important factors, such as obesity.

Measurement of BP

For several reasons, in the clinic, it is generally preferable to measure the patient's BP using mercury BP devices, rather than oscillometric machines. Firstly, values used to create the normative curves for BP in youth were gathered using mercury BP devices, and it is advisable to remain consistent for accurate comparative purposes. No such normative curves exist for oscillometric devices due to insufficient data. Secondly, oscillometric machines have been proven less accurate for patients with increased vascular stiffness [97], although oscillometric measures are not subject to the human error inherent in the mercury BP device values. Therefore, it is not surprising that the two methods of measurement agree on BP status classification in only 60% of cases [98].

It is important to appreciate that casual BP measurements (those done in the clinic) may not be ideal, because within an individual child, BP is highly variable between each clinic visit, and even between each measurement [99]. This problem may be partially addressed through the use of 24-hour ambulatory BP monitoring (ABPM). Patients wear the ABPM device and an arm cuff for a full 24 hours, with the cuff inflating and recording the patients' BP every 15 to 30 minutes, day and night. Considered the gold standard for BP measurement, ABPM data is not tainted by in-clinic factors that impinge on the quality of the data collected; this is especially important in the diagnosis of white-coat hypertension (hypertension only when in a medical setting) and masked hypertension (normal office BP, elevated ambulatory BP). Masked hypertension in particular is associated with end-organ damage, particularly with regards to LVM and cIMT, increasing the patient's risk for cardiovascular events [100, 101]. The nighttime ABPM measurements are valuable for measuring the amount of nocturnal BP dipping. Lack of nocturnal dipping is associated with cardiac abnormalities, including increased aortic stiffness and LVH [102-105].

Widespread use of the ABPM method to evaluate BP in children would represent a significant improvement in the quality of BP data collected: 24-hour ABPM accurately

diagnosed arterial hypertension in twice as many pre-pubertal children than did casual BP measurement [106]. ABPM values are correlated more closely with LVMI than casual BP measurements [107]. Consistently elevated ABPM SBP (load, or proportion of abnormal BP values >50%) is more predictive of abnormal LVMI and LVH, than is abnormal DBP [107, 108].

The ABPM also collects information regarding BP variability, data that would be otherwise inaccessible. Studies have found that increased BP variability (especially nighttime variability) is correlated with LVMI [109, 110], however this may be because of the strong association between blood pressure variability and BMI [111]. Nevertheless, research on the relationship between BP variability and LVMI is sparse in the pediatric population. Further investigation of this topic would help to make ABPM data a more powerful tool in predicting cardiovascular damage.

Current treatment recommendations

Naturally, one strategy to alleviate LVH is through the reduction of BP levels. However, through a combination of under-screening and under-appreciation of its importance, elevated BP in children is frequently either not diagnosed or not addressed; an estimated 25% of hypertensive children go undiagnosed [112]. Lack of action early on in these children's lives may have serious implications for their future health. One report found that BP levels in children as young as 9 years old could predict atherosclerosis in later years [113] (their risk was reduced, although not eliminated, if the children transitioned to normotensive adults).

Several strategies can be employed to address the disparity between incidence and detection. Firstly, the definition of hypertension is inherently flawed. Demarcated using normative curves (>95th percentile), the data used to generate those curves are specific to the period of time and the population from which the data was gathered, and may not apply to current pediatric populations. Furthermore, a diagnosis of hypertension is not immediately recognizable to a clinician, nor does it have intrinsic meaning. It would be helpful for physicians to be equipped with definitive age-, height-, sex-, and race-specific cut-offs for hypertension that reflect organ damage downstream of high BP, and that remain static in the face of population changes.

The longitudinal data required to formulate such thresholds are difficult to collect, and current data may not be adequate. However, inadequacies in treatments for those identified as

having hypertension can be addressed. Children with hypertension, but without LVH, should first be treated via therapeutic lifestyle changes. Such changes, including decreased sodium intake and screen time, and increased physical activity, have been shown to reduce BP and improve cardiovascular risk factors in pre-pubertal children [47]. For hypertensive children with LVH, pharmacological therapy is mandated and often has beneficial results [15]. Numerous studies have established that BP-lowering therapy reduces LVM and other cardiovascular indicators among hypertensive patients, in some cases to a remarkable extent [114]. This translates into beneficial outcomes. A recent study showed that for every reduction of 2 mmHg in mean SBP, stroke mortality and coronary heart disease mortality dropped by 7% and 10%, respectively [115]. Some researchers have proposed that treatment of BP-mediated LVH should be based around ACE inhibitors and/or diuretics, to reduce afterload [53, 116].

In addition to physiological alterations, it is important for physicians to consider patients' psychological state, which may be partially responsible for their high BP levels. Persistent mental stress induces sympathetic nervous system activation, elevating BP, and contributing to the development of LVH [117]. The contribution is even greater in children with a genetic predisposition to hypertension [118].

One of the primary impediments to the fight against hypertension in youth is the lack of screening. The natural question that arises is, should physicians be conducting screening, and if yes, to what extent? The conflicting opinions on this matter prompted the initiation of a large-scale study to compare the effectiveness of three approaches to BP-screening in youth: no intervention, "screen-and-treat", and population-wide strategies. It found that population-wide policy interventions, such as salt-reduction or increased physical education classes were extremely cost-effective. BP screening targeted at adolescents with certain conditions that make them at-risk, such as LVH or HTN - a "high-risk only" approach - resulted in less attractive cost-effectiveness ratios than population-wide screening [119].

In contrast, the U.S. Preventative Services Task Force recommends against all BP screening in children, citing insufficient evidence of its benefits. Moreover, the Task Force argues that screening is accompanied by significant harms, including the psychological burdens of false-positive diagnoses, and that there have been few longitudinal studies providing evidence that high BP in childhood predicts cardiovascular disease in adulthood [120]. In addition, the Task Force contends that rates of hypertension maintenance from childhood to adulthood vary

from estimates of 19% to 65% [120], so an expensive intervention program may not result in a significant reduction of hypertensive adults. There is also the problem of follow-up; even when hypertension is diagnosed in pediatric patients, BP is not regularly taken at check-up visits [121].

Conclusion

Adiposity is well known to be associated with elevated LVM. Data support that BP is associated with LVM as well, although it is likely that the BP-LVM relation is affected by other factors, including lifestyle, physical and genetic factors.

A large amount of research is currently being done on the topic of LVM in youth, yet there is a need for further clarification at every level: at the level of the physiology and the tissue, to determine the sequential progression from normal heart physiology to LVH; at the clinical level, to resolve which factors predispose an individual to elevated LVM or LVH; at the level of procedure and policy, to validate treatments and medications, and to clarify whether BP screening has significant advantages in preventing long-term cardiovascular damage.

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Chapter 4: Determinants and risk factors of blood pressure throughout childhood: a prospective cohort study

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4.1 Foreword to this manuscript chapter

The manuscript in this chapter uses the data from a longitudinal study (the QUALITY study), which has overarching aims to study determinants and outcomes of adiposity. The QUALITY study comprises 3 study visits, with the latest visit (V3) having occurred when subjects were 15 to 17 years old. Casual blood pressure (BP) is measured at each of these visits; these are the BP data used for the current manuscript. Of note, data from the third visit (V3) were unavailable at the time of writing (in the final stages of data cleaning and collation). I have therefore completed all aspects of the manuscript and performed all planned analyses using data from V1 (8-10 years old) and V2 (11-13 years old). The V3 data will be available in October 2017, after which I will complete all analyses including the V3 visit data and revise the manuscript, obtain feedback from authors, and submit the manuscript to *Pediatric Nephrology*, *Pediatrics,* or *Hypertension,* depending on the significance and impact of the final results.

The methods of the study have been presented at the Annual McGill Biomedical Graduate Conference (AMBGC) and the Canadian Society of Nephrology (CSN) Annual General Meeting, and preliminary results have been presented at the biannual QUALITY Day in

May 2017 [1-3]. Feedback from those conferences, and from the numerous conversations with the listed authors and with colleagues, was incorporated into this manuscript.

Though 1 to 3% of children are reported to have hypertension, and several risk factors associated with hypertension in children have been identified, the determinants of BP itself and how BP determinants and risk factors for elevated BP *change* throughout childhood, remain unclear. This study addresses that knowledge gap by assessing the associations between a number of factors and BP at 3 visits, using the same cohort of children at each visit.

4.2 Manuscript 2

Abstract

Background and objectives

Certain physical measures, such as height and weight, are known to contribute to blood pressure (BP) in children [4]. However, there is a lack of knowledge as to how these and other risk factors of BP change throughout childhood. Also unclear, is the extent to which increased adiposity, a major contributor to hypertension in youth, predicts future elevated BP in children.

Design, setting, participants, & measurements

We conducted a longitudinal cohort study of children at-risk for obesity, from Montreal and Quebec City, Canada. Inclusion criteria were having at least one obese parent, and not suffering from any serious medical condition. The study included three visits: V1 (age 8-10 years, n = 631), V2 (age 11-13 years, n = 564), and V3 (age 15-17 years). During visits, data were collected on the patients' anthropometric, biochemical, and lifestyle factors, and the parents' BMI and history of hypertension. Univariable and multivariable linear regressions were performed to evaluate the relation between selected factors and systolic and diastolic BP. Univariate analyses were used to evaluate the association between V1 BMI and V2 BP.

Results

At V1 and V2, the mean age of participants was 9.6 years and 11.7 years, respectively; approximately 40% were overweight or obese at each visit. In univariable analysis, all adiposity indicators were significantly associated with BP levels, as were the biochemical measures and several of the parental factors, especially those of the mother. In the multivariable analysis, no factors were consistently significant. Nevertheless certain trends in the associations were apparent. Screen time in boys and BMIz in girls were significantly associated with BP. In

addition, triglycerides and mother's BMI were associated with BP in both genders. At V2, smoking was significantly associated with girls' BP. When examining how factors at V1 were associated with BP at V2, in both genders, mother's BMI was associated with both systolic and diastolic BP, and VO2 max was associated with diastolic BP. BMIz in the upper quintile of the cohort was significantly associated with future higher BPz levels.

Conclusion

Several physical, lifestyle and familial factors are associated with BP during childhood, however, these associations are different between boys and girls and some change over time. In particular, adiposity and hereditary factors were significantly associated with systolic and diastolic BP, in both genders, and late childhood adiposity was associated with future early adolescent BP.

Introduction

Elevated blood pressure (BP) in children is associated with significant cardiac and renal damage [5, 6]. Moreover, there is substantial evidence that both BP levels [7] and end-organ damage [8] in children persist into adulthood. A number of cross-sectional studies have been conducted to identify factors associated with elevated BP at a single time-point in children and adolescents [9, 10]. In particular, extensive data have elucidated the strong relationship between adiposity and BP, which exists in normal and obese children [11]. Further, both obesity and elevated BP each have cumulative effects on end-organ damage as the conditions persist over time [12]. The extent to which adiposity impacts on BP over time and how the presence of obesity may predict BP levels later in time in children, remains unclear.

Several factors other than adiposity are well-known to influence BP. Fasting serum lipids, such as LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C), and insulin have been found to be associated with elevated BP or obesity in youth [13, 14]. Age and gender have modulatory effects on the sympathetic and parasympathetic branches of BP control during early adulthood [15]. In particular, the onset of puberty accelerates the rate of BP increases [16]. Puberty is a time during which several aspects of male and female physiology begin to diverge [16, 17], and may influence the effects of adiposity, serum lipids, and hereditary factors on BP. Physiological differences and associations of BP determinants with BP levels may be influenced by extrinsic factors such as socioeconomic status, diet, and activity level.

Together, all of the aforementioned variables present a complicated web of risk-factor interactions. Few recent studies have used longitudinal data from the same cohort to determine the long-term effects of risk factors on BP, and how the importance of these risk factors on BP levels changes throughout childhood. The present study uses data collected at three points in time, to evaluate the relationship between adiposity and several other risk factors and determinants with BP, in children over time. In addition, we explored the extent to which earlier childhood adiposity and other factors are associated with future BP levels.

Methods

Study design & cohort

The Quebec Adiposity and Lifestyle Investigation in Youth study (QUALITY) is a prospective, longitudinal cohort study, whose aim is to analyze the determinants of metabolic syndrome in children at-risk for obesity[18]. The QUALITY cohort consists of Caucasian children who were recruited from elementary schools in the greater Montreal and Quebec City areas. The study consists of three visits conducted between 2005-2008 (visit 1 [V1], age 8-10 years), 2007-2011 (visit 2 [V2], age 11-13 years), and 2012-2016 (visit 1 [V3], age 15-17 years). In order to be eligible, children had to have at least one obese biological parent (either body mass index [BMI] >30 kg/m^2 or waist circumference >88 cm in women and >102 cm in men)[18]. Children were excluded at study onset if they had diabetes, were taking antihypertensive medication or steroids, had serious psychological or cognitive problems, or were suffering from an illness that precluded performing study procedures. Visits were conducted at Centre Hospitalier Universitaire (CHU) Sainte-Justine in Montreal or Hôpital Laval in Quebec City. The protocol was approved by the Ethics Review Boards at both centres. Parental consent and participant assent was obtained prior to performing all study activities.

Study visit procedures and variable descriptions

Figure A1 in the appendix describes the QUALITY study visit day, as reported in previous publications [18]. At all three visits, participants underwent extensive evaluations including: anthropometry measurements, body composition, accelerometer data, fitness test, oral glucose tolerance test (OGTT), blood samples, parent and child lifestyle questionnaires, and casual blood pressure, the primary outcome of this study. These evaluations are described below.

Anthropometry & pubertal assessment

All measurements were recorded with the patient wearing light indoor clothing without shoes, and with pockets emptied. Weight measurements were taken using an electronic scale, and the values were recorded to the nearest 0.1kg. Height measurements were taken at the patient's maximal inspiration, using a stadiometer. Values were recorded to the nearest 0.1cm. Each measure was taken twice, with another measurement taken if the two values differed by more than 0.2kg for weight or 0.2cm for height. For each measure, the mean of the two closest values was used in the analyses. Trained nurses assessed pubertal maturation, as measured by Tanner stage (1-5) based on pubic hair development in boys, and breast and pubic hair development in girls. Puberty was defined as Tanner stage >1.

Adiposity

Several adiposity measures were performed. BMI was calculated (weight/height^2 (kg/m^2)). Sex and age-specific BMI percentiles and z-scores were calculated [19, 20]. Patients were categorized as overweight (85th-95th percentile) or obese ($\geq$95th percentile). Waist circumference (WC) was measured at the midpoint between the last rib and the iliac crest, using a standard tape measure.

A dual-energy X-ray absorptiometry (DEXA; Prodigy Bone Densitometer System, DF+14664; GE Lunar Corporation, Madison, Wisconsin, USA) was performed as previously described [21] and the data used to compute percentage central fat (CF%). Measurements were taken, beginning at the patient's head and moving down to the feet. CF% was calculated as (trunk fat mass/total mass)*100.

Physical activity and fitness

Daily physical activity (PA) was assessed using an Actigraph LS 7164 activity monitor. Patients wore the accelerometer on the wrist for 7 days, and activity data was stored in 1-minute segments. In each segment, data was recorded as "counts", with increasing values connoting increased patient acceleration during the time segment. Depending on the count, the patient's activity level was classified as sedentary, light, moderate, or vigorous. The moderate-and-vigorous physical activity (MVPA) is the summation of the number of "moderate activity" minutes and the number of "vigorous activity" minutes. The MVPA was averaged across the

valid days of accelerometer wear (minimum 4 days of data, each with minimum 10 hours of wear time). Non-wear time was defined as a period of ≥60 minutes of 0 counts, allowing for up to 2 consecutive minutes when the count ranged from 0–100 counts (very light physical activity).

Screen time was assessed by patient-completed questionnaire; it is the tabulation of the patient's daily hours of television viewing, video game use, and leisure computer time.

Fitness level was assessed by the patient's performance in an adapted standard incremental exercise test on an electromagnetic bicycle to volitional exhaustion. Peak oxygen consumption (VO2 max) was calculated, and was standardized to lean body mass. VO2 max was only considered valid if at least one of the following criteria was present: (1) the respiratory exchange ratio (CO2 production to O2 consumption) was greater than 1.0; (2) the patient had a heart rate of 185 beats/min or higher.

Insulin sensitivity and lipid profile

Patients underwent a 2-hour oral glucose tolerance test (OGTT) after a 12-hour overnight fast. Each patient was given an oral glucose bolus of 1.75 g/kg of body weight (maximum 75g), and blood was drawn immediately before the test, and at 30-, 60-, 90-, and 120-minute intervals. Plasma insulin concentrations were measured using the automated Access 2 immunoassay system (Beckman Coulter, Inc). Plasma glucose concentrations were measured with the automated Synchron LX20 (Beckman Coulter, Inc., glucose oxidase method). Analyses were performed at the CHU Sainte-Justine Clinical Biochemistry laboratory.

Two indices of insulin dynamics were derived: the homeostatic model assessment of insulin resistance (HOMA-IR) and the Matsuda insulin sensitivity index (Matsuda ISI). HOMA-IR is a measure of fasting insulin resistance that uses the results from the fasting blood draw: [(fasting insulin mU/L) x (fasting glucose mmol/L) / 22.5]. Matsuda ISI uses data from the OGTT, and is calculated as: 10,000 / √[fasting insulin x fasting glucose) x (mean OGTT insulin x mean OGTT glucose)] [22].

A fasting blood sample was collected in an EDTA tube and centrifuged at 4^{o}C for 10 minutes, 4000 rpm. Triglycerides and total cholesterol levels were measured using enzymatic assays [23]. High density lipoprotein cholesterol (HDL-C) levels were measured using a detergent that specifically solubilizes HDL-C, and low density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation [24]: LDL-C (in mmol/L) = (total

cholesterol – HDL-C – triglycerides) / 2.22.

Environment & lifestyle

We defined season the visit was conducted in as "wintertime" (November-March) or "summertime" (April-October). Smoking status was determined by patient-reported smoking of cigarettes at least once per month. Poverty status was determined by parent-reported household income below the 2015 Canadian low-income cut-offs (LICOs) [25].

Blood pressure

Blood pressure (BP) was measured on the right arm, with the cuff size selected using mid-arm circumference. During cuff inflation, the patient was in a seated position, with the back supported. Measurements were taken 30 minutes after lunch, after the patient had voided, and had rested with the cuff on the arm for at least 5 minutes. Measurements were recorded using an automated oscillometric device (Dinamap XL, model CR9340; Critikon Co., Tampa, Florida, USA), which was regularly calibrated using a mercury sphygmomanometer. Five BP readings were taken, at 1-minute intervals. During that time, the patient was encouraged to relax and refrain from talking. The first two BP readings were dropped, and the average of the last three measures was used to determine systolic and diastolic BP (SBP and DBP). Percentiles and z-scores for average SBP and DBP were calculated using age/height/sex-specific normative values from the National High Blood Pressure Education Program (NHBPEP) Fourth Report [26]. Normal BP was defined as SBP and DBP less than the 90^{th} percentile; pre-hypertension was SBP or DBP greater than the 90^{th} and less than the 95^{th} percentiles, or SBP >120 mmHg or DBP >80 mmHg in adolescents; hypertension was SBP or DBP greater than or equal to the 95^{th} percentile or >140 mmHg or DBP >90 mmHg.

Statistical analysis

Means (standard deviation), medians (interquartile range), or counts (percent) were used to describe characteristics. T-tests and Chi-square tests were performed to compare characteristics between sex groups and between study visits.

Association of BP determinants and risk factors with BP

Univariable analyses were performed to evaluate the associations of each BP determinant and risk factor with SBP and DBP, by sex and by study visit (simple linear regression and Pearson correlation for continuous variables and t-tests for binary variables).

In order to evaluate the independent associations of different BP determinants and risk factors with SBP and DBP, we performed multivariable linear regression analyses in boys and girls separately and at each of the study visits. We evaluated for collinearity between all of the BP determinants and risk factors. When collinearity was present, we excluded collinear variables and retained only one for the multivariable analysis. The same multivariable analysis (i.e., including the same variables) was performed for V1 and V2. Since the proportion of subjects who smoked only became significant at V2, we performed separate additional multivariable analysis including smoking status for V2.

Association of V1 BP determinants and risk factors for predicting BP at V2

In order to evaluate which factors at V1 were most predictive of V2 BP, we performed a backward, manual stepwise elimination multiple linear regression, for boys and girls separately. Beginning with the full model of V1 variables, variables with p-value ≥ 0.20 were eliminated from the model one by one until all variables retained within the model had an association with BP with a p-value <0.20.

Secondary analysis

Because of the strong association of adiposity with BP, we examined the relationship between V1 BMI z-score (BMIz) with V2 BP z-score (BPz) in more detail. We divided subjects into quintiles of BMI (1^{st} quintile being the lower 5^{th} BMI values in the group; 5^{th} quintile being the highest), based on their BMIz at V1. Boxplots of SBP and DBP z-scores (SBPz, DBPz) across BMIz quintiles were generated, by gender and study visit.

For all multivariate models, no interaction terms were included. Beta-coefficients and, in some cases, associated p-values were reported. All analyses were done using STATA/SE 12.1 statistical software (StataCorp, Inc.).

Results

Patient characteristics

Table 1 shows patient characteristics at V1 (n=631 families, mean patient age 9.6 years, Table 1) and V2 (n=564 families, mean age 11.7 years). At both visits, more girls than boys were pubertal (V1: 36.1% *vs.* 9.3%; V2: 82.1% *vs.* 53.7%). At V1 and V2, 43.6% and 39.7% of participants were classified as overweight or obese, respectively. V2 BP z-scores (BPz) were significantly greater in boys than in girls; in girls, DBPz at V1 was higher than at V2. All other BP z-scores were not significantly different by sex group or study visit (Table 1). Several adiposity and fitness measures, such as VO2 max and MVPA, were significantly higher in boys than girls, at V1 and V2 (Table 1). HOMA-IR and Matsuda ISI increased and decreased respectively at V2 *vs.* V1, reflecting greater insulin resistance in the patients over time (Table 1). No patients were cigarette smokers at V1, whereas at V2, 5.4% of boys and 7.2% of girls smoked.

Univariable analyses of BP versus risk factors and determinants

Table 2 shows univariable associations between factors and SBP and DBP z-scores, stratified by sex and study visit.

Age/development

Overall, Table 2 shows that at both study visits, there was no consistent association between age at visit, Tanner stage >1 or being born premature, with BP levels.

Adiposity/physical condition

Weight z-score, BMI z-score (BMIz), WC, CF%, abdominal fat, and obesity status (all measures of adiposity) were generally associated with higher BP levels at both visits in boys and girls. These associations were somewhat less evident for DBPz in girls at V2 (last column Table 2). BMIz was most strongly correlated with BP, compared to other adiposity measures (correlation r ranging from 0.16 to 0.32, Table 2). Overall, lean body mass (LBM) had the weakest associations with BP compared to other adiposity measures (r ranging from -0.01 to 0.25). The strength of associations between adiposity measures and BP were generally stronger with SBPz *vs.* DBPz at both visits (Table 2, comparing beta coefficients between SBPz and DBPz).

Higher MVPA was associated with lower BP, but this correlation was only statistically significant at V1 with DBPz. Higher screen time per day was overall correlated with higher BP, however this association was stronger and statistically significant mainly at V2. VO2 max was inconsistently associated with SBPz (Table 2).

Insulin resistance and lipid profile

Higher HOMA-IR and Matsuda ISI were significantly associated with higher and lower BP, respectively, at both visits, in boys and girls (Table 2). The associations of insulin resistance indices with BP were generally stronger in males, stronger in V1, and stronger with SBPz than with DBPz (Table 2). Higher HDL-C levels were correlated with lower BP, with associations being generally stronger in females (Table 2). LDL-C levels were inconsistently associated with higher BP. Triglyceride levels were also predominantly associated with higher BP; at V1, this association was consistently stronger in males (Table 2).

Familial factors

History of hypertension in the patient's mother was significantly associated with higher BP in females at V1, however hypertension history in the father was not significantly associated with any measures of BP in either sexes (Table 2). Mother's BMI was associated with all BP levels for males, at V1 and V2, with a stronger association at V1. In contrast, mother BMI was only associated with female SBPz at V1. Father BMI was inconsistently associated with BP measures for both genders (Table 2).

Environment & lifestyle

Winter season (*vs.* summer) was associated with higher BP at both visits, however this association was only statistically significant for SBPz (Table 2). Smoking status was associated with higher BP for girls at V2, but not for boys. Poverty status was not associated with any BP measures (Table 2).

<u>Multivariable analysis of BP determinants and risk factors at V1 and V2</u>

There was significant collinearity for several BP determinants and risk factors. All adiposity variables were highly correlated ($r \geq 0.70$); only BMIz was included in the multivariable

analyses, as it is most commonly used in clinical care and was consistently more strongly associated with BP in our cohort. Lean body mass was excluded from the multivariable models because it was highly correlated with VO2 max (r = 0.84) and BMIz (r = 0.54), and was used to scale VO2 max. Age was excluded because the range of patient ages at each visit was narrow. HOMA-IR was excluded because it reflects a similar physiological assessment (insulin sensitivity/resistance) as Matsuda ISI, and Matsuda ISI is more strongly associated with directly measured insulin resistance [27, 28]. Poverty status and father's history of hypertension were excluded from the models because they were not significantly associated with BP in univariable analyses.

Table 3 shows multivariable associations between selected factors and SBPz and DBPz, stratified by sex and study visit. Table A2 in the appendix shows the multivariable associations, using the same factors as in the Table 3, but with the inclusion of smoking status (from V2).

Pubertal status had stronger associations with BPz in both genders at V2 than at V1, but were never statistically significant. BMIz associations with BPz were uniformly stronger in girls than in boys, particularly at V2.

In boys, screen time was much more strongly associated with BPz at V2 than at V1. The opposite was true in girls, with stronger associations for screen time at V1 than at V2 (Table 3).

Matsuda ISI was negatively associated with BPz and associations in girls were stronger than in boys, with the exception of male V1 SBPz. HDL and LDL cholesterol were inconsistently associated with BP in both sexes, but BPz–LDL-cholesterol associations were stronger in boys than in girls. In both genders, triglyceride levels had stronger associations with BPz at V1 than at V2 (Table 3). Mother's history of hypertension was not included in the girls' V2 models because of extreme collinearity, however at V1, girls' mothers' histories of hypertension were more strongly associated with BPz than boys'. In contrast, overall, the associations between mother's BMI and BPz were stronger in boys than girls (Table 3). Winter season appeared to have stronger associations with SBPz than DBPz, although the differences were not substantial(Table 3).

In the model including smoking status (Table A2), there were no substantial changes among the other variables' beta coefficients. Smoking status was significantly associated with female SBPz and DBPz ($\beta = 0.82$ and 0.52, respectively), although not significantly associated with male BPz values.

Longitudinal associations between V1 Factors and V2 BP

Table 4 shows multivariable linear regressions using factors from V1 to predict SBPz and DBPz at V2, stratified by sex. In girls, higher V1 BMIz was predictive of higher V2 SBPz (adjusted $p<0.05$, Table 4) and marginally predictive of DBPz ($p=0.07$, Table 4). In boys, V1 BMIz was marginally predictive of V2 SBPz ($p=0.07$). Higher V1 triglycerides were significantly associated with higher V2 SBPz and DBPz in boys only (Table 4). In boys only, higher Mother's BMI was significantly associated with SBPz and DBPz at V2; father's V1 BMI was marginally associated V2 SBPz in boys. Higher V1 VO2 max was associated with lower V2 DBPz in males and females, but this association only achieved statistical significance in girls (Table 4). History of hypertension in the mother was only significantly associated with female V2 SBPz (Table 4). V1 Matsuda ISI was associated with V2 DBPz in girls only.

Secondary analysis: relationships between V1 adiposity and V2 BP

The boxplots in Figure 1 A–D show the medians and IQRs of SBPz and DBPz at V2, stratified by sex and V1 BMIz quintiles. In three of the four boxplots (male and female SBPz and male DBPz), the V2 BPz was significantly higher in the highest V1 BMI quintile compared to the lowest BMI quintile (Figure 1).

Discussion

This study examines the influence of a number of factors on SBP and DBP in children at-risk for obesity from the ages of 8-10 years old to 11-13 years. Overall, we found that associations of different BP determinants and risk factors with BP were different between boys and girls and that the strength of associations for certain factors changed with age. This suggests that future research evaluating BP determinants in youth and aimed at predicting higher future BP should consider boys and girls separately and account for physiologic changes that occur over time throughout childhood.

Adiposity is known to be one of the most important contributing factors to elevated BP. [29, 30]. Sorof et al. found that the prevalence of hypertension was 3 times greater in obese teenagers than non-obese teenagers [31]. Data from QUALITY and other studies have shown that several measures of adiposity, including BMI, WC, CF%, and total fat are highly correlated with BP and with each other [21, 32, 33]. We found that in the multivariable models, BMIz was

positively associated with BP z-scores even when controlling for other factors, but that the associations were generally stronger in females. The reason for this discrepancy between genders is unclear, however it may be the result of interplay between female-specific hormones, such as estrogen, and fat. This finding may also be affected by differences in physical activity between sexes; at both visits, average MVPA in girls was significantly lower than in boys. Therefore, while BMIz and its effects on BP may not have been different across sex, other risk factors may have been more influential in lowering BP. This is supported by the differences in beta-coefficients for BMIz between the univariable and multivariable models. The differences in the beta-coefficients for BMIz were much greater for boys than for girls (cumulative difference of 0.40 for girls, and 0.17 for girls), reflecting a stronger influence of the other variables on the BMIz-BP relationship for boys.

BMIz at age 8-10 years old, was not a significant factor associated with future BPz for either gender, nor were any other anthropometric measures of adiposity. The Bogalusa Heart Study, as well as many other studies have found that adiposity, including BMI and WC, are associated with both SBP and DBP [34]. In our cohort, however, the mean V2 SBPz was much lower than the V2 DBPz (approximately -0.75 *vs.* -1.05), which could explain why BMIz was only associated with the latter.

In the univariable analyses, pubertal status was associated with higher BP. This is consistent with previous findings that BP increases at an accelerated rate in adolescents during and after puberty [16]. However, pubertal status was no longer significantly associated with BP in the multivariable models, suggesting that other factors (e.g., screen time, physical activity) may be confounding the association of pubertal status with BP, similar to what we found with BMIz. We did not find that a history of preterm birth was associated with BP. This diverges from previous literature showing that preterm birth is associated with permanent changes in vascular function, which throughout childhood and adolescence, contribute to elevated BP and increased risk for hypertension [35-37]. However, our cohort did not have substantial numbers of children with a history of severe prematurity, which may have prevented our ability to fully explore the relationship of prematurity with long-term BP outcomes.

Higher screen time per day has been shown in previous studies to be positively associated with BP in children [10]. In our study, we found that screen time was significantly associated with BP at V2, but for the most part, not at V1. Also, the associations in boys were slightly

stronger than in girls. The significance did not persist in the multivariable models, although association magnitude remained higher in boys than girls and stronger at V2 than at V1. We surmise that as the patients aged, they had more access to television and computers and that near pubertal age, video games are also more popular among boys and girls, which may have accounted for the difference across genders.

In univariable analyses, MVPA was only significantly associated with DBP at V1; when other variables were included in multivariable analyses, MVPA showed no association with BP. Similarly, in the univariable models, VO2 max was *positively* associated with only SBP at V1, but was not associated with BP in the multivariable models. This is in contrast to studies that have found that low VO2 max is associated with lower short-term and long-term BP [38, 39].

Insulin sensitivity is closely related to adiposity and BP in children and adolescents [40]. Lower physical activity and increased adiposity are correlated with decreased insulin sensitivity [41], and low insulin sensitivity is associated with moderate to severe BP elevation [42]. This is thought to be a function of the relation between insulin and the sympathetic nervous system: a decrease in insulin sensitivity results in increased circulating glucose and sympathetic activity [43], and ultimately, may develop into type II diabetes. We found that Matsuda ISI was inversely associated with BP levels in the univariable analyses. However, in the multivariable analysis results, the associations of Matsuda ISI with BP were not consistently significant.

In the univariable analyses, HDL-C ("good" cholesterol) was negatively associated and LDL-C ("bad" cholesterol) was positively associated with BP levels. This was expected, however, for unclear reasons, both measures were more strongly associated in boys than in girls. The multivariate models of HDL-C and LDL-C yielded few significant relationships with BP.

Triglyceride levels are a criteria for diagnosis of metabolic syndrome in children [44]. Our results show that triglycerides were positively associated with BP. In multivariable models, associations with BP were not significant. Nevertheless, V1 triglycerides were associated with V2 male SBPz and DBPz. The association of triglycerides with future BP may reflect the cumulative longitudinal effects on the heart and blood vessels.

Children with a family of history of hypertension are prone to suffering from persistent BP elevation [45]. As to which parent's BP is more influential in determining the offspring's BP is a matter of debate [46-48]. We found that the strength of influence depended on the gender of both the parent and the offspring. Of note, hypertension prevalence in mother and father rose

significantly from V1 to V2. History of paternal hypertension was not associated with any BP in univariable analyses. In girls, maternal hypertension was positively and independently associated with patient BP measures at V1. Maternal BMI was associated only with boys' BP at V1 and V2. Paternal hypertension and BMI were not associated with offspring BP measures. In multivariable models, almost all these associations disappeared. When evaluated longitudinally, mother's BMI was the only variable that predictive of all forms of future BP, in boys and girls. Studies have shown that maternal weight during gestation can affect offspring BP, but there are few investigations on the effects of the mother's post-gestational weight. Parental influences are more complex and cannot be attributed to a single cause, as they influence offspring both genetically and through shared environmental factors; this is particularly true for offspring BP and BMI [49, 50]. This is an especially important consideration given that most of the children in this cohort lived with both parents at the beginning of the study. We speculate that the association between mother BMI and offspring BP may be confounded by environmental dietary effects. The variable "poverty status" encompasses environmental risk factors to some degree as poverty is associated with a number of factors: positively associated with smoking, adiposity, screen time, and family stress but negatively associated with access to recreational facilities and health services, and nutrition [51, 52]. In future studies, in order to more fully account for the environmental effects, additional variables, such as diet and home location (urban *vs.* suburban, high-crime *vs.* low-crime, etc.) should be considered.

SBPz tended to be significantly higher during wintertime in the univariable models, which may be due to increased sympathetic nervous system activation [53]. Seasonality was not significantly associated with BP in the multivariable models.

In both the univariate models and multivariable models including smoking status, we found that smoking was significantly associated with girls' V2 BP. Smoking is known to correlate strongly with BP in adolescents of both genders [54], so our findings to the contrary may be because our V2 cohort contained very few smokers (5.4% of boys, 7.2% of girls).

There were several limitations to this study. First, our sample size, though considerably larger than several previously published studies, may not have been large enough to adequately evaluate some BP determinants and risk factors (e.g., smoking). Moreover, because this was a cohort of patients who were generally healthy, there may not have been enough subjects with substantially abnormal values on several of the factors evaluated (e.g., Matsuda index,

triglycerides) to meaningfully evaluate associations with abnormal BP. In addition, few subjects had high BP, as defined by current guidelines. It is possible that strengths of associations may differ in studies of cohorts with a higher percentage of patients with abnormal BP. This also forced us to evaluate our outcome, BP, as a continuous variable, rather than use the binary outcome, hypertension *vs.* non-hypertension.

As this was a longitudinal study performed over the course of more than a decade, it was performed at various times by several different researchers, nurses, and technicians. We did not test for inter-tester reliability or test-retest reliability, as the questions answered by the patients were completed using a the same written questionnaire throughout. Nevertheless, measurements such as waist circumference and echocardiogram values are dependent on tester proficiency and consistency, which may have varied to a very limited degree. There was also missing data on some variables. We used case-wise deletion to analyze our results, which reduced our sample size. An alternative method would be to use multiple imputation, and we could not think of reasons that patients with incomplete data would differ from other patients. Once all data are acquired, we will perform a comparison of those with versus without missing data. We did not perform an evaluation of effect modification (evaluating whether some variables have different associations with BP depending on the different levels of another variable). Future analyses will include evaluation for effect modification, focusing on the adiposity-BP relationship, since adiposity remained one of the strongest determinants of BP in our cohort, even in multivariable analyses.

This longitudinal study focused on children and young adolescents at-risk for hypertension and with a disproportionate prevalence of obesity. In summary, many of the evaluated variables, the majority of which relate to adiposity, were significantly associated with BP in the univariable models. However when other variables were included, many associations become non-significant. This suggests that in most cases, elevated BP is the result of many contributing factors. Future BPz in the children was predicted by a small number of factors, composed of lipid measures, aerobic capacity, and parental values. Most predominant was BMIz; BMIz quintile was a strong predictor of future BPz, in boys and girls.

Adiposity is itself a cardiovascular risk factor and contributes to the development of other risk factors, including hypertension. While the progression of such factors to pathology (i.e. myocardial infarction or stroke) are not often observed in children, minor cardiac and blood

vessel injury can occur and get progressively worse throughout childhood and adolescence. This study provides evidence that adiposity and adiposity-associated variables from several categories (anthropometric, biochemical, hereditary, environmental/lifestyle) should be closely monitored among youth, especially those at-risk for developing hypertension.

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Manuscript 2 tables and figures

Table 1. Patient characteristics, blood pressure and determinants at V1 and V2, stratified by gender.

Characteristic	V1 (8-10 years old), n = 631		V2 (11-13 years old), n = 564	
	Male (n = 344)	Female (n = 287)	Male (n = 313)	Female (n = 251)
Age / Development				
Age, mean (SD)	9.6 (0.9)	9.6 (0.9)	11.7 (0.9) †	11.6 (1.0) †
Pubertal, n (%)	32 (9.3)	104 (36.1) *	168 (53.7) †	206 (82.1) *†
Born prematurely, n (%)	17 (4.9)	17 (5.9)	16 (5.1)	16 (6.4)
Blood Pressure				
Systolic BP z-score, mean (SD)	-0.68 (0.71)	-0.75 (0.71)	-0.69 (0.76)	-0.83 (0.80) *
Systolic Prehypertension, n (%)	2 (0.6)	3 (1.2)	2 (0.6)	3 (1.2)
Systolic Hypertension, n (%)	0 (0.0)	1. (0.4)	0 (0.0)	0 (0.0)
Diastolic BP z-score, mean (SD)	-1.07 (0.43)	-1.02 (0.45)	-1.02 (0.44)	-1.09 (0.50) *†
Diastolic Prehypertension, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Diastolic Hypertension, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Adiposity / Physical Condition				
BMI, mean (SD)	19.4 (4.3)	19.7 (4.4)	21.0 (4.9) †	21.2 (4.9) †
BMI z-score, mean (SD)	0.72 (1.04)	0.70 (1.12)	0.70 (1.10)	0.66 (1.07)
BMI Categories, n (%)				
Overweight	71 (20.6)	51 (17.7)	53 (16.9) †	45 (17.9)
Obese	73 (21.2)	70 (24.3)	72 (23.0) †	54 (21.5) †
Central Fat %, mean (SD)	39.9 (5.4)	41.7 (5.5) *	41.7 (4.8) †	43.5 (4.8) *†
MVPA, mean (SD)	30.4 (18.7)	18.9 (11.6) *	50.1 (24.7) †	33.9 (17.9) *†
Waist circumference, mean (SD)	67.4 (12.2)	67.5 (12.1)	73.1 (13.8)	71.5 (12.5)
Screen time, mean (SD)	3.0 (2.0)	2.4 (1.9) *	3.71 (2.36) †	3.12 (2.07) *†
VO2 Max, mean (SD)	1595.7 (295.7)	1420.6 (283.4) *	1999.0 (412.3) †	1754.3 (361.0) *†
Insulin resistance and lipid profile				
HOMA-IR, mean (SD)	0.93 (0.62)	1.18 (0.83) *	1.34 (0.88) †	1.65 (1.20) *†
Matsuda ISI, mean (SD)	3.35 (2.09)	2.69 (1.87) *	2.47 (1.65) †	2.09 (1.53) *†
Parental Values				
Mother BMI, mean (SD)	29.7 (6.8)	29.1 (6.4)	30.1 (6.5)	29.2 (5.8)
Mother HTN, n (%)	4 (1.2)	8 (2.8)	101 (29.4) †	84 (29.2) †
Father BMI, mean (SD)	30.4 (5.3)	31.0 (5.7)	31.2 (5.5) †	31.2 (5.8)†
Father HTN, n (%)	21 (6.1)	19 (6.6)	157 (45.6) †	144 (50.0) †
Other Factors				
Seasons				
Winter, n (%)	199 (57.9)	166 (57.6)	176 (56.2)	141 (56.2)
Summer, n (%)	145 (42.2)	122 (42.4)	137 (43.8)	110 (43.8)
Smoking status, n (%)	0 (0.0)	0 (0.0)	17 (5.4)	18 (7.2)
Poverty status, n (%)	30 (11.3)	21 (9.8)	18 (6.8) †	15 (7.4)

T-tests were conducted between genders at each visit, and between visits within the same gender.
* = Cross-gender comparison (e.g. Male V1 *vs.* Female V1) significant at $p < 0.05$
† = Cross-visit comparison (e.g. Male V1 *vs*. Male V2) significant at $p < 0.05$
Abbreviations: *BMI* = body mass index; *MVPA* = moderate and vigorous physical activity; *HOMA-IR* = homeostatic model assessment-insulin resistance; *Matsuda ISI* = Matsuda insulin sensitivity index

Table 2 – Univariable associations of blood pressure determinants and risk factors at V1 and V2, stratified by gender.

Characteristic	**V1 (8-10 years old), n = 630**				**V2 (11-13 years old), n = 564**			
	Systolic BP z-score (p-value)		Diastolic BP z-score (p-value)		Systolic BP z-score (p-value)		Diastolic BP z-score (p-value)	
	Male (n = 344)	Female (n = 287)	Male (n = 344)	Female (n = 287)	Male (n = 313)	Female (n = 251)	Male (n = 313)	Female (n = 251)
Age / Development								
Age	**β = 0.12 (0.01) r = 0.15**	β = 0.03 (0.57) r = 0.03	β = 0.025 (0.33) r = 0.05	**β = -0.06 (0.02) r = -0.13**	β = -0.02 (0.61) r > -0.01	β = 0.04 (0.50) r = 0.06	β = -0.03 (0.29) r = -0.06	β = -0.03 (0.29) r = -0.05
Tanner > 1 (mean BP Yes vs. No)	(-0.52 vs. -0.69) p = 0.10	(-0.72 vs. -0.77) p = 0.27	(-1.07 vs. -1.07) p = 0.48	**(-1.08 vs. -0.99) p = 0.05**	(-0.68 vs. -0.72) p = 0.34	(-0.81 vs. -0.94) p = 0.16	**(-1.06 vs. -0.97) p = 0.03**	(-1.07 vs. -1.19) p = 0.08
Born Prematurely (mean BP Yes vs. No)	(-0.54 vs. -0.68) p = 0.21	(-0.76 vs. -0.75) p = 0.48	(-1.01 vs. -1.07) p = 0.28	(-1.00 vs. -1.03) p = 0.42	(-0.54 vs. -0.70) p = 0.14	(-1.00 vs. -0.82) p = 0.23	(-1.01 vs. -1.02) p = 0.43	(-1.26 vs. -1.08) p = 0.08
Height z-score	β < 0.01 (0.95) r < 0.01	β = 0.02 (0.61) r = 0.03	β > -0.01 (0.96) r > -0.01	β > -0.01 (0.97) r > -0.01	β < 0.01 (0.98) r > -0.01	β = -0.06 (0.28) r = -0.06	β = -0.04 (0.13) r = -0.09	β = -0.05 (0.13) r = -0.09
Adiposity / Physical Condition								
Weight z-score	**β = 0.17 (0.00) r = 0.24**	**β = 0.11 (0.00) r = 0.18**	**β = 0.09 (0.00) r = 0.22**	**β = 0.07 (0.00) r = 0.17**	**β = 0.14 (0.00) r = 0.20**	**β = 0.18 (0.00) r = 0.26**	**β = 0.06 (0.01) r = 0.14**	β = 0.04 (0.17) r = 0.09
BMI z-score	**β = 0.19 (0.00) r = 0.28**	**β = 0.13 (0.00) r = 0.21**	**β = 0.11 (0.00) r = 0.26**	**β = 0.08 (0.00) r = 0.20**	**β = 0.17 (0.00) r = 0.23**	**β = 0.24 (0.00) r = 0.32**	**β = 0.09 (0.00) r = 0.21**	**β = 0.07 (0.01) r = 0.16**
Obese (mean BP Yes vs. No)	**(-0.34 vs. -0.77) p = 0.00**	**(-0.52 vs. -0.83) p = 0.00**	**(-0.85 vs. -1.13) p = 0.00**	**(-0.89 vs. -1.07) p = 0.00**	**(-0.39 vs. -0.78) p = 0.00**	**(-0.42 vs. -0.95) p = 0.00**	**(-0.91 vs. -1.05) p = 0.01**	(-1.03 vs. -1.10) p =0.14
Waist Circumference	**β = 0.02 (0.00) r = 0.31**	**β = 0.01 (0.00) r = 0.24**	**β = 0.01 (0.00) r = 0.27**	**β = 0.01 (0.02) r = 0.18**	**β = 0.01 (0.00) r = 0.23**	**β = 0.02 (0.00) r = 0.36**	**β = 0.01 (0.00) r = 0.18**	**β = 0.01 (0.03) r = 0.14**
Moderate and Vigorous Physical Activity	β > -0.01 (0.06) r = -0.11	β > -0.01 (0.29) r = -0.07	**β > -0.01 (0.00) r = -0.19**	**β = -0.01 (0.03) r = -0.14**	β > -0.01 (0.20) r = -0.08	β > -0.01 (0.42) r = -0.06	β > -0.01 (0.10) r = -0.11	β > -0.01 (0.96) r > -0.01
Central Fat %	**β = 3.56 (0.00) r = 0.27**	**β =2.99 (0.00) r = 0.23**	**β = 1.77 (0.00) r = 0.22**	**β = 1.72 (0.00) r = 0.21**	**β = 4.11 (0.00) r = 0.22**	**β = 5.01 (0.00) r = 0.30**	**β = 1.39 (0.01) r = 0.16**	β = 1.14 (0.08) r = 0.11
Lean Body Mass	**β < 0.01 (0.00) r = 0.25**	**β < 0.01 (0.01) r = 0.15**	β < 0.01 (0.06) r = 0.10	β < 0.01 (0.85) r = -0.01	**β < 0.01 (0.00) r = 0.19**	**β < 0.01 (0.05) r = 0.14**	β < 0.01 (0.94) r = -0.01	β < 0.01 (0.40) r = -0.04
Screen Time / Day	β = 0.03 (0.12) r = 0.08	β = 0.04 (0.11) r = 0.09	**β = 0.02 (0.05) r = 0.11**	β = 0.01 (0.59) r = 0.03	**β = 0.05 (0.01) r = 0.14**	**β = 0.05 (0.05) r = 0.13**	**β = 0.03 (0.00) r = 0.17**	**β = 0.03 (0.03) r = 0.14**

Insulin resistance and lipid profile								
HOMA-IR	**β = 0.42 (0.00) r = 0.36**	**β = 0.26 (0.00) r = 0.31**	**β = 0.22 (0.00) r = 0.31**	**β = 0.11 (0.00) r = 0.20**	**β = 0.27 (0.00) r = 0.31**	**β = 0.23 (0.00) r = 0.36**	**β = 0.12 (0.00) r = 0.23**	**β = 0.08 (0.00) r = 0.21**
Matsuda ISI	**β = -0.09 (0.00) r = -0.25**	**β = -0.08 (0.00) r = -0.22**	**β = -0.04 (0.00) r = -0.18**	**β = -0.05 (0.00) r = -0.22**	**β =-0.09 (0.00) r = -0.19**	**β = -0.13 (0.00) r = -0.27**	**β = -0.05 (0.00) r = -0.19**	**β = -0.07 (0.00) r = -0.23**
HDL Cholesterol	**β = -0.39 (0.01) r = -0.14**	**β = -0.38 (0.03) r = -0.13**	**β = -0.28 (0.00) r = -0.17**	**β = -0.34 (0.00) r = -0.18**	β = -0.24 (0.15) r = -0.09	**β = -0.55 (0.01) r = -0.18**	**β = -0.27 (0.01) r = -0.16**	**β = -0.32 (0.01) r = -0.17**
LDL Cholesterol	β = 0.10 (0.14) r = 0.08	**β = 0.17 (0.02) r = 0.14**	**β = 0.10 (0.02) r = 0.13**	β = 0.08 (0.07) r = 0.11	β = 0.05 (0.51) r = 0.03	β = 0.14 (0.12) r = 0.09	**β = 0.10 (0.03) r = 0.13**	β = 0.04 (0.50) r = 0.04
Triglycerides	**β = 0.49 (0.00) r = 0.25**	**β = 0.30 (0.00) r = 0.18**	**β = 0.24 (0.00) r = 0.21**	**β = 0.20 (0.00) r = 0.20**	**β = 0.34 (0.00) r = 0.19**	**β = 0.42 (0.00) r = 0.22**	**β = 0.22 (0.00) r = 0.21**	β = 0.12 (0.11) r = 0.10
Parental Values								
Mother HTN (mean BP Yes vs. No)	(-0.84 vs. -0.67) p = 0.33	**(-0.32 vs. -0.77) p = 0.05**	(-0.84 vs. -1.07) p = 0.15	**(-0.62 vs. -1.03) p = 0.01**	(-0.70 vs. -0.69) p = 0.32	(-0.93 vs. -0.81) p = 0.21	(-1.05 vs -1.01) p = 0.19	(-1.15 vs. -1.07) p = 0.18
Father HTN (mean BP Yes vs. No)	(-0.46 vs. -0.69) p = 0.08	(-0.81 vs. -0.75) p = 0.37	(-1.04 vs. -1.07) p = 0.37	(-0.86 vs. -1.04) p = 0.05	(-0.74 vs. -0.66) p = 0.24	(-0.85 vs. -0.82) p = 0.34	(-1.04 vs. -1.01) p = 0.31	(-1.07 vs. -1.10) p = 0.31
Mother BMI	**β = 0.14 (0.01) r = 0.14**	**β = 0.02 (0.00) r = 0.17**	**β = 0.10 (0.00) r = 0.16**	β > -0.01 (0.71) r = -0.02	**β = 0.02 (0.00) r = 0.19**	β = 0.01 (0.19) r = 0.10	**β = 0.01 (0.00) r = 0.18**	β = 0.01 (0.45) r = 0.06
Father BMI	β = 0.01 (0.40) r = 0.05	β < 0.01 (0.55) r = 0.04	β < 0.01 (0.62) r = 0.03	**β = 0.01 (0.01) r = 0.15**	β = 0.02 (0.09) r = 0.13	β = 0.02 (0.07) r = 0.15	β = 0.01 (0.35) r = 0.07	β = 0.01 (0.46) r = 0.06
Environmental & Lifestyle								
Seasonality (mean BP Yes vs. No)	(-0.64 vs. -0.70) p = 0.20	**(-0.66 vs. -0.83) p = 0.02**	(-1.05 vs. -1.08) p = 0.24	(-0.98 vs. 1.06) p = 0.06	**(-0.60 vs. -0.76) p = 0.03**	**(-0.71 vs. -0.93) p = 0.02**	(-1.00 vs. -1.03) p = 0.26	(-1.07 vs. -1.10) p = 0.34
Smoker (mean BP Yes vs. No)	N/A	N/A	N/A	N/A	(-0.83 vs. -0.68) p = 0.21	**(-0.45 vs. -0.86) p = 0.02**	(-1.01 vs. -1.02) p = 0.47	**(-0.85 vs. -1.11) p = 0.02**
Poverty Status (mean BP Yes vs. No)	(-0.57 vs. -0.73) p = 0.12	(-0.83 vs. -0.72) p = 0.26	(-0.99 vs. -1.10) p = 0.09	(-1.12 vs. -1.02) p = 0.16	(-0.55 vs. -0.76) p = 0.13	(-0.64 vs. -0.88) p = 0.13	(-0.88 vs. -1.04) p = 0.07	(-1.10 vs. -1.10) p = 0.50

Simple linear regression and Pearson correlation tests (continuous variables, with beta coefficients (p-value) and correlation coefficients *r* shown) or t-tests (binary variables, mean BP z-score in each group shown, with p-value).
***Values with p < 0.05 are shown in bold.**

Table 3 – Multivariable associations of blood pressure determinants and risk factors at V1 and V2, stratified by gender (βs shown).

Characteristic	Systolic BP z-score		Diastolic BP z-score		Systolic BP z-score		Diastolic BP z-score	
	Male V1 (n = 344)	**Male V2** (n = 313)	**Male V1** (n = 344)	**Male V2** (n = 313)	**Female V1** (n = 287)	**Female V2** (n = 251)	**Female V1** (n = 287)	**Female V2** (n = 251)
Age / Development								
Pubertal Status (p-value)	0.0531 (0.73)	0.1751 (0.37)	-0.0009 (0.99)	0.0548 (0.62)	-0.0044 (0.97)	0.0682 (0.84)	0.0834 (0.23)	-0.2524 (0.21)
Adiposity / Physical Condition								
BMI z-score	0.0347 (0.57)	0.0552 (0.62)	0.0352 (0.36)	-0.0147 (0.82)	0.0546 (0.37)	0.1553 (0.19)	**0.0751 (0.05)**	0.0567 (0.42)
MVPA	-0.0008 (0.78)	**0.0084 (0.05)**	-0.0013 (0.43)	0.0005 (0.84)	-0.0034 (0.47)	-0.0038 (0.46)	-0.0037 (0.20)	0.0016 (0.61)
Screen Time / Day	0.0094 (0.68)	0.0490 (0.06)	0.0160 (0.26)	**0.0404 (0.01)**	0.0137 (0.65)	0.0004 (0.99)	0.0106 (0.58)	0.0115 (0.69)
VO2 Max	0.0003 (0.18)	0.0002 (0.53)	-0.0001 (0.35)	-0.0021 (0.19)	-0.0001 (0.49)	<0.0001 (0.89)	**-0.0004 (0.01)**	-0.0002 (0.36)
Insulin resistance and lipid profile								
Matsuda ISI	**-0.0574 (0.03)**	-0.0185 (0.76)	-0.0114 (0.49)	-0.0219 (0.53)	-0.0495 (0.11)	-0.0956 (0.10)	-0.0342 (0.08)	-0.0622 (0.07)
HDL Cholesterol	-0.0186 (0.92)	0.4566 (0.30)	**-0.2618 (0.03)**	-0.1708 (0.50)	0.0290 (0.90)	0.8454 (0.09)	-0.0667 (0.64)	0.1369 (0.64)
LDL Cholesterol	-0.1009 (0.27)	-0.3345 (0.07)	-0.0175 (0.76)	-0.1032 (0.32)	0.0712 (0.40)	0.0351 (0.85)	0.0040 (0.94)	0.0680 (0.53)
Triglycerides	**0.2945 (0.05)**	0.2764 (0.20)	0.1565 (0.09)	0.1072 (0.39)	**0.2755 (0.05)**	0.1299 (0.53)	**0.1859 (0.03)**	-0.0473 (0.70)
Parental Values								
Mother HTN	-0.2938 (0.46)	0.4770 (0.52)	0.2181 (0.38)	-0.2055 (0.63)	0.2735 (0.43)	---	0.4093 (0.06)	---
Mother BMI	**0.0162 (0.03)**	0.0213 (0.13)	0.0073 (0.12)	0.0090 (0.27)	0.0146 (0.07)	-0.0014 (0.92)	-0.0083 (0.10)	-0.0016 (0.85)
Father BMI	-0.0088 (0.32)	0.0071 (0.61)	-0.0006 (0.92)	-0.0051 (0.52)	-0.0037 (0.68)	0.0156 (0.42)	0.0048 (0.39)	-0.0043 (0.70)
Miscellaneous								
Season (wintertime)	**0.2108 (0.03)**	0.3274 (0.05)	0.0502 (0.41)	0.1080 (0.27)	0.0232 (0.82)	0.2658 (0.15)	0.0008 (0.99)	0.0700 (0.52)
Adjusted R^2	0.1195	0.0803	0.0756	0.0709	0.0887	0.0397	0.1669	-0.0049

***Values with p-value < 0.05 are shown in bold.**

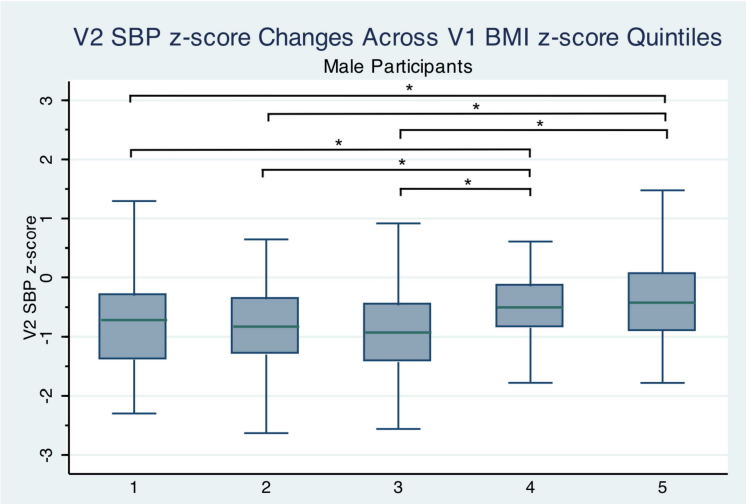
Table 4. Longitudinal multivariable associations between factors at V1 and BP at V2, stratified by gender and SBP/DBP

	Stepwise Multiple Regression			
V1 Variable	β	SE	p	Adjusted R^2
Male SBPz V2				
Triglycerides	**0.496**	**0.113**	**0.000**	
HDL-C	0.301	0.161	0.063	
BMI Mother	**0.021**	**0.006**	**0.001**	
BMI Father	**0.018**	**0.008**	**0.02**	
				0.112
Female SBPz V2				
HDL-C	**-0.483**	**0.212**	**0.023**	
Matsuda ISI	**-0.053**	**0.026**	**0.044**	
HTN Mother	**1.153**	**0.546**	**0.036**	
BMI Mother	**0.016**	**0.008**	**0.046**	
				0.077
Male DBPz V2				
VO2 Max	-0.002	< 0.001	0.054	
Triglycerides	**0.239**	**0.074**	**0.001**	
BMI Mother	**0.016**	**0.004**	**0.000**	
				0.096
Female DBPz V2				
VO2 Max	-0.002	< 0.001	0.070	
Triglycerides	-0.242	0.145	0.098	
BMI Mother	**-0.038**	**0.019**	**0.047**	
				0.031

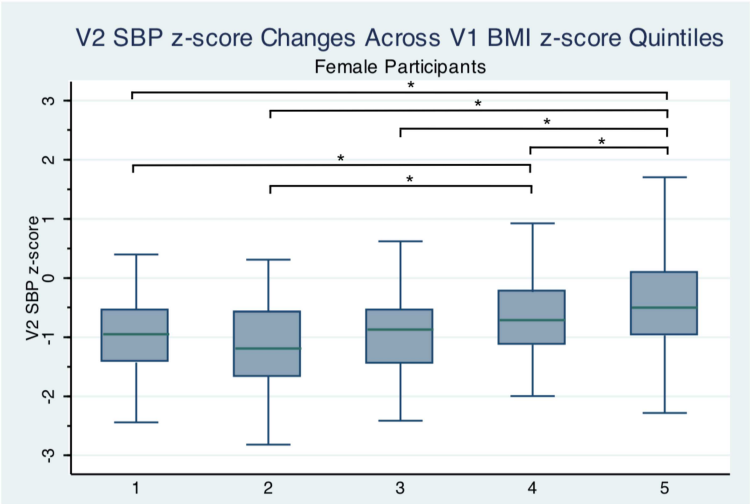
Backwards-stepwise elimination procedure used until all remaining variables had p-value < 0.20. Variables shown are those in the final model.
Variables with p < 0.05 are shown in bold.

Figure 1. Boxplots of V2 BP z-scores at each V1 BMI z-score quintile, stratified by gender and SBPz & DBPz

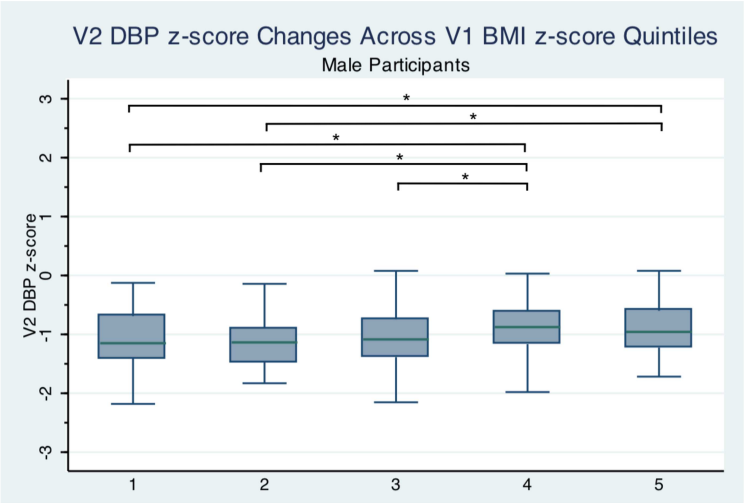
A.



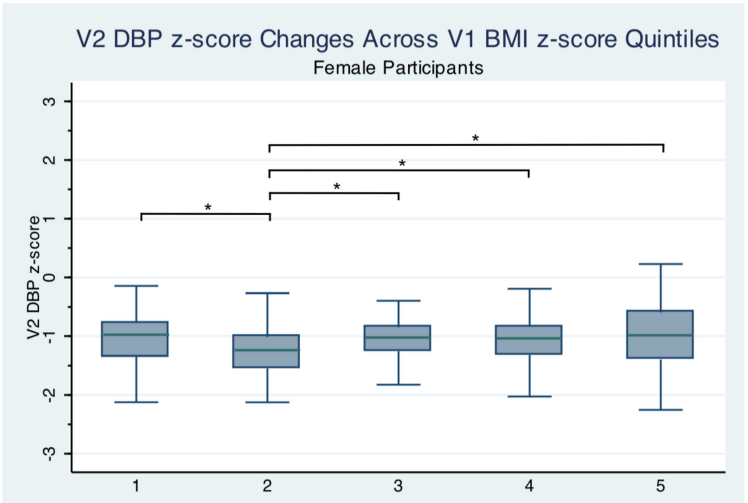
B.



C.



D.



Box plots (middle line is median, upper and lower box borders are the 25th and 75th percentiles, and the whiskers are 1.5 IQR in length) of BMIz quintiles across V2 BPz. P-values are the results of parametric tests of comparing pairs of groups.

Manuscript 2 appendix

Figure A1. Schedule of QUALITY visit day

QUALITY Study Schedule

7 :00 : Patient arrives for study visit

Welcome and information regarding the program of the day

Application of topical anaesthetic cream for adolescent

Weight, height, skin fold thickness, waist and hip circumferences, Tanner staging for adolescent.

Fasting blood tests, 2h-OGTT, questionnaires

11:00 : Lunch for adolescents

Assessment of adolescent`s body composition (DXA)

Adolescent`s BP and explanations about dietary recall

Exercise testing for adolescent (at least 2h after lunch) and explanations regarding accelerometer

15:00: End of the day

Table A2. Multivariable models evaluating associations between factors and BPz and including smoking status for V2, stratified by gender and SBPz & DBPz

Characteristic	Systolic BP z-score		Diastolic BP z-score		Systolic BP z-score		Diastolic BP z-score	
	Male V1 (n = 344)	**Male V2** (n = 313)	**Male V1** (n = 344)	**Male V2** (n = 313)	**Female V1** (n = 287)	**Female V2** (n = 251)	**Female V1** (n = 287)	**Female V2** (n = 251)
Age / Development								
Pubertal Status (p-value)	0.0531 (0.73)	0.1950 (0.32)	-0.0009 (0.99)	0.0487 (0.67)	-0.0044 (0.97)	0.1552 (0.63)	0.0834 (0.23)	-0.1899 (0.32)
Adiposity / Physical Condition								
BMI z-score	0.0347 (0.57)	0.0438 (0.70	0.0352 (0.36)	-0.0111 (0.86)	0.0546 (0.37)	0.1730 (0.13)	**0.0751 (0.05)**	0.0694 (0.30)
MVPA	-0.0008 (0.78)	**0.0085 (0.04)**	-0.0013 (0.43)	0.0004 (0.86)	-0.0034 (0.47)	-0.0021 (0.67)	-0.0037 (0.20)	0.0028 (0.34)
Screen Time / Day	0.0094 (0.68)	0.0512 (0.05)	0.0160 (0.26)	**0.0398 (0.01)**	0.0137 (0.65)	-0.0163 (0.73)	0.0106 (0.58)	-0.0006 (0.98)
VO2 Max	0.0003 (0.18)	0.0002 (0.44)	-0.0001 (0.35)	-0.0002 (0.18)	-0.0001 (0.49)	0.0001 (0.82)	**-0.0004 (0.01)**	-0.0001 (0.40)
Insulin resistance and lipid profile								
Matsuda ISI	**-0.0574 (0.03)**	-0.0178 (0.77)	-0.0114 (0.49)	-0.0221 (0.53)	-0.0495 (0.11)	-0.1027 (0.07)	-0.0342 (0.08)	**-0.0673 (0.04)**
HDL Cholesterol	-0.0186 (0.92)	0.4482 (0.31)	**-0.2618 (0.03)**	-0.1682 (0.51)	0.0290 (0.90)	0.8005 (0.10)	-0.0667 (0.64)	0.1047 (0.71)
LDL Cholesterol	-0.1009 (0.27)	-0.3200 (0.08)	-0.0175 (0.76)	-0.1078 (0.31)	0.0712 (0.40)	-0.0356 (0.84)	0.0040 (0.94)	0.0172 (0.87)
Triglycerides	**0.2945 (0.05)**	0.2714 (0.21)	0.1565 (0.09)	0.1088 (0.39)	**0.2755 (0.05)**	0.0698 (0.73)	**0.1859 (0.03)**	-0.0905 (0.44)
Parental Values								
Mother HTN	-0.2938 (0.46)	0.4709 (0.52)	0.2181 (0.38)	-0.2036 (0.64)	0.2735 (0.43)	---	0.4093 (0.06)	---
Mother BMI	**0.0162 (0.03)**	0.0226 (0.12)	0.0073 (0.12)	0.0086 (0.30)	0.0146 (0.07)	-0.0033 (0.81)	-0.0083 (0.10)	-0.0030 (0.71)
Father BMI	-0.0088 (0.32)	0.0068 (0.62)	-0.0006 (0.92)	0.0050 (0.53)	-0.0037 (0.68)	0.0127 (0.50)	0.0048 (0.39)	-0.0064 (0.55)
Miscellaneous								
Season (wintertime)	**0.2108 (0.03)**	0.3062 (0.08)	0.0502 (0.41)	0.1146 (0.25)	0.0232 (0.82)	0.3199 (0.08)	0.0008 (0.99)	0.1088 (0.30)
Smoking status	---	0.5887 (0.43)	---	-0.1830 (0.67)	---	**0.8156 (0.03)**	---	**0.5857 (0.01)**
Adjusted R^2	0.1195	0.0751	0.0756	0.0587	0.0887	0.0992	0.1669	0.0931

Values with p-values < 0.20 were included; **values with p-values < 0.05 are shown in bold**
Smoking status only included in V2 models (highlighted in yellow)

4.3 Post-reflections of manuscript 2

Knowing the determinants of BP in youth, and knowing how they change throughout childhood and adolescence will enable researchers and clinicians to more accurately evaluate a patient's risk for developing hypertension at a specific age, and treat the patient accordingly. The above manuscript only included two visits when patients were aged 8-10 and 11-13, respectively, but the final manuscript will include a third visit at age 15-17. With that data, we expect to be able to discern trends of association strengths across time. We will use more complex longitudinal analyses (including mixed models or generalized estimating equations, allowing for repeated measurements over time), once all data are acquired. A next step in research would be to compare the associations found in our study to those in a cohort of children at the general population level of risk (those without an obese parent) to see how much familiality played a role in the observed associations. Finally, future research targeting the risk factors we identify, for predicting and altering management of patients, with the goal of long-term BP reduction, should be performed.

Chapter 5: The associations between blood pressure and left ventricular mass and intima media thickness in adolescents

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5.1 Foreword to this manuscript chapter

The manuscript in this chapter uses the data from the QUALITY-BP study, which is ancillary to the previously discussed QUALITY study. The present study's aims are to investigate the relationships between blood pressure (BP) and markers of end-organ damage, namely enlargement of the left ventricle and the presentation of albuminuria (indicative of renal damage) when participants are in late adolescence. The patients had their casual and ABPM BP measured, underwent echocardiograms and blood and urine sample analyses, and completed comprehensive lifestyle questionnaires. Of note, questionnaire data were only available for the 29 patients who underwent study visits since February 2017. Therefore, in order to maintain a sufficiently large sample size for this thesis analysis, questionnaire-derived variables, such as amount of sleep, physical activity, and drug use, were not included in the multivariable models.

In addition, the blood and urine sample data were not available at the time of writing. I have therefore focused on the associations between BP and left ventricular mass (LVM) and carotid intima media thickness (cIMT), two measures of cardiac damage. Once all of the patient visits have been completed (September 2017), the blood and urine analysis data will be cleaned

and collated, and will be available for use in Fall 2017. At that time, I will complete the rest of the analyses, revise the manuscript, obtain feedback from all authors and submit the final manuscript to either *Pediatric Cardiology*, *Pediatrics,* or *Hypertension,* depending on the significance and impact of the final results.

In youth, elevated BP is known to be associated with markers of end-organ damage, the most common of which is left ventricular hypertrophy (LVH); up to 41% of hypertensive children and adolescents present with LVH [1]. This study evaluates the associations between LVH and cIMT and a number of other physical measures, including BP, in an at-risk adolescent population. The BP used is derived from ABPMs, the gold standard for robust BP measurement in youth.

5.2 Manuscript 3

Abstract

Background and objectives

Elevated left ventricular mass (LVM) and carotid intima media thickness (cIMT) are often the result of a progressive strain on the heart through unhealthy lifestyle practices and poor physical condition. The most common contributors to abnormal LVM and cIMT are high adiposity and high blood pressure (BP). Yet it has not been established whether daytime BP, nighttime BP, or both contribute to cardiac pathologies. It is also unclear the extent to which different methods of scaling LVMI affect its association with BP.

Design, setting, participants, & measurements

We conducted a single-center cross-sectional cohort study of children at-risk for obesity, from 2016 to 2017. 134 patients aged 15 to 19 years were recruited from a concurrently performed longitudinal study (inclusion and exclusion criteria: had to have at least one obese parent and not be suffering from any serious medical condition). A study visit was conducted throughout one morning, during which data were collected on the patients' anthropometric, biochemical, and lifestyle factors, and echocardiogram was performed. Univariable and multivariable linear regressions were performed between selected factors and the patients' LVMI, cIMT, and BP.

Results

The mean age of participants was 17.2 years, and approximately 36% were overweight or obese. In the univariable analyses, only boys' adiposity variables were significantly associated with

LVMI. No variables from either gender were associated with BP or cIMT. In the multivariable analyses, in which boys and girls were grouped together, BMIz and lean body mass were significantly associated with LVMI, while BP was associated with cIMT. Correlations between BP and LVMI (scaled by height$^{2.7}$, BSA, or LBM) were substantially different depending on the scaling factor used.

Conclusion

Our study suggests that adiposity is associated with LVMI and BP is associated with cIMT, regardless of the period in which BP was examined (daytime, nighttime, or 24-hours). Method of scaling LVMI significantly alters findings on associations with BP. Future studies should focus on determining which LVMI scaling factor is most appropriate and most indicative of cardiac damage in adolescents.

Introduction

Elevated blood pressure (BP) is associated with organ damage throughout childhood and adolescence. The most common forms of damage among youth with hypertension are cardiac abnormalities, especially left ventricular hypertrophy (defined in children as LVM >95th percentile) and increased carotid intima media thickness (cIMT), present in 41% and 39% of hypertensive adolescents, respectively [2]. Obesity, which is accompanied by increased metabolic demands, unhealthy lifestyles, and various potentially severe health problems, is also strongly associated with LVH and increased cIMT [3, 4]. Among hypertensive and obese individuals, practicing healthy habits, such as exercising regularly, can greatly reduce the propensity to develop cardiac abnormalities [5, 6]. LVH and elevated cIMT put young patients at a significantly greater risk of suffering future morbidity and mortality, such as strokes and myocardial infarctions [7, 8].

When evaluating the left ventricular mass (LVM) of patients to determine if it is excessively elevated, one must account for the patient's body size. LVM increases with body growth, and particularly with adiposity [9]. Three factors are commonly used to index LVM: height$^{2.7}$, body surface area (BSA), and lean body mass (LBM). LBM has been suggested to be a more robust and representative scaling factor for LVM [10]; however, its clinical use is limited because in order to determine LBM, the patient must undergo a bioelectric impedance analysis or a duel-energy X-ray absorption test. Foster et al. have generated an equation that calculates a

patient's LBM using his/her age, height, weight, and BMI z-score. The equation was validated in a population of children and adolescents that spanned the adiposity spectrum [11].

Ambulatory BP monitoring (ABPM) is an essential, and often underutilized, tool to measure BP in clinical settings. ABPM is more informative than casual BP screening [12], and is a more powerful predictor of future morbidity and mortality [13, 14]. It also allows researchers to distinguish between day and nighttime BP, each of which can have different contributions to cardiac outcomes [15].

The aim of this study is to evaluate the associations of daytime, nighttime, and 24 hour BP, BMI and LBM, with LVMI and cIMT in a cohort of adolescents at risk for obesity. We hypothesized that BP, BMI, and LBM will all be consistently associated with these measures of cardiac damage. We also aim to compare the methods of LVMI scaling and to understand the extent to which scaling method affects the relation between LVM and BP.

Methods

Study design & cohort

The Quebec Adiposity and Lifestyle Investigation in Youth Blood Pressure (QUALITY-BP) study is a prospective cross-sectional study, with an aim to evaluate the associations between elevated blood pressure (BP) and markers of end-organ damage in adolescents. Participants in QUALITY-BP were recruited from the QUALITY cohort, a longitudinal three-visit study that was conducted from 2005-2016. In order to participate in the original QUALITY study (at inception in 2005), each child had to have at least one obese biological parent. Obesity in the parent was defined using either BMI (>30 kg/m^2) or waist circumference (women: >88cm; men: >102cm) thresholds. Children were excluded from the study if they had types 1 or 2 diabetes, were taking antihypertensive medication or steroids, had serious psychological or cognitive problems, or were suffering from an illness that precluded administration of any of the study measurements. After the third QUALITY study visit (2016, aged 17 to 19 years old), the adolescents were contacted by phone for recruitment into the QUALITY-BP study, which was conducted from 2016-2017 at Centre Hospitalier Universitaire (CHU) Sainte-Justine in Montreal, Canada. All patients who completed the third QUALITY study were eligible for participation in QUALITY-BP. The QUALITY-BP study protocol was approved by the Institutional Ethics Review Board at the McGill University Health Centre and CHU Sainte-

Justine. Prior to any study activities, all participants under the age of 18 provided informed assent and their parents signed written consent forms, and those patients above 18 provided written consent.

Study visit procedures and variable descriptions

Figure A1 in the appendix describes the QUALITY-BP study visit day. Before the visit, consenting patients performed a 24-hour urine collection. At the start of the visit, patients were given $20 for their participation in the study. On the day of the study visit, the subjects underwent tests and procedures described below.

24-hour urine collection

In the week prior to the study visit, willing participants were sent a container and instructions detailing how to perform a 24-hour urine collection. Patients were told to conduct their normal daily activities, and to store the container of urine at $4^{o}C$ (in their refrigerator) when not actively depositing urine. The patients brought the urine collection with them on their visit day. Upon their arrival to CHU Sainte-Justine on the morning of the visit, the urine was immediately stored in a refrigerator at the hospital, and was kept there until the time of analysis. Urine albumin, creatinine, sodium, and potassium concentrations were measured at the CHU Sainte-Justine Biochemistry laboratory.

Anthropometry & adiposity

Weight measurements were taken using an electronic scale, and the values were recorded to the nearest 0.1kg. Height measurements were taken at the patient's maximal inspiration, using a stadiometer, and values were recorded to the nearest 0.1cm. BMI was calculated (weight/height^2 (kg/m^2)), and sex and age-specific BMI percentiles and z-scores were calculated [16, 17]. Overweight was defined as BMI percentile $\geq 85^{th}$ percentile and $<95^{th}$ percentile; obesity was defined as BMI $\geq 95^{th}$ percentile.

Waist circumference (WC) was measured using a standard tape measure, at the midpoint between the lowest rib and the iliac crest. Hip circumference (HC) was measured at the widest part of the buttocks. Values for both WC and HC were recorded to the nearest 0.1cm.

All of the patients' measurements were recorded with the patient wearing only light indoor clothing without shoes, and with pockets emptied. Each measure was taken twice, with a third measurement taken if the two values differed by more than 0.2kg or 0.2cm. For each evaluation, the mean of the two closest values was used in the analyses.

Because DEXAs were not performed on this cohort, lean body mass (LBM) was estimated using an equation created by Foster et al. [11]. The equation uses age, height, weight, and BMI z-score to predict patient LBM, and has been validated in the QUALITY cohort (data not shown) and independently in a separate cohort of children [11].

Patient questionnaire & FFQ

Patients were asked to complete a lifestyle questionnaire that appraised their daily activity and fitness, drug and cigarette use, and sleep habits. In addition, patients filled out a food frequency questionnaire (FFQ), to assess how often they consume various foods containing high amounts of sodium. A nutritionist at McGill University (Montreal, Canada) calculated the sodium content in a standard serving of each food type, and using that information, the patient's average daily consumption of sodium was calculated.

Echocardiogram

Patients underwent an echocardiogram (2D imaging, M-mode, and Tissue Doppler Imaging), and aorta and carotid artery ultrasounds. Echocardiogram and ultrasound measures were taken using a Philips IE33 cardiac ultrasound scanner (Bothell, Washington, USA) according to American Society of Echocardiography (ASE) guidelines, by one of three technicians. The images were then reviewed by a trained technician at the CHU Sainte-Justine Medical Imaging Department, who was blind to subject data.

Left ventricular mass (LVM) was calculated using the M-mode values as per methods outlined by the ASE [18]; LVM index (LVMI) was calculated using the LVM at diastole, scaled by height$^{2.7}$ [19]. This is the most common method of indexation, particularly among cohorts in which obesity is prevalent [20]. The other parameters, such as cIMT, were assessed using the appropriate software, and were not normalized.

Blood and urine sample

After the echocardiogram, blood and urine samples were collected. At the time of the blood draw, the patients had been fasting for at least 12 hours. The blood was drawn into EDTA-tubes, processed within 20 minutes, and subsequently stored at -80^{o}C. Plasma cystatin C, aldosterone, and renin were measured using validated assays.

The urine sample was centrifuged at 4000 rpm, room temperature, for 10 minutes after which it was processed. Urine albumin and creatinine concentrations were measured. All analyses were performed at the CHU Sainte-Justine Biochemistry laboratory.

Casual BP

After a light snack, patients' casual BP was measured. Patients were seated in a straight-backed chair, and fitted with the appropriate cuff size, measured using mid-arm circumference. The cuff was placed on their right arm for 5 minutes prior to BP measurement. Measurements were recorded using an automated oscillometric device (Dinamap XL, model CR9340; Critikon Co., Tampa, Florida, USA), which was regularly calibrated using a mercury sphygmomanometer. Five measurements were taken at 1-minute intervals, during which time the patient was encouraged to refrain from talking and using his/her cellular phone. The first two BP readings were dropped, and the average of the last three measures was used as the systolic and diastolic BP (SBP and DBP). For patients below the age of 18 years, percentiles of the average SBP and DBP were calculated, using age/height/sex-specific normative values from the National High Blood Pressure Education Program (NHBPEP) Fourth Report [21]. In these patients, hypertension was defined as SBP or DBP $\geq 95^{th}$ percentile. In patients above the age of 18 years, hypertension was defined as SBP > 140 mmHg or DBP > 90 mmHg. After the measurements on the right arm were completed, casual BP was measured once on the patient's left arm.

ABPM

The patient was then taught how to use the ambulatory blood pressure monitor (ABPM) (Spacelabs 90217A, Snoqualmie, WA, USA). Patients were asked to wear the ABPM device for a 24-hour period in the week following the visit. The cuff inflated every 30 minutes, day and night. Patients were given a pamphlet, in which they recorded the time at which they: went to sleep, woke up, when/if they consumed caffeinated beverages, and when/if they engaged in

intense physical activity, throughout the 24 hour period. They were also given a pre-paid envelope to return the device once they had completed the recording. Two measurements were taken during the visit day using the ABPM device, as a demonstration of its use, and to determine the patient's baseline ABPM-acquired BP.

Once the ABPM device was returned by mail to CHU Sainte-Justine, the ABPM data was uploaded to the 92506 ABP Report Management System. Using the self-reported sleep-wake times from the patient's pamphlet, the BP values were divided into daytime and nighttime measures. If the patient returned the device but not the information pamphlet, he/she was called back and asked about his/her normal sleep-wake times. If the patient was not reachable by phone, only 24-hour BP was calculated and used for analyses.

Statistical analysis

Patient characteristics

Means (with standard deviations), medians (with interquartile ranges or minimum & maximum values), or absolute counts (with percentages) were used to describe the patient physical characteristics, BP, and cardiac geometry and function. T-tests (for variables with $n \geq 25$) or Mann-Whitney U tests (for variables with $n < 25$) were performed to compare the values between genders (significance set at p-value < 0.05).

Associations of cardiac measures and patient characteristics with BP

Univariable analyses were performed to evaluate the associations of various patient cardiac, anthropometric, and adiposity-related characteristics with LVMI, cIMT, and BP: simple linear regressions and Pearson correlation tests were performed to evaluate associations between each variable and outcome. In evaluating the associations of each cardiac measure, including LVMI and cIMT, with SBP and DBP, sex-stratified correlation analysis was performed, evaluating different BP measurement methods from ABPM, separately (24-hour, daytime, nighttime, casual).

In order to evaluate the independent associations of different factors such as BP, with cardiac abnormalities, we performed separate multivariable linear regression analyses using either LVMI or cIMT as the outcome. Collinearity was not severe in the variables considered ($r < 0.60$ for all variables). The same multivariable analyses (i.e., including the same variables) were

performed but using the different BP measurement methods from ABPM (24-hour, daytime, nighttime, casual), in order to determine if the factors associated with LVMI and cIMT outcomes were the same at each time period. We also performed one set of analyses that included a factor assessing the presence of nighttime BP dipping ("Non-Dip").

Separate multivariable regressions were performed in order to determine the associations between several patient characteristics and LVMI and cIMT (Table 4). Individual regressions were done, including either 24-hour BP (Table 4A), daytime BP (4B), nighttime BP, or nighttime dipping (4C), and each was stratified by SBP and DBP. In order to maximize the sample size, male and female patients were combined into a single cohort, and gender was added as a variable in the model. BMIz, LBM, age, and gender were included in the multivariable models (Table 3). BMIz and LBM are commonly used indices of adiposity, and are known to be associated with cardiac outcomes; age can influence the cardiac geometry and the degree to which damage is observed; gender is an important consideration in the interpretation of cardiac measures. The other variables may have also contributed to elevated LVMI and cIMT, but their sample sizes were too small (n <15) to be included in the models.

All results regarding urinary indices, 24-hour urine collections, and FFQ are not included, as the data are still being compiled. For all multivariate models, no interaction terms were included. Beta-coefficients and p-values were reported. All analyses were done using STATA/SE 12.1 statistical software (StataCorp, Inc.).

Results

Patient characteristics:

Table 1A shows selected anthropometry, adiposity, and lifestyle characteristics in the cohort. The average age for boys and girls was 17.1 and 17.4 years, respectively. Both the age and the percentage of participants over 18 (boys: 28.6%; girls: 42.9%) were significantly greater in girls. In contrast, height and LBM were greater in boys than in girls. No other variable was significantly different across sex groups. The average BMI was 24.9, which corresponds to approximately the 72nd percentile (as per CDC, WHO) [16, 17]. 35.8% of participants were overweight or obese (Table 1A).

Table 1B shows the office and ABPM BP measures. None of the BP types was significantly different across sex groups. The ABPM SBP and DBP means were 112 mmHg and

65 mmHg respectively, well below the adult ambulatory hypertension thresholds of 135 and 85 mmHg. Few participants suffered from ambulatory (5.7%), masked (1.8%), or white-coat hypertension (1.8%). However, the majority of participants (>85%) had insufficient nighttime BP dipping (Table 1B).

Table 1C shows the characteristics relating to cardiac geometry and function. LVMI scaled by BSA was significantly different between boys and girls, although LVMI scaled by height$^{2.7}$ and by LBM were not. Left ventricular internal diameter, interventricular septum, aorta root diameter, and left atrium diameter were all significantly greater in boys than in girls. The values for all other variables were relatively equivalent for both sex groups (Table 1C).

Correlations between BP and patient characteristics with LVMI and cIMT

Table 2 shows the Pearson correlations between 24-hour SBP and DBP and cardiac measures, stratified by sex. Tables A1-3 in the appendix show the correlations with cardiac measures using daytime, nighttime, and office SBP and DBP. There was significant variation in the correlation of variables with LVMI, depending on the index used (Table 2). In the case of LVM indexed using height$^{2.7}$ as the scaling factor, the correlations with 24-hour BP were negative in boys, but positive in girls. With LVM indexed using BSA, the correlations with 24-hour BP were mostly positive, but were stronger for SBP than DBP. LVM indexed using calculated LBM showed no consistent pattern of association with BP (Table 2).

The average and maximum carotid intima media thickness (cIMT) were positively correlated with BP, although the correlations were stronger with SBP than with DBP. The associations of BP and other important geometric and function-related cardiac measures are also shown (Table 2). Most of the analyses resulted in substantial differences in the correlation coefficients for boys *vs.* girls and/or SBP *vs.* DBP (Table 2). Moreover, in Tables A1-3, we see that in general the correlations for most of the variables were stronger with daytime BP than nighttime BP, and even stronger with casual BP.

Univariable Regression

Table 3 shows simple linear regression and Pearson correlation coefficients between the physical and lifestyle characteristics listed in Table 1 and LVMI and cIMT. Table A4 in the appendix shows the simple linear regressions for associations between patient characteristics and BP.

Several of the anthropometric and adiposity variables were significantly positively associated with LVMI in boys, but were not significantly associated with LVMI in girls (Table 3). Similarly, many variables were not associated with cIMT. Neither age nor waist and hip circumference were significantly associated with LVMI or cIMT. The lifestyle variables also showed no pattern of consistent association with either LVMI or cIMT (Table 3).

With regard to the associations between general patient characteristics and BP (Table A4), age was significantly associated with DBP for both genders, but not with SBP.

Multivariable regression models predicting LVMI

In the 24-hour and daytime BP models, only higher BMIz and LBM were significantly associated with LVMI (Tables 4A & 4B). Magnitudes of association with LVMI of BMIz were greater than any other variable included, and the associations were slightly stronger in models including SBP *vs.* DBP. LBM was negatively associated with LVMI, and had stronger associations in the daytime BP models than the 24-hour BP models.

In the nighttime model, only BMIz was a significant predictor of cIMT (Table 4C). In the nighttime dipping model, in which non-dipping was included as a binary variable (Non-Dip) BMIz and LBM were both significantly associated with cIMT. The adjusted R^2 for the models ranged from 0.31 to 0.37, indicating strong abilities for the models to predict LVMI (Table 4C).

Multivariable regression models predicting cIMT

For the 24-hour and daytime BP models predicting cIMT, only BP was significantly associated with cIMT (Tables 5A & 5B). In both the 24-hour and daytime models, SBP was more strongly associated with cIMT than DBP was (Tables 5A & 5B). For the nighttime BP and non-dipping models, no variables were significant, and the adjusted R^2 values were close to 0 (Table 5C).

Discussion

This study evaluates the associations between adolescent BP and cardiac damage, namely LVMI and cIMT. We found that LVMI was most strongly associated with adiposity, whereas cIMT was associated with BP. There were no substantial differences between the results using daytime, nighttime, or 24-hour BP.

The average and maximum cIMT were positively associated with BP, which affirms previous research showing a strong link between ABPM measures and cIMT [22]. A longitudinal Finnish study found that SBP, but not DBP, in adolescence predicted future cIMT, due to BP inducing progressive alterations in arteries and contributing to the development of atherosclerosis [23]. We also found that the associations for SBP were stronger than for DBP.

BMIz was associated with boys' LVMI. This finding is corroborated by a plethora of data suggesting that adiposity, directly and indirectly contributes to increased LVMI in children [24-26] (for further discussion on this topic, see Chapter 3). In fact, Yoshinaga et al. found that BMI was *the only* significant predictor of LVMI (although in their study LVM was normalized using height raised to different allometric powers: 3.1 for boys, 1.9 for girls) [27]. It is unclear why this association was not present for the girls in our cohort as well. In the multivariable associations, BMIz again emerged as a significant factor associated with LVMI. Of note, however, in the multivariable models, boys and girls were analyzed jointly in order to have a larger cohort population, so we did not differentiate between associations in boys *vs.* girls.

In addition, LBM was significantly associated with BP in most of our models. Of note, LBM calculation utilizes BMIz, though LBM and BMIz did not show excessive collinearity. However, the fact that both were significant predictors of BP was not surprising given that they are both measures of adiposity.

In the univariable analyses, cIMT was not associated with any of the variables tested. This is surprising in light of the many studies showing associations between BMI and cIMT [23, 28]. The sample sizes for these analyses were fairly low; it is therefore worth noting that while we rarely found significant associations, the associations were mostly in the directions expected. BP was the most consistently significant variable in the daytime and 24-hour cIMT models.

Analyses were also performed between selected patient characteristics and 24-hour BP (Table A4). We found that weight was more strongly associated with SBP than with DBP. However, we also found that BMIz was negatively associated with male BP, but positively associated with female BP, which is contrary to usual findings in a pediatric population [29]; we would expect BMIz to be strongly and positively associated with BP in both genders. Our limited sample size may have led to imprecision of our estimates of association.

As predicted, more sleep hours was associated with lower 24-hour BP. This may be because of the physiological effects of sleep or because of a prolonged nocturnal dipping effect

in the patients. Remarkably, physical activity was found to be *positively* associated with BP in girls. This could be the result of patients using the ABPM during periods of exercise, although it is unclear why the association is female-specific. Regular cigarette use was negatively associated with BP. The effects of smoking on BP are unclear (see Chapter 2) but it does reduce anxiety in users, which may serve to lower BP in the short term.

We also found that, as expected, associations of variables with LVMI had different trends, depending on the scaling factor used. The degree to which this occurred highlights the importance of further studies to determine the most appropriate scaling factor.

All mean LVMIs were within the healthy range of values, with no patient deemed to have LVH, according to adult thresholds. In LVMI scaled for LBM only, girls had a significantly greater LVMI than boys, while the LVMIs calculated using other methods did not differ significantly across gender. It is important to note that the various LVMI scaling factors used in this study are not equally robust; neither scaling by height nor BSA adequately accounts for obesity, whereas LBM does [11]. Nevertheless, only LVMI scaled by height$^{2.7}$ was included in the multivariable analyses because of its widespread use in clinical and research settings.

In examining the associations between the cardiac measures and 24-hour BP, each of the LVMIs had distinctly different association patterns: LVMI (height$^{2.7}$) was negatively associated with BP in boys and positively with BP in girls; LVMI (BSA) was positively associated with all BP values, and much more strongly with SBP than DBP; and LVMI (LBM) was negatively associated with BP in girls, but positively with BP in boys. The reason for the differences within each LVMI is unclear, although they may be because of the differences in scaling factor values between genders (e.g. LVM is significantly greater in boys *vs.* girls). Inconsistences *between* the indices are expected, although they make diagnoses of LVH more ambiguous. Analyses by Mahgerefteh et al. have shown that rates of LVH change depending on the criteria used to diagnose it [30]. There is still no consensus on which scaling factor is most appropriate for widespread use in pediatric populations.

Absence of nighttime dipping was fairly common, with 57% and 80% of patients having insufficient drops in their SBP and DBP, respectively while sleeping. Non-dipping is normally found relatively frequently in adolescents with elevated adiposity; a study on obese adolescents found that 50% of participants had systolic non-dipping [31]. Alternatively, the inflation of the ABPM device during the night may have disrupted the patient's sleep, resulting in elevated BP.

The main limitations of this study were related to the sample size. Our sample size of 134 patients, 111 of whom underwent echocardiograms, made it difficult to obtain precise results. In addition, for the questionnaire-derived data, including all lifestyle variables, the sample size was limited to only 29 patients, effectively preventing us from including those variables in the multivariable models, because that would have drastically reduced our sample size. Very few of our patients had systolic or diastolic hypertension, so that hypertension could not be used as a binary measure. Also, z-scores were unavailable for certain variables that should be normalized, e.g. height, weight, LBM, and LVMI. Only BMI z-scores were available. In future analyses, all of the aforementioned variables will be converted to population z-scores, allowing for more meaningful interpretation of the data and comparisons between genders.

While other studies have evaluated the relationships between BP and LVM, ours is noteworthy for several reasons: (i) our cohort is at-risk for hypertension and obesity, having had at least one obese parent at the time of QUALITY recruitment. (ii) We included lean body mass (LBM) in our analyses. Normally, to determine LBM, researchers must perform bioimpedance analysis or dual-energy X-ray absorptiometry, tests that are complex and often unavailable. We used a recently developed equation that uses only widely-available anthropometric variables to calculate LBM. (iii) We used ABPM-derived BP values in our analyses. Most of the studies on this topic use casual BP, which can be tainted by the patients' anxiety of the healthcare administrators and the hospital setting. The ABPM evaluates BP in the patients' usual setting, and allowed us to examine how daytime, nighttime, and 24-hour BP each contribute individually to cardiac pathologies.

To conclude, BMIz and LBM were significantly associated with LVMI in boys, while BP was a significantly associated with cIMT in both genders. These associations were present regardless of whether we were using 24-hour, daytime, or nighttime BP in our analyses. There were also significant differences in the strengths and directions of associations of each calculated LVMI ($height^{2.7}$, BSA, and LBM) with BP, although that topic was not fully explored in this study. Future studies should evaluate the each LVMI scaling method for predicting outcomes, in order to determine which is the most accurate for clinical use.

Manuscript 3 references

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Manuscript 3 table and figures

Table 1. Patient characteristics, stratified by gender

1A. General physical and lifestyle characteristics.

Characteristic	Male	Female
Age	n = 77	n = 57
Age, mean (SD)	17.1 (1.21)	17.4 (1.18)*
≥ 18 years old, n (%)	22 (28.6)	24 (42.9)*
Anthropometry & Adiposity	n = 77	n = 57
Height, mean (SD)	174.0 (9.35)	168.6 (9.89)*
Weight, mean (SD)	74.9 (18.3)	71.2 (19.5)
BMI, mean (SD)	24.8 (6.07)	24.9 (5.21)
BMI z-score, mean (SD)	0.54 (1.22)	0.69 (0.98)
Overweight individuals, n (%)	11 (14.3)	11 (19.6)
Obese individuals, n (%)	17 (22.1)	9 (16.1)
Calculated Lean Body Mass, mean (SD)	55.7 (10.9)	45.5 (9.1)*
Hip & Waist Circumference	n = 16	n = 14
Waist circumference, median (IQR)	79.1 (74.1 to 95.5)	74.7 (71.6 to 79.1)
Hip circumference, median (IQR)	100.6 (97.4 to 109.3)	100.0 (95.2 to 101.5)
Lifestyle	n = 15	n = 14
Sleep (hours/day), median (IQR)	8.0 (7.5 to 9)	8.68 (8.46 to 9.07)
60+ min sessions of activity / week, median (min/max)	4 (1 to 7)	2.5 (1 to 6)
Active (>2 sessions / week), n (%)	12 (80)	7 (50)
Smoked in past month, n (%)	3 (20.0)	1 (7.1)

T-tests or Mann-Whitney U tests were conducted on the values between genders.
* = Inter-gender comparison (Male *vs.* Female) significant at $p < 0.05$

1B. Blood pressure characteristics.

Characteristic	Male	Female
Casual Blood Pressure	n = 15	n = 14
Systolic BP, median (IQR)	113.3 (105.7 to 120.7)	108.3 (104.7 to 112)
Diastolic BP, median (IQR)	61.7 (55.7 to 66)	63.5 (59.3 to 69.3)
ABPM Blood Pressure		
24-hour, mean (SD)	n = 56	n = 43
SBP	112.4 (8.0)	110.9 (7.4)
DBP	64.2 (5.5)	64.9 (6.2)
Daytime, mean (SD)	n = 58	n = 49
SBP	117.3 (8.1)	115.4 (8.0)
DBP	69.0 (5.9)	69.3 (6.6)
Nighttime, mean (SD)	n = 59	n = 47
SBP	104.3 (9.5)	103.5 (7.6)
DBP	56.2 (5.8)	57.3 (6.3)
ABPM Hypertension & Dipping		
24-hour HTN, n (%)	n = 56	n = 43
SBP	1 (1.8)	1 (2.3)
DBP	0 (0.0)	1 (2.3)
Daytime HTN, n (%)	n = 58	n = 49
SBP	1 (1.8)	2 (4.1)
DBP	1 (1.8)	2 (4.1)
Nighttime HTN, n (%)	n = 59	n = 47
SBP	3 (5.2)	3 (6.7)
DBP	3 (5.2)	2 (4.4)
White-Coat HTN, n (%)	n = 30	n = 27
	0 (0.0)	1 (3.9)
Masked HTN, n (%)	n = 30	n = 25
	0 (0.0)	1 (3.9)
Absence of Nighttime Dipping, n (%)	n = 56	n = 44
SBP	37 (66.1)	23 (52.3)
DBP	47 (83.9)	38 (86.4)

T-tests or Mann-Whitney U tests were conducted on the values between genders. No pairs were significantly different.
Casual BP data were only available for 29 participants at the time of writing. When the rest of the data is ready to be used, it will be included in the analyses and the n will be substantially higher.

1C. Cardiac geometry and function characteristics.

Characteristic	Male	Female
Cardiac Geometry	n = 69	n = 52
LVMI (Ht^2.7), mean (SD)	27.0 (5.4)	27.5 (4.0)
LVMI (BSA), mean (SD)	63.3 (8.3)	62.9 (9.3)
LVMI (LBM), mean (SD)	2.18 (0.27)	2.52 (0.37)*
LVID, mean (SD)	48.3 (2.9)	46.8 (3.6)*
LVPW, mean (SD)	7.96 (1.49)	7.85 (0.87)
IVS, mean (SD)	7.22 (0.85)	6.95 (0.88)*
AO, mean (SD)	27.1 (3.1)	26.0 (2.6)*
OG, mean (SD)	32.4 (3.4)	30.8 (3.6)*
Beta Stiffness, mean (SD)	9.97 (2.56)	9.47 (2.63)
cIMT avg, mean (SD)	0.46 (0.08)	044 (0.07)
cIMT max, mean (SD)	0.57 (0.08)	0.56 (0.08)
Cardiac Function		
EF (teich), mean (SD)	59.3 (4.4)	59.2 (4.5)
MV E/A, mean (SD)	1.97 (0.39)	1.86 (0.42)
E/e' lat, mean (SD)	4.75 (0.96)	4.98 (0.89)

T-tests or Mann-Whitney U tests were conducted on the values between genders.
* = Inter-gender comparison (Male *vs.* Female) significant at $p < 0.05$

Abbreviations: LVMI = left ventricular mass index; LVID = left ventricular internal diameter; LVPW = left ventricular posterior wall; IVS = interventricular septum; AR = aorta root diameter; LA = left atrium diameter; cIMT Avg = average carotid intima-media thickness (post wall); cIMT Max = maximum carotid intima-media thickness (post wall); EF = ejection fraction; MV E/A = mitral valve E peak/mitral valve A peak; E/e' lat = early mitral inflow velocity/mitral annular early diastolic velocity

Table 2. Correlations between cardiac measures and 24-hour BP, stratified by gender and SBP & DBP.

	Male		Female	
Characteristic	24-hour SBP	24-hour DBP	24-hour SBP	24-hour DBP
Cardiac Geometry	n = 65	n = 50	n = 40	n = 40
LVMI (height$^{2.7}$)	r = -.01	r = -.23	r = .17	r = .02
LVMI (BSA)	**r = .25**	r = .02	r = .05	r = -.07
LVMI (LBM)	r = .14	r = -.01	r = -.01	r = -.04
LVIDd	r = .27	r = -.04	r = .08	r = -.20
LVPWd	**r = .28**	r = .12	**r = .30**	r = .10
IVSd	r = .18	r = .13	r = .15	r = -.06
AR	**r = .29**	**r = .32**	r = .12	r = .03
LA	r = .13	r = .00	r = .07	r = -.19
Beta Stiffness	r = .17	**r = .27**	**r = .33**	**r = .35**
cIMT avg	**r = .35**	r = .06	**r = .33**	**r = .31**
cIMT max	**r = .33**	r = .10	r = .23	r = .19
Cardiac Function				
EF (teich)	r = -.02	r = .01	r = .00	r = -.04
MV E/A	r = .23	r = -.11	r = .01	r = -.17
E/e' lat	r = -.02	r = -.12	r = .14	r = .06

Values with p < 0.05 are shown in bold.

Table 3. Univariable associations between physical and lifestyle variables and SBP & DBP, stratified by gender.

	Male		Female	
Characteristic	24hr SBP	24hr DBP	24hr SBP	24hr DBP
Age	n = 72	n = 72	n = 52	n = 52
Age	1.13 (0.15) r = 0.17	**1.37 (0.01)** **r = 0.31**	0.63 (0.49) r = 0.10	**1.35 (0.05)** **r = 0.27**
≥ 18 years old	1.74 (0.41)	**3.03 (0.03)**	2.70 (0.21)	2.50 (0.13)
Anthropometry & Adiposity	n = 72	n = 72	n = 52	n = 52
BMI	-0.13 (0.42) r = -0.10	-0.20 (0.06) r = -0.23	0.30 (0.14) r = 0.21	0.03 (0.83) r = 0.03
BMI z-score	-0.61 (0.43) r = -0.09	**-1.05 (0.04)** **r = -0.24**	1.96 (0.06) r = 0.26	0.02 (0.98) r < 0.01
Overweight individuals	-0.72 (0.79)	-1.21 (0.50)	0.80 (0.77)	-1.04 (0.62)
Obese individuals	-3.07 (0.19)	-2.78 (0.07)	0.59 (0.84)	-0.13 (0.95)
Calculated Lean Body Mass	0.15 (0.10) r = 0.20	0.01 (0.90) r = 0.02	0.15 (0.20) r = 0.18	-0.04 (0.65) r = -0.06
Hip & Waist Circumference	n = 12	n = 12	n = 12	n = 12
Waist circumference	-0.06 (0.70) r = -0.13	0.03 (0.73) r = 0.11	0.23 (0.36) r = 0.29	-0.09 (0.71) r = -0.12
Hip circumference	-0.01 (0.97) r = -0.01	0.10 (0.51) r = 0.21	0.31 (0.28) r = 0.34	-0.07 (0.80) r = -0.08
Lifestyle	n = 12	n = 12	n = 12	n = 12
Sleep (hrs/day)	-1.91 (0.49) r = -0.22	-0.56 (0.72) r = -0.12	-3.02 (0.19) r = -0.40	-1.52 (0.51) r = -0.21
60+ min sessions of activity / week	1.52 (0.29)	0.21 (0.80)	1.96 (0.08)	1.72 (0.12)
Active (>2 sessions / week)	0.15 (0.98)	-0.29 (0.94)	**7.10 (0.05)**	5.29 (0.14)
Smoked in past month	-0.46 (0.94)	-1.55 (0.62)	-0.76 (0.91)	-2.64 (0.70)

Simple linear regression and Pearson correlation tests, p-values shown in brackets.
Hip and waist circumferences and lifestyle variables were only available for 24 participants at the time of writing.
Values with p < 0.05 are shown in bold.

Table 4. Univariable associations between physical and lifestyle variables and LVMI and cIMT, stratified by gender.

	Male		Female	
Characteristic	LVMI (height$^{2.7}$)	cIMT	LVMI (height$^{2.7}$)	cIMT
Age	n = 69	n = 68	n = 51	n = 50
Age	-0.244 (0.66) r = -0.055	-0.008 (0.33) r = -0.119	0.019 (0.97) r = 0.005	- 0.007 (0.43) r = -0.114
≥ 18yo	0.895 (0.53)	-0.034 (0.09)	-0.297 (0.80)	-0.014 (0.50)
Anthropometry & Adiposity	n = 69	n = 68	n - 52	n = 52
BMI	**0.657 (0.00)** **r = 0.78**	-0.001 (0.49) r = -0.085	0.171 (0.15) r = 0.205	< 0.000 (0.96) r = -0.007
BMI z-score	**3.072 (0.00)** **r = 0.718**	-0.004 (0.55) r = -0.074	0.949 (0.09) r = 0.241	0.004 (0.71) r = 0.054
Overweight individuals	-0.031 (0.99)	0.041 (0.16)	2.334 (0.07)	0.021 (0.41)
Obese individuals	**8.163 (0.00)**	-0.017 (0.43)	1.277 (0.44)	-0.018 (0.52)
Calculated Lean Body Mass	**0.239 (0.00)** **r = 0.487**	0.001 (0.44) r = 0.095	-0.050 (0.46) r = -0.105	< 0.001 (0.94) r = 0.011
Hip & Waist Circumference	n = 12	n = 12	n = 12	n = 12
Waist circumference	0.021 (0.80) r = 0.081	< 0.001 (0.61) r = 0.162	-0.007 (0.96) r = -0.014	0.001 (0.48) 0.229
Hip circumference	0.026 (0.85) r = 0.06	0.001 (0.63) r = 0.156	0.040 (0.83) r = 0.066	< 0.001 (0.85) 0.063
Lifestyle	n = 12	n = 12	n = 12	n = 12
Sleep (hrs/day)	-0.850 (0.58) r = -0.179	-0.031 (0.07) r = -0.538	0.086 (0.95) r = 0.019	-0.015 (0.23) r = -0.378
60+ min sessions of activity / week	**1.460 (0.04)**	0.012 (0.21)	1.217 (0.09)	0.001 (0.89)
Active (>2 sessions / week)	2.128 (0.57)	0.075 (0.07)	**5.187 (0.01)**	-0.007 (0.78)
Smoked in past month	-1.713 (0.65)	-0.045 (0.31)	2.669 (0.54)	---

Simple linear regression and Pearson correlation tests, p-values shown in brackets.
Hip and waist circumferences and lifestyle variables were only available for 24 participants at the time of writing.
Values with p < 0.05 are shown in bold.

Table 5. Multivariable associations between selected variables and LVMI and cIMT, stratified by 24-hour, daytime, and nighttime BP.

5A. 24-hour BP

Variable / LVMI ($ht^{2.7}$)	β	SE	P	Adjusted R^2
24-hour SBP (n = 89)				
24-hour SBP	0.04	0.05	0.42	
BMIz	3.07	0.47	**0.00**	
Age	0.3	0.32	0.35	
LBM	-0.11	0.06	**0.05**	
Gender	-1.33	0.99	0.18	
				0.326
24-hour DBP (n = 89)				
24-hour DBP	-0.05	0.07	0.43	
BMIz	2.98	0.47	**0.00**	
Age	0.41	0.33	0.22	
LBM	-0.10	0.06	0.08	
Gender	-1.23	0.99	0.22	
				0.326

Variable / cIMT (avg)	β	SE	P	Adjusted R^2
24-hour SBP	< 0.01	< 0.01	**0.00**	
BMIz	-0.01	0.01	0.25	
Age	-0.01	0.01	0.06	
LBM	< 0.01	< 0.01	0.38	
Gender	< 0.01	0.02	0.82	
				0.120
24-hour DBP	< 0.01	< 0.01	**0.04**	
BMIz	-0.01	0.01	0.19	
Age	-0.01	0.01	0.07	
LBM	< 0.01	< 0.01	0.13	
Gender	< 0.01	0.02	0.80	
				0.036

5B. Daytime BP

Variable / LVMI ($ht^{2.7}$)	β	SE	P	Adjusted R^2
Daytime SBP (n = 97)				
Daytime SBP	0.04	0.04	0.34	
BMIz	2.95	0.45	**0.00**	
Age	0.38	0.30	0.21	
LBM	-0.12	0.05	**0.03**	
Gender	-1.23	0.96	0.20	
				0.320
Daytime DBP (n = 97)				
Daytime DBP	< 0.00	0.06	0.98	

Variable / cIMT (avg)	β	SE	P	Adjusted R^2
Daytime SBP	< 0.01	< 0.01	**0.00**	
BMIz	-0.01	0.01	0.15	
Age	-0.01	0.01	0.15	
LBM	< 0.01	< 0.01	0.21	
Gender	0.02	0.02	0.40	
				0.114
Daytime DBP	< 0.01	< 0.01	**0.02**	

BMIz	2.91	0.46	**0.00**		BMIz	-0.01	0.01	0.12	
Age	0.40	0.31	0.20		Age	-0.01	0.01	0.13	
LBM	-0.12	0.05	**0.03**		LBM	< 0.01	< 0.01	0.08	
Gender	-1.27	0.96	0.19		Gender	0.01	0.02	0.45	
				0.314					0.314

5C. Nighttime BP and nighttime dipping

Variable / LVMI ($ht^{2.7}$)	β	SE	P	Adjusted R^2	Variable / cIMT (avg)	β	SE	P	Adjusted R^2
Nighttime SBP (n = 95)									
Nighttime SBP	< 0.00	0.05	0.91		Nighttime SBP	< 0.01	< 0.01	0.09	
BMIz	2.76	0.46	**0.00**		BMIz	-0.01	0.01	0.12	
Age	0.47	0.32	0.15		Age	-0.01	0.01	0.18	
LBM	-0.08	0.06	0.21		LBM	< 0.01	< 0.01	0.38	
Gender	-0.74	1.00	0.46		Gender	0.01	0.02	0.71	
				0.327					0.019
Nighttime DBP (n = 95)									
Nighttime DBP	-0.05	0.07	0.46		Nighttime DBP	< 0.00	< 0.01	0.99	
BMIz	2.76	0.46	**0.00**		BMIz	-0.02	0.01	0.09	
Age	0.54	0.33	0.10		Age	-0.01	0.01	0.38	
LBM	-0.08	0.06	0.19		LBM	< 0.01	< 0.01	0.29	
Gender	-0.74	1.00	0.47		Gender	0.01	0.02	0.74	
				0.331					-0.012
Nighttime Dip (n = 95)									
Nighttime Dip (Y/N)	2.55	1.02	**0.01**		Nighttime Dip (Y/N)	-0.01	0.02	0.72	
BMIz	3.19	0.45	**0.00**		BMIz	-0.01	0.01	0.12	
Age	0.35	0.3	0.25		Age	-0.01	0.01	0.10	
LBM	-0.12	0.05	**0.03**		LBM	< 0.01	< 0.01	0.09	
Gender	-1.36	0.95	0.16		Gender	0.01	0.02	0.68	
				0.371					0.010

Multiple linear regressions. **Values with p < 0.05 are shown in bold.**

Manuscript 3 appendix

Figure A1. Schedule of QUALITY-BP visit day

QUALITY BP Visit Schedule (Morning)

7:00	Welcome, information regarding the visit day, signing the consent form
7:15	Measurement of height and weight
7:30	Echocardiogram
8:30	Drawing of blood and urine samples
8:45	Snack
9:00	BP Measurement
9:15	Questionnaire on health and food frequency
	Explanation on utilization of ABPM
10:00	End of visit

Table A2. Correlations between cardiac measures and daytime SBP & DBP, stratified by gender.

	Male		Female	
Characteristic	Daytime SBP	Daytime DBP	Daytime SBP	Daytime DBP
Cardiac Geometry	n = 62	n = 62	n = 48	n = 48
LVMI (height$^{2.7}$)	r = -.09	r = -.22	r = .27	r = .13
LVMI (BSA)	r = .24	r = .06	r = .16	r = .05
LVMI (LBM)	r = .20	r = .10	r = .09	r = .07
LVIDd	r = .22	r = -.12	r = .13	r = -.12
LVPWd	r = .16	r = .05	**r = .35**	r = .17
IVSd	r = .08	r = .02	r = .23	r = .04
AR	**r = .27**	r = .23	r = .19	r = .14
LA	r = -.01	r = -.13	r = .18	r = -.04
Beta Stiffness	r = .10	r = .21	**r = .29**	**r = .38**
cIMT avg	**r = .32**	r = .09	**r = .36**	**r = .39**
cIMT max	**r = .29**	r = .12	r = .27	r = .26
Cardiac Function				
EF (teich)	r = .11	r = .15	r = .05	r = .03
MV E/A	**r = .34**	r = -.04	r = -.01	r = -.17
E/e' lat	r = .05	r = -.03	r = .17	r = .09

Table A3. Correlations between cardiac measures and nighttime SBP & DBP, stratified by gender.

	Male		Female	
Characteristic	Nighttime SBP	Nighttime DBP	Nighttime SBP	Nighttime DBP
Cardiac Geometry	n = 53	n = 53	n = 43	n = 43
LVMI (height$^{2.7}$)	r = -.11	r = -.13	r = .19	r = .01
LVMI (BSA)	r = .19	r = .07	r = .00	r = -.16
LVMI (LBM)	r = .08	r = .02	r = .01	r = -.07
LVIDd	r = .21	r = -.04	r = -.07	**r = -.33**
LVPWd	r = .25	r = .14	r = .20	r = .05
IVSd	r = .13	r = .09	r = .06	r = -.13
AR	**r = .28**	**r = .34**	r = .03	r = -.11
LA	r = .12	r = .06	r = -.08	r = -.28
Beta Stiffness	r = .16	r = .17	**r = .31**	r = .21
cIMT avg	r = .22	r = -.06	r = .05	r = .03
cIMT max	r = .22	r = .02	r = .10	r = .01
Cardiac Function				
EF (teich)	r = .09	r = .08	r = -.16	r = -.16
MV E/A	r = .12	r = -.20	r = .10	r = -.11
E/e' lat	r = -.16	r = -.16	r = .11	r = .08

Table A4. Correlations between cardiac measures and casual SBP& DBP, stratified by gender.

	Male		Female	
Characteristic	Casual SBP	Casual DBP	Casual SBP	Casual DBP
Cardiac Geometry	n = 12	n = 12	n = 13	n = 13
LVMI (height$^{2.7}$)	r = .26	r = -.37	r = .38	r = .13
LVMI (BSA)	r = .19	r = -.23	r = .17	r = -.07
LVMI (LBM)	r = .05	r = -.31	r = .06	r = -.15
LVIDd	r = .05	r = -.07	r = .39	r = .28
LVPWd	r = .49	r = -.05	r = .48	r = .32
IVSd	**r = .67**	r = .31	r = .08	r = -.19
AR	r = -.36	r = -.21	r = .48	r = .52
LA	r = .07	r = -.24	r = .36	r = .30
Beta Stiffness	r = -.08	r = .11	r = .17	r = .24
cIMT avg	**r = .68**	r = .45	r = -.12	r = .02
cIMT max	r = .46	r = .45	r = -.31	r = -.04
Cardiac Function				
EF (teich)	r = .06	r = -.28	r = -.13	r = .03
MV E/A	r = .06	r = .03	r = -.13	r = -.30
E/e' lat	r = -.15	r = -.19	r = .11	r = .14

Values with p < 0.05 are shown in bold.

5.3 Post-reflections for manuscript 3

Our findings regarding the adiposity-LVMI and BP-cIMT relationships are important, as they suggest that perhaps cIMT should be considered a useful measure of BP outcomes in clinical care. However, cIMT is harder to measure accurately and incorporating it into common clinical practice would require a significant amount of knowledge translation strategies.

Also, in order to use LVM in-clinic and compare the values of various patients, LVM must be scaled properly; there are several scaling methodologies that have different strengths and yield different results. Future research should thoroughly compare the LVMI scaling methods, and determine which is the most appropriate for use in the pediatric population.

Chapter 6: Conclusions

Hypertension and pre-hypertension in adults are diagnosed according to BP levels that have been shown to be associated with end-organ damage [1]. However, in pediatric populations, hypertension is defined based on normative values, with the 85th and 95th percentiles used as the thresholds for pre-hypertension and hypertension, respectively. We contend that it would be more clinically relevant and more accurate to use an outcome-based definition, similar to adults, wherein hypertension thresholds are tied to end-organ damage.

Outcome-based hypertension definitions must be age-, sex-, and possibly race-specific, as those variables are known to create divergences in BP [2, 3]. If outcome-based hypertension definitions are created and circulated among physicians, a diagnosis of hypertension would be more meaningful for physicians, and would allow for a standardized approach to treating patients with elevated BP. Ultimately, it would translate into better care for pediatric patients, and reduce the risk that they develop significant life-threating end-organ damage.

To begin to formulate such definitions requires two things: (1) to understand the factors that contribute to elevated BP at different time points throughout childhood and adolescents, and (2) to determine which BP levels induce organ damage. Our QUALITY and QUALITY-BP studies were important steps towards both of those objectives. In the former study, the participants had at least one obese parent, making them at risk for developing hypertension. While disproportionately high percentages of participants were obese or overweight, very few were actually hypertensive. This constrained us to the use of BP as a continuous outcome, instead of having a binary hypertension variable, making our results able to predict BP more specifically, but less clinically relevant. There are a number of factors associated with BP, some expected, some unexpected. Adiposity is well-known to contribute to BP, through both direct and indirect mechanisms [4-8] [9], however other factors such as familiality are less well understood (especially, as in our study, when the strength of familiality associations differs between the mother and the father). Further, we found that seemingly innocuous variables, such as what season it is, could influence patient BP. These factors should be known to physicians, so that they can minimize false diagnoses of hypertension. Also the presence of one or more risk factors in a patient should trigger increased surveillance of the patient's BP at follow-up appointments. Interestingly, the factors changed depending on the patient's age. More conclusive trends will be seen once the visit 3 data is available for analysis.

In the QUALITY-BP study, we considered the other side of the equation: the associations between BP and cardiac damage in adolescents. We found that both BMIz and LBM were significantly associated with LVMI, and BP was significantly associated with cIMT. LVMI and cIMT are important indicators of cardiac pathology, and are predictive of future cardiac events and mortality. While BMIz is easily calculated, LBM was previously ascertainable only by complicated procedures. We applied equation-derived LBM and in the future, I will perform a validation study using V1 and V2 data to compare equation-derived LBM to DEXA-derived LBM. These results can also be used to direct patient care; adolescents who present with excessive BMIz, LBM, or BP should undergo echocardiograms and/or cardiac screening. While this path of care is already recommended for elevated BP, it is not yet standard when treating obese patients. A future challenge is to define what is "excessive" for each of those factors in relation to cardiac measures. In addition, studies should evaluate whether other factors influence cardiac geometry at earlier times in childhood.

The literature review (Chapter 3) revealed that according to some studies, there may be an independent relationship between BP and LVMI. This is corroborated in the QUALITY-BP study, in which BP does not emerge as a significantly predictor for elevated LVM. Our review also highlighted the numerous factors that may impact the BP-LVM relationship. Future research should determine the capacity for each of the variables discussed to independently contribute to LVMI in youth.

The U.S. Preventative Services Task Force is opposed to the implementation of ubiquitous screening for BP in children [10]. We know from studies of other diseases, such as colorectal cancer, that prevalent screening can be an effective preventative tool in at-risk populations [10]. There is ample evidence, in this thesis and in the scholarly literature that elevated BP in youth is associated with poor short- and long-term outcomes. In addition, medications are available that can control BP without inducing significant adverse effects. So, while universal screening may be inefficient, targeted screening should be expanded broadly to include patients who present with any of the established risk factors for hypertension.

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