Blood Pressure Responses to Physical and Mental Challenges in Adolescent Males and Females

by

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ABSTRACT

Enhanced blood pressure (BP) reactivity from stressors predicts hypertension, a disease more common in men than women throughout their reproductive age. Visceral obesity and genetic predisposition are major risk factors for hypertension. Here, we investigated whether visceral fat (VF) and one of the best-established loci of hypertension, *CYP17A1*, contribute to the sex difference in BP reactivity. In a community-based sample of 596 adolescents, we measured beat-by-beat BP during posture and math-stress tests, quantified visceral fat with magnetic resonance imaging, and genotyped all subjects at *CYP17A1*. Adolescent males versus females demonstrated greater BP reactivity to physical and mental stressors. In males but not females, VF was associated with higher BP reactivity to active standing and a specific variant of *CYP17A1* was associated with higher BP reactivity to math stress. VF and *CYP17A1* contribute to the observed sex-differences in BP reactivity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACE – angiotensin-converting enzyme

ACh – acetylcholine

ACTH – adrenocorticotropic hormone

AGT – angiotensinogen

AMBP – ambulatory blood pressure monitoring

Ang – Angiotensin

ANS – autonomic nervous system

AR – androgen receptor

ATP – adenosine triphosphate

AT_1/AT_2 – angiotensin receptor 1 or 2

BP – blood pressure

BMI – body mass index

BSA – body surface area

cAMP – cyclic adenosine monophosphate

CNS – central nervous system

CO – cardiac output

CRH – corticotropin-releasing hormone

CRP – C-reactive protein

CYP17 – cytochrome P-450c17

DBP – diastolic blood pressure

DHEA – dehydroepiandrosterone

DNA – deoxyribonucleic acid

DXA – dual-energy x-ray absorptiometry

E – epinephrine

eNOS – endoplasmic nitric oxide synthase

ER – endoplasmic reticulum

ERα/ERβ – estrogen receptor alpha or beta

FFA – free fatty acid

FFM – fat free mass

FM – fat mass

GH – growth hormone

GnRH – gonadotropin-releasing hormone

GWAS – genome-wide association study

h^2 – heritability

HDL – high-density lipoprotein

HPA – hypoathalmic-pituitary-adrenal axis

HR – heart rate

IBI – inter-beat interval

IL-1/IL-6 – interleukin 1 or 6

iNOS – inducible nitric oxide synthase

LBNP – lower body negative pressure

LC/NE – locus ceruleus-norepinephrine system

LDL – low-density lipoprotein

MAP – mean arterial pressure

MRI – magnetic resonance imaging

MSD – mean successive difference

NADPH – nicotinamide adenine dinucleotide phosphate

NE – norepinephrine

NO – nitric oxide

NOS – nitric oxide synthase

PNS – parasympathetic nervous system

POMC – pro-opiomelanocortin

PP – pulse pressure

PPARγ - peroxisome proliferator-activated receptor gamma

PR-A/PR-B – progesterone receptor A or B

QTL – quantitative trait loci

RAAS – Renin-angiotensin-aldosterone system

RNS – reactive nitrogen species

ROS – reactive oxygen species

SBP – systolic blood pressure

SF – subcutaneous fat

SHR – spontaneously hypertensive rat

SNS – sympathetic nervous system

SNP – single nucleotide polymorphism

SV – stroke volume

SYS – Saguenay Youth Study

TBF – total body fat

TNF-α – tumour necrosis factor alpha

TPR – total peripheral resistance

VF – visceral fat

VSMC – vascular smooth muscle cell

1. INTRODUCTION

Hypertension is a known risk factor for cardiovascular disease, the main cause of death worldwide[1]. The prevalence of hypertension is growing throughout the world, affecting 65 million people, one third of the adult population, in the United States alone[2]. In Canada, the prevalence is similar, with 27% of Canadians being hypertensive[3]. Despite its high incidence, hypertension remains one of the most modifiable risk factors for cardiovascular disease in Canada and globally[4, 5]. Understanding the mechanisms of this growing health problem is essential in developing effective prevention and treatment measures.

The pathogenesis of hypertension has its origins in childhood[6, 7]. Thus pediatric cardiovascular research has focused on identifying risk factors that may play a role in the early pathophysiology of hypertension. Blood pressure (BP) reactivity during childhood and adolescence is an important area of research because it is a strong predictor of hypertension in adulthood. High BP responses to physical and mental challenges enhance the pressure load on the vessels, heart and kidneys, leading to their structural and functional changes that in turn contribute to chronic BP elevation. Like resting BP, these BP responses are influenced by risk factors such as sex, obesity and genetic predisposition. Adolescence is a period of preclinical hypertension when the initial stages of high BP become apparent, without being complicated by confounding variables such as medication. Identifying early signs and symptoms of hypertension in this age group provides an opportunity for prompt intervention.

In this introduction (Chapter 1), I first define BP reactivity and recovery from stress and then describe the major physiological systems that regulate BP, with an emphasis on those involved in controlling short-term BP responses to stressors: the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal axis (HPA axis). Next, I outline ways to assess BP reactivity and recovery and the wide variety of stressors used to test these parameters, along with a brief description of the physiological responses generated by each stressor. I then explore the relationship between enhanced BP

reactivity and delayed BP recovery and the future development of hypertension. In the remainder of the introduction, I examine how sex, obesity and genetics modulate these BP responses, concluding with the aims of my research.

Chapter 2 of my thesis consists of the methods, describing in detail the study participants and how the assessments were carried out. My findings are then presented in two manuscripts: Manuscript 1 examines the role of visceral fat (VF) in BP responses to physical and mental challenges in adolescent males and females (Chapter 3), and Manuscript 2 describes how a *CYP17A1* variant is associated with BP reactivity to mental stress in adolescent males (Chapter 4). The thesis concludes with a brief discussion and future directions (Chapter 5).

1.1 BLOOD PRESSURE REACTIVITY

1.1.1 DEFINING BLOOD PRESSURE REACTIVITY

Broadly speaking, cardiovascular reactivity is the change in a cardiovascular parameter in response to an aversive or challenging stressor, while cardiovascular recovery is the change in the parameter once the stressor is removed. Any variable measuring cardiovascular function can be assessed; these can be divided into those that reflect predominantly changes in vascular resistance (*eg*. systolic blood pressure [SBP], diastolic blood pressure [DBP]) and those that reflect mainly changes in cardiac function (*eg*. heart rate [HR], cardiac output [CO]). The most commonly assessed parameters for cardiovascular reactivity and recovery are blood pressure (BP) and HR.

BP is a measure of the force exerted by the blood on any unit area of the vessel wall[8]. BP is defined according to phases of the cardiac cycle: SBP is the pressure during contraction of the heart, systole, and DBP is the pressure during relaxation of the heart, diastole. The difference between SBP and DBP is given as pulse pressure (PP). Mean arterial pressure (MAP) is the mean blood pressure during a

single cardiac cycle, and can be estimated by multiplying CO and total peripheral resistance (TPR). Due to pressure changes throughout the cardiac cycle, MAP is not simply the mean of SBP and DBP but can be estimated as: MAP = DBP + ⅓ (SBP-DBP). Throughout medical history, BP has been recorded using a mercury manometer and, as a result, the units of BP measurement are *millimeters of mercury* (mm Hg)[8]. TPR is a measure of the resistance, or impediment to blood flow, of the entire systemic circulation. It cannot be measured directly so it is calculated as the pressure difference in the vasculature over the rate of blood flow, and is measured in *peripheral resistance units* (PRU)[8].

HR, typically expressed in *beats per minute* (bpm), is the number of heartbeats per unit time. HR can vary rapidly in response to both extrinsic and intrinsic control mechanisms. It is inversely related to interbeat interval (IBI). Mean successive difference (MSD) is the average of the difference between successive IBIs. Stroke volume (SV) is the volume of blood being pumped by the heart with each beat and is an important determinant of CO. CO is the volume of blood being pumped by the heart each minute (SV x HR), while venous return is the quantity of blood returning to the heart each minute from the veins. Cardiac index (CI) is CO adjusted for body surface area (BSA), *CO per metre^2 of BSA*. The heart has an intrinsic control mechanism allowing it to pump out the same amount of blood that flows into the right atrium from the veins, an effect termed the *Frank-Starling law of the heart*. As more blood flows into the heart, the chambers of the heart are stretched. This activates mechanoreceptors in the walls of the atria and causes a greater degree of overlap between actin and myosin filaments in the cardiac muscle of the ventricles, and, as a result, the heart contracts with more force[8]. Thus, this mechanism ensures venous return matches CO.

1.1.2 PHYSIOLOGICAL REGULATION OF BLOOD PRESSURE REACTIVITY

There are several physiological systems involved in regulating BP (Figure 1.1). Those most likely to influence short-term BP responses, like BP changes over the course of minutes studied here, are the

ANS and the HPA axis. The ANS includes various cardiovascular autonomic reflexes (the baroreceptor, atrial, chemoreceptor and exercise reflexes) that induce shifts in autonomic balance, with some altering BP within seconds (*eg.* baroreceptors, chemoreceptors). Another system likely to have only modulatory effects on acute BP changes is the renin-angiotensin-aldosterone system (RAAS), which affects sodium and water balance in the kidney and therefore primarily controls BP over the long-term (over hours or days). In addition oxidative stress, inflammation and endothelial dysfunction contribute to functional and structural changes to the endothelium that alter BP and impede normal BP regulation.

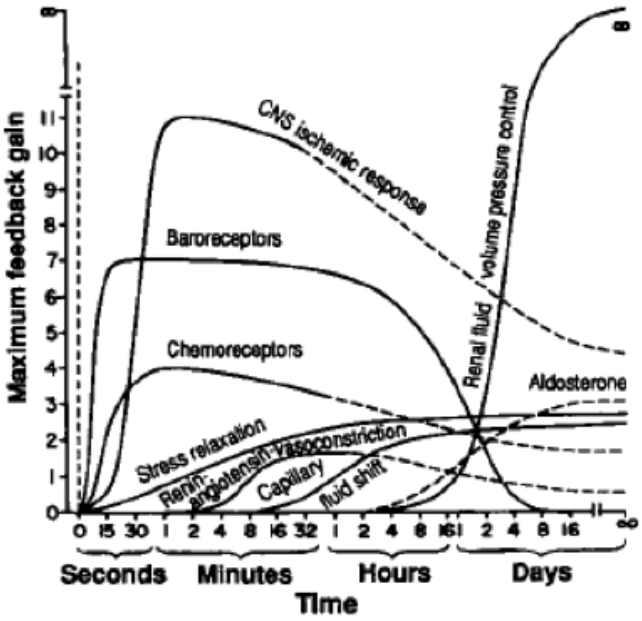

Figure 1.1. Approximate times of different BP control systems after a change in BP.
The degree of activation is shown for various pressure control mechanisms after a sudden change in BP (indicated by the vertical dashed line), expressed in terms of feedback gain, which is the ratio of the correction of the BP change to the remaining uncorrected change in BP. If BP returns to normal, the feedback gain is infinity. Several systems based on neural receptors (baroreceptors, chemoreceptors, and CNS ischemic response) respond within seconds, others exert effects within minutes (RAAS, hormonal systems), while the kidney-fluid volume system takes hours to days. Reproduced from Guyton AC. Blood pressure control – special role of the kidneys and body fluids. Science 1991; 252:1813-6. Permission granted (http://www.sciencemag.org/site/about/permissions.xhtml).
CNS = central nervous system, RAAS = renin-angiotensin-aldosterone system.

1.1.2.1 Autonomic nervous system

The autonomic nervous system (ANS) regulates the body's involuntary physiological functions, including BP[8]. The ANS is divided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), and is regulated by major central nervous system (CNS) sites in the spinal cord, brain stem, hypothalamus and cortex[8]. In simple terms, the SNS mobilizes the body's systems in response to stressful situations (*e.g.,* increases HR) while the PNS acts primarily to conserve energy and restore resources (*e.g.,* digestion, absorption). Stimulation of either branch of the ANS can have excitatory or inhibitory effects depending on the target organ. In most cases, activation of one is accompanied by inhibition of the other, a concept known as sympatho-vagal balance[9]. For example, sympathetic stimulation of the heart increases HR, while parasympathetic activation lowers HR. However, in other organs, one system predominates over the other[8]. Such is the case with systemic blood vessels, which are predominantly regulated by sympathetic activity and constrict with activation. The SNS commonly initiates a widespread reaction throughout the entire body, especially when fear or pain is involved (*eg.* the *fight or flight* "stress" response); in contrast, the PNS response is more often highly specific, targeting only certain organs[8]. Organs innervated by both the SNS and PNS include the heart, eye, bronchi, urinary bladder and reproductive organs[10].

Efferent signals from either branch of the ANS originate in the CNS, travel through a preganglionic neuron to the autonomic ganglia, and then leave the ganglia through a postganglionic neuron to reach the effector organ[8]. At the ganglionic synapse, both sympathetic and parasympathetic preganglionic neurons release the neurotransmitter acetylcholine (ACh), which binds to cholinergic nicotinic receptors of the postganglionic neuron[8]. Binding of ACh opens voltage-gated sodium and potassium channels, depolarizing the post-ganglionic neuron above the threshold required to generate an action potential[10].

The ANS and PNS differ in several key ways with respect to their anatomy, use of neurotransmitters and type of receptors on the target organs (Figure 1.2). The SNS has short preganglionic

neurons originating from regions T1 to L2 of the vertebral column, which innervate paravertebral sympathetic chains of ganglia or prevertebral ganglia (the celiac plexus and the superior and inferior mesenteric plexus)[8]. These synapse with long postganglionic nerves, which release norepinephrine (NE) that binds to α- and β-adrenergic receptors (*i.e.* adrenoceptors) on the effector organs[8]. Adrenoceptors are G-protein coupled receptors that open or close ion channels via secondary messengers. NE can have excitatory or inhibitory effects depending on the receptor subtype. The target organs innervated by postganglionic neurons originating in paravertebral ganglia include the systemic vasculature, heart and bronchi; those innervated by prevertebral ganglia include the kidney and intestine[8].

One exception to the mechanism described above is the SNS activation of the adrenal medulla. Sympathetic preganglionic neurons directly innervate the adrenal gland without passing through a ganglion[8]. Preganglionic neurons release the neurotransmitter ACh, which binds to nicotinic receptors on specialized secretory cells. These cells, which are actually modified neuronal cells analogous to postganglionic nerve fibers, secrete E and NE into the circulation[8]. Sympathetic stimulation of the adrenal medulla results in a surge of catecholamines in the circulation (on average, 80% E and 20% NE)[8]. Once these catecholamines reach organs, they can stimulate adrenoceptors in a manner analogous to direct sympathetic stimulation. For example, E and NE can act on β_1-adrenoceptors in the heart to increase contractility, with the effects lasting 5 to 10 times longer than NE released directly from sympathetic postganglionic neurons, as circulating catecholamines are removed from the body more slowly[8]. Circulating NE increases the activity of the heart and causes vasoconstriction of nearly all blood vessels, greatly elevating BP; conversely, E has a considerable excitatory effect on β-receptors of the heart, increasing CO, and only a modest vasoconstrictive effect on blood vessels in muscles[8].

In contrast to the SNS, the PNS has long preganglionic neurons that originate in the midbrain (cranial nerve III), pons (cranial nerve VII), medulla (cranial nerve IX and X) and the sacral parasympathetic nucleus consisting of segments S2 to S4[8]. Parasympathetic preganglionic neurons terminate in ganglia located within or near effector organs where they synapse with very short

postganglionic neurons[8]. Parasympathetic postganglionic nerves secrete the same neurotransmitter, ACh, at their nerve endings as preganglionic nerves[8]. ACh binds to cholinergic muscarinic receptors located on the effector organs. Like adrenoceptors, muscarinic receptors are G-protein coupled receptors that have either stimulatory or inhibitory effects depending on the specific G-protein stimulated. Postganglionic neurons that synapse with preganglionic neurons of cranial nerves III, VII and IX innervate the eye, the lacrimal, nasal, submandibular and sublingual glands, and the parotid gland, respectively[8]. Postganglionic nerves that synapse with cranial nerve X (*i.e.* the vagus nerve) innervate intramural ganglia of the heart, the sinoatrial and atrioventricular nodes, and visceral organs of the thorax and abdomen. The majority of parasympathetic nerve fibers (about 75%) are in the two vagus nerves[8]. Postganglionic neurons that synapse with sacral preganglionic neurons innervate the lower abdomen and pelvic viscera[8].

Neural regulation of the cardiovascular system via the ANS occurs through the balance of sympathetic and parasympathetic outflows in response to the body's demands[9]. The shift in SNS or PNS dominance is regulated by the cardiovascular control centre, located in the nucleus ambiguus of the medulla oblongata. The ANS regulates BP through its effects on the vasculature, heart and kidneys. In certain situations, these effects occur through various fast acting systems known as reflexes, which serve to maintain homeostasis for the brain (*eg.* in response to a change in pressure, oxygen availability or metabolic demands).

ANS & the vasculature – regulation of vasomotor tone and BP:

The vasculature is made up of arteries, arterioles, capillaries, venules and veins. These blood vessels are responsible for supplying the tissues with oxygenated blood and removing waste and CO_2. Blood vessels are composed of three tissue layers. The innermost layer, known as the *tunica intima*, is composed of a single layer of endothelial cells (the endothelium) and a thin internal elastic lamina. This is surrounded by the *tunica media*, which is comprised primarily of vascular smooth muscle cells that

constrict or dilate the vessel[8]. This layer displays the greatest amount of variation among the vessel types. The *tunica adventitia*, the outermost layer, is made up of elastic connective tissue and contains nerve endings, which innervate the vessel to cause dilation or constriction of the smooth muscle, and the *vaso vasorum*, which supplies blood to the vessel tissue. An external elastic lamina separates the tunica media from the tunica adventitia.

Neural regulation of BP occurs primarily through its effects on vessel radius. The vasomotor centre of the medulla oblongata controls vasoconstriction and vasodilation through sympathetic innervation of blood vessels. It continuously transmits signals to sympathetic vasoconstrictor nerves to maintain a partial state of contraction in blood vessels, called *vasomotor tone*[8]. In the vasculature, NE released from post-ganglionic nerves acts on α_1-, α_2- and β_2-adrenoceptors. Both α_1- and α_2-adrenoceptors are located in the coronary arteries, which constrict in response to NE. α_1-adrenoceptors are also located in the smooth muscle of blood vessels in the skin, sphincters of the gastrointestinal tract, brain and kidneys (*i.e.* renal arteries). Sympathetic stimulation of these blood vessels results in vasoconstriction. α_2-adrenoceptors are primarily involved in vasoconstriction of veins[10]. Activation of both α_1- and α_2-receptor subtypes results in vasoconstriction, though via divergent signalling pathways. Stimulation of α_1-adrenoceptors, which are coupled to the G_q protein, starts a signalling cascade resulting in an influx of calcium ions, leading to vasoconstriction. Similarly, activation of α_2-adrenoceptors, coupled to the inhibitory G_i protein, inactivates adenylate cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP)[10]. When this enzyme is inactivated, it leads to a decrease in cAMP and results in the constriction of blood vessels. Stimulation of β_2-adrenoceptors results in vasodilation. β_2-adrenoceptors are found in small coronary arteries, the hepatic artery and arteries to skeletal muscle[10]. Sympathetic stimulation of these arteries results in increased perfusion of these tissues. Stimulation of β_2-adrenergic receptors, coupled to the stimulatory G protein, G_s, activates adenylate cyclase, leading to an increase in cAMP and causing vasodilation[10].

Autonomic regulation of BP is the fastest control mechanism; sympathetic stimulation can double MAP within 5 to 15 sec while inhibition can reduce it to half in as little as 10 sec[8]. Conversely, parasympathetic stimulation has negligible effects on blood vessels, except in specific regions, such as the blush area of the face[8]. Thus, the regulation of vasomotor tone and BP is accomplished almost entirely by the sympathetic branch of the ANS. However, both sympathetic and parasympathetic activity can influence BP indirectly via their effects on HR and myocardial contractility.

ANS & the heart – regulation of heart rate, conduction velocity and contractility:

The heart is the organ responsible for pumping blood throughout the circulatory system. Blood from the venous systemic circulation flows into the right atrium, which contracts to eject blood into the right ventricle. A strong ventricular contraction ejects blood into the pulmonary arteries, which carries deoxygenated blood to the lungs where gas exchange occurs. The pulmonary veins return oxygenated blood to the left atrium, which pumps blood into the left ventricle. The left ventricle elicits a forceful contraction to pump blood into the aorta and throughout the rest of the systemic circulation, supplying the organs and brain with oxygenated blood.

The heart contains specialized auto-rhythmic cells, known as pacemaker cells, that control the rhythmic contraction of the heart[8]. These are located in the sinoatrial node, atrioventricular node and the *His-Purkinje* system. The sinoatrial node exhibits the fastest rate of self-excitability, generating approximately 100 action potentials per minute, and initiates contraction of the heart at this rate[8]. The sinoatrial node overrides the atrioventricular node and *His-Purkinje* system because their rate of spontaneous firing is much lower (40 to 60 and 15 to 40 action potentials per minute, respectively)[8]. Therefore, without input from the central nervous system, the natural HR would be 100 *bpm*, in keeping with the intrinsic rhythm of the sinoatrial node, the primary pacemaker of the heart.

Electrical stimulation of the heart follows a conduction pathway, whereby the sinoatrial node generates an action potential that travels through both atria and into the ventricles, causing rhythmic contraction of the heart. The speed at which the action potential travels down its neural pathway (*i.e.* from the atria to the ventricles) is known as conduction velocity. Once the impulse reaches the atrioventricular node in the lower right atrium, there is a delay to allow filling of the ventricles from the atria. The signal is then conducted through the *bundle of His* and the *Purkinje fibers*, leading to a forceful contraction of the ventricles[8]. The wave of depolarization propagates to adjacent cells via gap junctions in the intercalated discs connecting adjacent cardiomyocytes, resulting in rapid, synchronous depolarization of the myocardium[8]. This property allows the heart to function as a single contractile unit.

In addition to its direct effects on the vasculature, the ANS can alter BP indirectly through its effects on the heart. The heart is supplied by both sympathetic and parasympathetic nerves, which can alter the heart's rhythm, conduction velocity and contractility. Stimulation of the PNS sends signals from the cardiovascular control centre in the nucleus ambiguus of the medulla oblongata to the heart via the vagus nerve[8]. The vagus nerve innervates mainly the sinoatrial and atrioventricular nodes, plus the junctional fibers between the atria and atrioventricular node[8]. ACh is released at the vagal nerve endings, which binds to M_2-muscarinic receptors. Stimulation of M_2-muscarinic receptors activates a G_i-protein, causing a decrease in cAMP in pacemaker cells, leading to inhibitory effects[10]. Parasympathetic stimulation of the heart has negative chronotropic and dromotropic effects, *i.e.*, it reduces excitability of the sinoatrial node and conduction velocity of the action potential through the atrioventricular node[8]. PNS stimulation increases the permeability of fiber membranes to potassium, allowing more ions to flow out of the cell. Increased intracellular negativity delays the potential of pacemaker cells from reaching depolarization threshold, by causing rapid hyperpolarization and slowing the rate of depolarization[8]. Thus, parasympathetic stimulation of the heart lowers resting HR from 100 *bpm* to approximately 70 *bpm.* Vagal stimulation induces rapid effects on the heart, producing a maximal effect within 400 ms; it therefore regulates the time between beats (IBI)[11]. The PNS also has modest negative inotropic effects, decreasing the contractility of the myocardium. Parasympathetic innervation of the atria decreases the

flow of calcium into cells of the atria during an action potential[8]. A reduction in atrial contractility reduces ventricular filling. While it was originally assumed that parasympathetic innervation of the heart resulted in negative ionotropic responses in the atria, it has recently been shown that this effect extends to the ventricles as well[12]. Strong PNS activity can decrease the strength of myocardial contractility by about 25%[8].

In contrast to the PNS, the SNS exerts excitatory effects on the entire heart. Sympathetic nerve fibres release NE at their nerve endings, which bind to G_s-protein-coupled β_1-adrenoceptors located throughout the heart. Stimulation of these β_1-adrenoceptors increases cAMP in the cell, resulting in stimulatory effects[10]. At the sinoatrial and atrioventricular nodes, NE binds to β_1-adrenoceptors causing an increase in sodium influx, resulting in the pacemaker cells reaching their depolarization threshold more rapidly. The increased rate of depolarization thereby increases the rate of impulse generation[8]. At the sinoatrial node, sympathetic stimulation has a positive chronotropic effect, increasing heart rate via the increase in rate of depolarization[8]. The SNS has a positive dromotropic effect as well, increasing conduction velocity by stimulating the conductive system of the heart[8]. Furthermore, sympathetic stimulation of the myocardium has a positive inotropic effect by increasing the rate of calcium influx into cardiomyocytes during action potentials, which results in increased contractility of the atria and ventricles[8]. Sympathetic stimulation therefore increases CO and ejection pressure, as a result of the increase in pumping force. The SNS exerts effects on the heart with a delay of around five seconds, meaning its effects are not as immediate as those of the PNS[8]. Sympathetic stimulation of the heart at rest results in a pumping level 30% greater than would be seen without sympathetic innervation[8].

ANS & the kidneys – regulation of water and sodium balance and production of renin:

The kidneys perform several homeostatic functions to regulate BP. They determine sodium and water balance, thereby altering blood volume. The kidneys also produce renin, an enzyme of the renin-

angiotensin-aldosterone system (RAAS) (discussed in 1.1.2.2), which influences both vascular tone and the regulation of sodium and water balance. The kidneys are vital to the long-term regulation of blood pressure.

The kidneys are comprised of millions of structural and functional subunits called nephrons. Each nephron is made up of a renal corpuscule, consisting of a glomerulus and a Bowman's capsule, and a renal tubule, divided into a proximal end, a loop of Henle and a distal end[8]. The kidneys receive blood from the left and right renal arteries, which branch directly from the descending aorta. Each renal artery sequentially segments into smaller branches that eventually feed into the afferent arterioles[8]. The glomerulus receives blood from the afferent arterioles and filters water and solutes out into the surrounding Bowman's capsule; the remaining blood flows out via the efferent arterioles. The resulting glomerular filtrate enters the renal tubules, where approximately 99% of the filtrate, consisting of water, ions and minerals, is reabsorbed by the tubular epithelium[8]. In particular, sodium passes from the tubular lumen to the tubular epithelium via active transport and facilitated diffusion. The remaining filtrate passes into a collecting duct system where metabolic waste products (*e.g.*, urea, creatinine) and excess ions (*e.g.*, chloride, hydrogen) get excreted into urine. Some water, electrolytes and other molecules, such as glucose and amino acids, are reabsorbed in the peritubular capillaries and return to the circulation[8].

The kidneys possess two negative feedback mechanisms to maintain renal blood flow and glomerular filtration rate at near constant levels[8]. Too "low" glomerular filtration causes afferent arteriole vasodilation, which leads to a rise in glomerular pressure and blood flow. Similarly, too few sodium and chloride ions in the filtrate cause the juxtaglomerular cells to release renin, which results in efferent arteriole vasoconstriction via angiotensin II. Both these mechanisms serve to return the glomerular filtration rate back to normal[8].

Additionally, BP can influence kidney function. Even a slight rise in BP can cause a considerable increase in urinary excretion of water (*pressure diuresis*) and sodium (*pressure natriuresis*). Pressure natriuresis and pressure diuresis are the most potent regulatory mechanisms of blood volume and

extracellular fluid volume[8]. A rise in BP causes a parallel rise in glomerular pressure, which increases the glomerular filtration rate and enhances fluid volume output. Conversely, when BP is low, renal sodium and water reabsorption increase, augmenting extracellular fluid volume. This raises blood volume, which increases venous return and CO. Increased CO, in turn, enhances the amount of blood flowing to tissues and increases TPR, as local vasculature constricts to return blood flow to normal[8]. These effects lead to an elevation of BP. In addition to the effects of BP, the local control mechanisms of the kidney can be overridden by autonomic or hormonal regulation.

Regarding the autonomic regulation, the kidney is innervated by the SNS via the renal plexus. Sympathetic fibres innervate afferent and efferent arterioles, renal tubules and juxtaglomerular cells[8]. The kidney, however, does not receive input from the PNS. When BP is low, acute sympathetic stimulation triggers vasoconstriction in the renal arteries, thereby reducing renal blood flow and limiting urinary output[8]. This serves to increase blood volume and return BP to normal, maintaining homeostasis. In the proximal renal tubules, sympathetic activation of receptors increases sodium reabsorption[13, 14]. Stimulation of β_1-adrenoceptors on the juxtaglomerular cells results in the secretion of renin, which increases BP through the activation of the RAAS[15, 16] that, among others, involves the release of aldosterone from the adrenal gland. Aldosterone acts on the kidney where it increases sodium reabsorption in the renal tubules, which is accompanied by water reabsorption through osmosis. This results in an increase in blood volume and elevation of BP[8]. Another hormone influencing BP via its effect on the kidney is vasopressin (also known as antidiuretic hormone). Vasopressin is secreted by the posterior pituitary gland in response to high sodium levels, which activate osmoreceptors in the hypothalamus[8]. Vasopressin increases water reabsorption in the renal tubules to maintain a normal concentration of sodium ions[8]. It increases blood pressure through its water-conserving effects on the kidneys; it is also a potent vasoconstrictor. Conversely, a low sodium ion concentration reduces the release of vasopressin, which lowers BP. Additional effects of aldosterone and vasopressin on BP are discussed in 1.1.2.2 and 1.1.2.3.

Cardiovascular autonomic reflexes – baroreceptor, atrial, chemoreceptor and exercise reflexes:

The cardiovascular autonomic reflexes, which include the baroreceptor, atrial, chemoreceptor and exercise reflexes, integrate the ANS influences on the vasculature, heart and kidneys, as I just described prior to this section.

Baroreceptor reflex: The baroreceptor system is a simple control mechanism that regulates BP[17]. It serves to dampen short-term BP fluctuations through a negative feedback system that preserves transcapillary pressure for maintaining vital tissues, such as the brain[18]. Baroreceptors are specialized mechanoreceptors stimulated by stretch that are distributed throughout the walls of the large systemic arteries of the thoracic and neck regions. They are most abundant in the bifurcation region of the carotid arteries, known as the carotid sinus, and in the aortic arch[8]. A rise in BP and subsequent stretch in the arterial wall activates baroreceptors, which transmit impulses from the glossopharyngeal nerve and the vagus nerve to the tractus solitarius of the medulla. This area of the brain stem responds to changes in the firing rate of action potentials from the baroreceptors[8]. Once these impulses reach the medulla, secondary signals excite the vagal centre and inhibit the vasomotor centre, causing parasympathetic stimulation in the heart and decreasing sympathetic activity in the heart, vasculature and kidney, respectively[17]. Parasympathetic activation immediately reduces HR and, after a few seconds delay, sympathetic withdrawal reduces heart contractility and vasomotor tone, leading to a reflex drop in BP[8]. Conversely, when BP is low, the baroreceptors become inactive resulting in a decrease in vagal centre activity and an increase in vasomotor centre activity from a lack of inhibition. The resulting cardiac parasympathetic withdrawal and sympathetic stimulation lead to an increase in HR and vascular resistance, thus, BP returns to normal.

The baroreceptor reflex is particularly vital to maintain constant BP in response to the upright posture. Active standing causes a transient drop in BP in the head and upper body, as blood is displaced to the lower body due to gravitational forces[19]. This fall in pressure inhibits the baroreceptors, eliciting an immediate increase in sympathetic outflow and inhibition of cardiac parasympathetic outflow to limit the

drop in arterial pressure in the head and thoracic region, resulting in BP recovery[20]. Contraction of postural muscles compresses blood vessels, resulting in an increase in venous return and CO, and activates the exercise reflex (described below) to further increase BP and HR via sympathetic activation and vagal withdrawal[20]. An immediate and effective compensatory BP response depends on the rapid adaptation of vascular smooth muscle to changes in diameter, the arterial baroreceptors to increase vasomotor tone and cardiac function, the skeletal muscle pump to maintain venous return and cardiac output and the release of neurohormones, such as catecholamines[19]. Failure to adequately maintain BP and cardiac output results in orthostatic hypotension and presyncope[21].

The baroreceptor system produces rapid BP changes that affect short-term BP regulation. Carotid sinus baroreceptors are stimulated by MAP over 60 mm Hg while aortic baroreceptors operate at MAP above 30 mm Hg. Given that normal MAP is around 100 mm Hg, slight pressure changes cause strong autonomic reflexes to maintain normal MAP[8]. Baroreceptors respond extremely rapidly to a change in pressure; the rate of impulse firing increases during systole versus diastole, and can be up to twice as high in response to a changing pressure (*eg.* fluctuating around 150 mm Hg) versus a stationary one (*eg.* stable at 150 mm Hg)[8]. Baroreceptors are able to actively "reset" to permit BP to rise appropriately during specific behaviours such that the operating range increases to higher BP levels without a reduction in reflex sensitivity[17]. Baroreflex resetting involves both neural and humoral mechanisms; it can be triggered reflexly, for example, by muscle contraction during exercise, or by neurohumoral regulation, for example, by circulating angiotensin II from the RAAS. The role of the baroreceptor system is debated in the long-term regulation of BP. Under unstressed conditions, arterial baroreceptors have little influence on long-term BP[17]. Thus, the baroreceptor system is only useful in regulating BP changes if they occur over the short-term. Other BP regulatory mechanisms, such as the atrial reflex, are important in long-term BP regulation.

Atrial reflex: Both the atria and pulmonary arteries possess low-pressure receptors within their walls that respond to stretch, similar to the baroreceptors of the large systemic arteries. These receptors detect increases in BP in the low-pressure areas of the circulatory system that are caused by increases in blood volume. Excess blood volume leads to greater venous return and CO and, therefore, greater systemic arterial pressure[8]. Low-pressure receptors play an important role in minimizing arterial pressure changes resulting from fluctuations in blood volume through their effects on the kidneys and HR.

Stretch of the atria resulting from a rise in venous return causes sympathetic withdrawal and a reflex dilation of the peripheral arterioles, most notably a potent relaxation of the afferent arterioles supplying the kidneys with blood. This is known as the *volume reflex*. The drop in afferent arteriolar resistance increases the glomerular capillary pressure, resulting in an increase in the filtration rate of fluid into the kidney tubules[8]. Simultaneously, signals are transmitted to the hypothalamus to minimize the secretion of vasopressin, which decreases water reabsorption in the kidneys[8]. Combined, these effects on kidney function cause rapid fluid loss through an increase in urine output, returning blood volume back to normal. This decrease in blood volume also serves to control MAP, especially on the venous side where most of the blood is held, through a decrease in venous return[8].

In addition to its effects on the kidneys, the atrial reflex also controls HR. An increase in atrial pressure causes HR to increase by as much as 75%[8]. This is partly due to the direct effect of increased atrial volume causing stretch at the sinus node; however, the majority of the reflex rise in heart rate is caused by the *Bainbridge reflex*[8]. The atrial stretch receptors transmit afferent signals through the vagus nerve to the medulla, which in turn transmits efferent signals through parasympathetic (inhibitory) and sympathetic (excitatory) fibers to increase HR and myocardial contractility. The atrial reflex control of HR is vital to preventing the accumulation of blood in the veins, aorta and pulmonary circulation.

Chemoreceptor reflex: The chemoreceptor reflex operates in a manner analogous to the baroreceptor reflex. Chemoreceptors are chemosensitive cells that detect a reduced availability of oxygen (hypoxia) or a build up of carbon dioxide (hypercapnea) or hydrogen ions in the blood[8]. Chemoreceptors are found in the carotid and aortic bodies and in the brain[17]. Carotid and aortic chemoreceptors excite nerve fibers adjacent to the baroreceptor fibers, passing through the glossopharyngeal and vagus nerves into the vasomotor centre of the medulla oblongata[8]. Each carotid or aortic body is supplied with abundant blood flow by a small artery, ensuring constant exposure to arterial blood. When arterial pressure drops below a critical level (*i.e.* below 80 mm Hg), the chemoreceptors are stimulated by hypoxia and hypercapnea resulting from diminished blood flow[8]. As a result, they transmit signals to the vasomotor centre, leading to a reflex rise in BP. Activation of the chemoreceptor reflex stimulates breathing, causes arousal and increases sympathetic outflow to the heart and blood vessels[17]. Sympathetic activation and vasoconstriction result in acute increases in BP and HR.

In addition to their affect on the vasomotor centre, the primary function of chemoreceptors is to regulate respiration. The afferent nerve fibers of the carotid and aortic bodies connect to the dorsal respiratory area of the medulla[8]. When oxygen from the arterial blood falls below normal, the chemoreceptors are strongly stimulated, thereby increasing respiration. The impulse rate is particularly sensitive to changes in the arterial pressure of oxygen in the range between 60 and 30 mm Hg, the range in which the hemoglobin saturation of oxygen decreases rapidly[8]. A decrease in arterial oxygen concentration stimulates respiration via chemoreceptors, but does not directly affect the respiratory centre. In contrast, an increase in either carbon dioxide or hydrogen ion concentrations has direct effects on the respiratory centre which are much more powerful than those mediated by the chemoreceptors, which are activated in a parallel manner.

Exercise reflex: The exercise reflex is another cardiovascular autonomic reflex; however, unlike other BP regulatory mechanisms that serve to maintain BP within a narrow range, the cardiovascular

response to exercise causes the requisite elevation in BP during times of heightened activity. Muscles require substantially more blood flow during heavy exercise in order to sustain their metabolic demands. This is achieved in two ways: first, through the local vasodilatory effect in muscle vasculature and, second, through simultaneous shifts in autonomic balance to increase BP.

The tremendous increase in muscle blood flow is caused primarily by local effects in the muscles acting on arterioles to cause vasodilation. During exercise, the blood vessels within active muscles dilate in response to the increase in metabolic demands of the muscle cells[8]. The higher rates of muscle cell metabolism cause the cells to deplete nutrient and oxygen availability very rapidly, resulting in local vasodilation and an increase in local blood flow. Oxygen deficiency causes vasodilation because vascular smooth muscle cells cannot maintain a state of contraction without oxygen[8]. The reduced availability of oxygen also stimulates the release of vasodilating substances (*eg.* adenosine, potassium ions, acetylcholine, adenosine triphosphate, lactic acid, carbon dioxide)[8]. During strenuous exercise, the adrenal medulla releases NE and E; E stimulates the β_2-adrenoceptors, found in blood vessels of skeletal muscle (and in the coronary arteries), leading to vasodilation and increased perfusion of active muscle (including the heart)[8]. In skeletal muscle, local vasodilation can increase local blood flow by as much as 20-fold, ensuring the active tissues receive sufficient amounts of extra oxygen and nutrients to sustain their level of function during vigorous activity[8].

In addition, blood flow to active muscles is increased through the simultaneous elevation of BP, mediated by the ANS[8]. Despite local vasodilation in muscles, the nervous system prevents BP from failing below normal and provides additional signals to increase BP[8]. During exercise, higher brain centres control the cardiopulmonary system, including HR, BP and ventilation, in a top down manner, a concept termed central command[22]. To meet the metabolic demands of contracting muscles, central command involves the synchronized activation of locomotor and cardiovascular systems, most likely via the insulate cortex, cingulate cortex and the midbrain[22]. At the onset of exercise, the brain transmits signals to muscles, to cause exercise, and to the vasomotor centre, to initiate mass sympathetic discharge

throughout the body and withdrawal of parasympathetic stimulation to the heart[8]. Increased sympathetic and decreased parasympathetic innervation of the heart raises HR and increases myocardial contractility, resulting in greater CO[23]. In the vasculature, sympathetic stimulation constricts most peripheral arteries to raise BP (except blood vessels in skeletal muscle, the heart (coronary arteries) and the brain (cerebral arteries), which require enhanced blood flow during exercise)[8]. Sympathetically-mediated contraction of the veins and other capacitance parts of the circulation, combined with the compression of many of the internal vessels by exercising muscles and the lower resistance in the vasculature of active muscles, increase the mean systemic filling pressure and promote venous return of blood to the heart[8]. This further raises CO and BP. Aside from sympathetic stimulation originating in higher brain centres, afferent feedback from contracting muscles also contributes to the shift in autonomic balance during exercise[22]. This reflex may be mediated by metabolic end products that act on sensory nerve endings in the muscle tissue, initiating signals that pass up the spinal cord to the vasomotor centre to excite the SNS[8]. These sympathetically mediated effects on the heart and vasculature raise HR, CO and BP instantaneously and are essential to keep up with the metabolic demands of working muscles. During most types of heavy exercise, MAP rises approximately 30 to 40 per cent resulting in a two-fold increase in blood flow to active muscles[8].

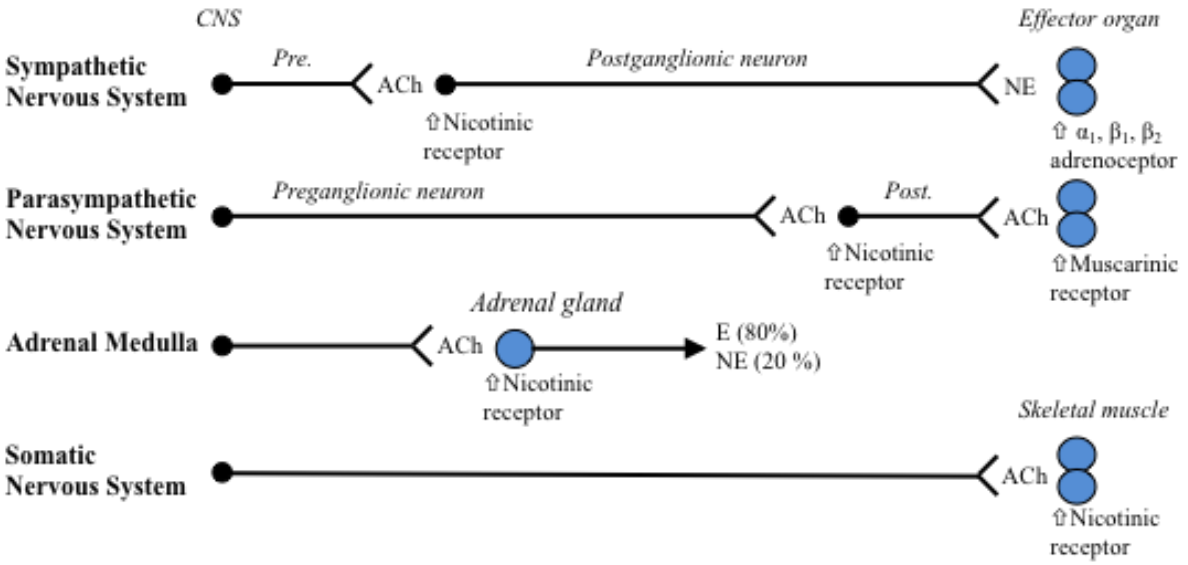

Figure 1.2. Organization of the autonomic nervous system
The sympathetic and parasympathetic branches of the autonomic nervous system differ in the length of pre- and post-ganglionic neurons, in neurotransmitters used (norepinephrine [NE] and acetylcholine [ACh]) and in the types of receptors stimulated on the effector organs (α- and β-adrenoceptors and muscarinic cholinergic receptors). Preganglionic neurons release ACh, which binds to nicotinic cholinergic receptors at the autonomic ganglia of both systems. Adapted from Costanzo, 1995[24].
ACh = acetylcholine, CNS = central nervous system, E = epinephrine, NE = norepinephrine.

1.1.2.2 Hypothalamic-pituitary-adrenal axis

The HPA axis is part of the neuroendocrine system that controls reactions to stress and regulates physiological processes, such as metabolism, reproduction, growth and immunity. Activation of the stress system occurs in response to challenging or threatening stimuli. When faced with excessive stress, an individual mounts a non-specific adaptive response: attention is enhanced, CO and respiration are accelerated, catabolism is increased and blood flow is redirected toward the brain, heart and muscles[25]. This adaptive response has some specificity toward the stressor that generates it (this is discussed more in 1.1.3.2). The stress response is initiated and maintained by the hypothalamus (the paraventricular nuclei) and brain stem (the locus ceruleus-norepinephrine system [LC/NE])[25]. During exposure to threatening stimuli, the brain exerts effects on all organs of the body via the HPA axis, together with the SNS and the adrenal medulla.

The HPA axis is a complex set of feedback interactions among the hypothalamus, the pituitary glands and the adrenal glands. The hypothalamus is directly connected to the anterior and posterior pituitary gland by the hypophyseal stalk and regulates their function using hormonal and nervous stimuli[8]. The hypothalamus controls secretion from the anterior pituitary by releasing stimulatory or inhibitory hormones into the median eminence; these are brought via the hypothalamic-hypophysial portal vessel to glandular cells of the anterior pituitary[8]. In contrast, the posterior pituitary is directly innervated by nerves originating from the supraoptic and paraventricular nuclei of the hypothalamus[8]. Hormones secreted by the pituitary gland are released directly into the circulation and carried to receptors on their target tissues, which, in this case, are the adrenal glands. The adrenal glands are situated on top of the kidneys and are principally responsible for releasing hormones in response to stress. The central adrenal medulla releases E and NE in response to sympathetic stimulation, while the outer adrenal cortex synthesizes corticosteroids from cholesterol. Corticosteroids include: (1) ***mineralocorticoids***, which affect electrolytes in extracellular fluid (aldosterone), (2) ***glucocorticoids***, which participate in whole body homeostasis and the body's response to stress (cortisol), and (3) ***androgenic hormones***, which mimic male sex hormones (dehydroepiandrosterone [DHEA]). Cortisol is the principal glucocorticoid in humans and the final product of HPA activation, described in more detail below.

Acute stress, whether physical or mental in origin, elicits a coordinated response between the SNS and the HPA axis, both of which modulate the cardiovascular system. Subjects with the greatest sympathetic response to stress also show the greatest cortisol response[26]. Stress increases sympathetic outflow to the vasculature and heart, which leads to elevation in BP, HR and CO. Simultaneously, activation of the HPA axis causes the release of vasopressin and corticotropin-releasing hormone (CRH) from the hypothalamus[25]. Vasopressin is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and released by nerve endings terminating in the posterior pituitary[8]. Vasopressin, as described above, is an antidiuretic hormone and potent vasoconstrictor, causing BP to increase[25]. CRH is a 41-amino acid peptide produced by specialized neurons in the hypothalamus, whose cell bodies are located in the paraventricular nucleus. CRH is secreted into the hypophysial portal system and carried to

the anterior pituitary[8]. These two peptides synergistically promote the secretion of adrenocorticotropic hormone (ACTH), a large 39-amino acid peptide, by the anterior pituitary[8]. ACTH is then transported by the circulation to the adrenal cortex, stimulating the production of glucocorticoids via the activation of *adenylate cyclase,* which increases cAMP, and *desmolase,* which converts cholesterol to pregnenolone (a precursor of corticosteroids)[8]. The increased adrenocortical secretion of cortisol occurs within three minutes of the onset of stress[8, 27]. Cortisol makes up approximately 95% of glucocorticoids secreted, although a small but significant amount of corticosterone is also produced[8]. At the same time, sympathetic stimulation of the adrenal medulla results in greater E and NE release. Glucocorticoids increase the sensitivity of vascular smooth muscle cells to E and NE through glucocorticoid receptors, potentiating their vasoconstrictive response[28]. Glucocorticoids also suppress the production of vasodilators, such as nitric oxide (NO), in endothelial cells[28]. Together, the sympathetically-mediated rise in catecholamine production and the local effect of glucocorticoids on vasomotor tone lead to further elevations in BP[25]. Glucocorticoids, the final effectors of the HPA axis, participate in the control of whole body homeostasis and regulate the stress response via negative feedback inhibition (described below).

In addition to their effects on BP, glucocorticoids have widespread effects throughout the body, influencing metabolism, reproduction, growth and immunity. Glucocorticoids stimulate the synthesis of glucose in the liver, increasing gluconeogenesis up to ten-fold, by mobilizing amino acids and fatty acids from the breakdown of muscle and adipose tissue[8]. Glucocorticoids also reduce glucose utilization by cells[8]. These effects cause a rise in blood glucose and promote insulin resistance[25]. Reproduction and growth are also profoundly inhibited by various components of the HPA axis (*eg.* CRH suppresses gonadotropin-releasing hormone [GnRH] and growth hormone [GR])[25]. Glucocorticoids inhibit the secretion of sex steroids and growth factors and make the target tissues resistant to these hormones[25]. For example, glucocorticoids prevent the formation of muscle and bone via their antagonistic effects on GR and sex steroids[25]. Moreover, cortisol has powerful anti-inflammatory and immunosuppressive effects[8]. These effects counteract inflammatory cytokines, which are potent activators of the central stress

response. For example, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin 6 (IL-6) can activate the HPA axis alone or synergistically[25].

Glucocorticoids play a key role in regulating the basal level of HPA activity and in terminating the stress response in a negative feedback loop (Figure 1.3). Cortisol has negative effects on: (1) the hypothalamus to decrease the formation of CRH and (2) the anterior pituitary gland to suppress the production of ACTH[8]. Additionally, cortisol directly reduces the cleavage of pro-opiomelanocortin (POMC) into ACTH and β-endorphins[8]. Given that circulating ACTH is the key regulator of glucocorticoid secretion by the adrenal cortex[25], this negative inhibition helps to regulate circulating cortisol levels. The plasma concentrations of cortisol, CRH and ACTH exhibit a diurnal rhythm; levels are high in the morning but low in the evening[8]. This negative feedback also limits the duration that tissues are exposed to glucocorticoids, thus, minimizing the catabolic, antireproductive, antigrowth and immunosuppressive effects of these hormones[25]. While these effects are beneficial in the short-term and enable the body to combat stressors, prolonged activation of the HPA and LC/NE system is damaging. For example, chronic activation of the stress system increases visceral adiposity, decreases lean body mass (LBM) and suppresses osteoblastic activity[25]. Furthermore, certain disorders are associated with HPA hyperactivity (*eg.* depression, anorexia), while others are related to HPA hypoactivity (*eg.* chronic fatigue syndrome)[25]. Thus, having either an excessive or a disrupted stress response is detrimental and can result in pathology.

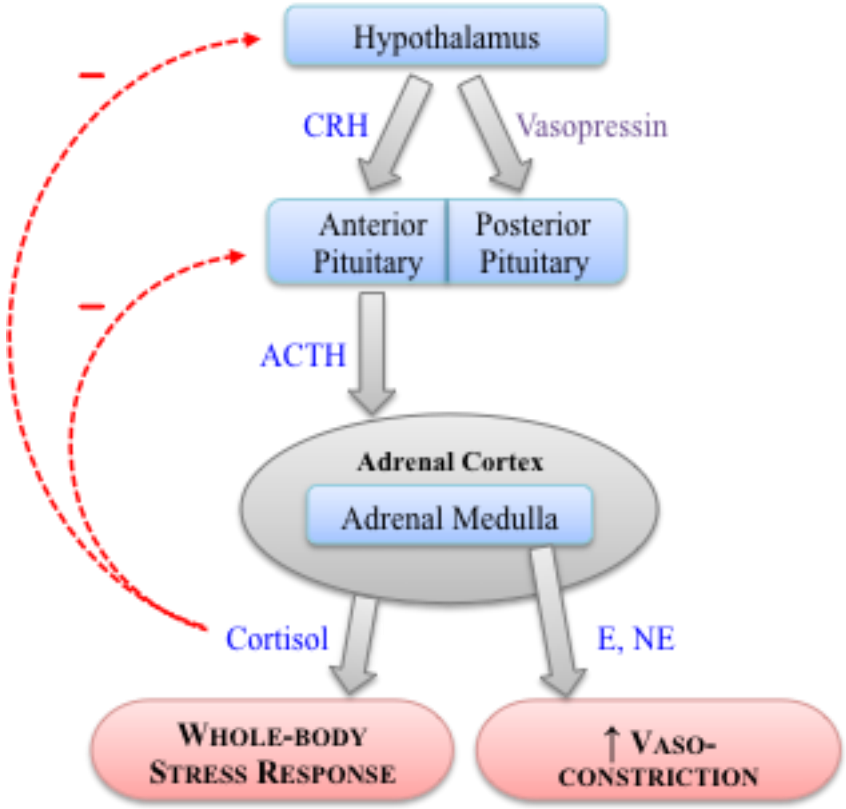

Figure 1.3. Organization of the hypothalamic-pituitary-adrenal axis
Stress-induced HPA activation leads to the secretion of vasopressin, a potent vasoconstrictor, and CRH from the paraventricular nucleus of the hypothalamus. CRH acts on the anterior pituitary, prompting the release of ACTH into the circulation. ACTH stimulates the adrenal gland to release E/NE from the adrenal medulla, resulting in increased vasoconstriction, and cortisol from the adrenal cortex, which controls the whole-body stress response and exerts negative feedback inhibition on the hypothalamus and anterior pituitary.
ACTH = adrenocorticotropic hormone, CRH = corticotropin releasing hormone, E = epinephrine, HPA = hypothalamic-pituitary-adrenal axis, NE = norepinephrine.

1.1.2.3 Renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system (RAAS) is a critical pathway for BP control and kidney function; it is a hormone system that regulates BP, natriuresis and blood volume, and controls regional blood flow and local vascular responses to stimuli. The RAAS is composed of various regulatory components and effector molecules, many of which have opposing to functions, that facilitate the dynamic control of vascular function (Figure 1.4)[29]. ***Renin*** is synthesized and stored by juxtaglomerular

cells in the wall of renal afferent arterioles. It is secreted in response to low renal perfusion pressure, tubular salt content, or sympathoactivation[8]. Upon entering the circulation, renin cleaves angiotensinogen, produced by the liver, to yield the 10-amino acid peptide ***angiotensin I (Ang I)***. Ang I can be cleaved by either the dipeptide carboxypeptidase angiotensin-converting enzyme (ACE) to form ***angiotensin II (Ang II)***, or alternatively, by an additional carboxypeptidase, ACE2, to give ***angiotensin (1-9) (Ang [1-9])***[30]. ACE is expressed primarily in the lungs and removes dipeptides from the C-terminal, while ACE2 is highly expressed in the endothelium, kidneys and heart and cleaves a single amino acid from the C-terminal of any given substrate[29]. Similar to ACE, the substrate affinity of ACE2 is not restricted to angiotensin peptides; for example, ACE2 is able to cleave the C-terminal amino acid from vasoactive peptides such bradykinin metabolites, apelin-13 and apelin-36[29, 30]. It is important to note that enzymes other than ACE and ACE2 are involved in the RAAS pathway (*eg.* chymases, endopeptidases, aminopeptidases)[30].

In the first pathway, the decapeptide Ang I is converted by ACE into Ang II, an 8-amino acid peptide[8]. Ang I has mild vasoconstrictive properties, while Ang II is a potent vasoconstrictor. Most cardiac and renal effects of Ang II are mediated by the angiotensin type 1 (AT_1) receptor, including vascular smooth muscle contraction, pressor responses and aldosterone secretion; however, Ang II binding to the angiotensin type 2 (AT_2) receptor induces a counter-regulatory vasodilation[30]. Ang II causes intense constriction of arterioles, increasing BP via increased vascular resistance, and mild constriction of veins, increasing venous return[8]. In addition to its effects on vascular tone, Ang II decreases pressure natriuresis, the excretion of salt and water in the kidneys. It constricts renal afferent arterioles, which limits blood flow through the kidneys, resulting in less fluid filtering through the glomeruli and into the tubules. This slows peritubular blood flow as well, allowing rapid reabsorption of water from the tubules via osmosis[8]. As a result, urine excretion is diminished. Ang II also has a weak effect on the tubules themselves, increasing their reabsorption of water and sodium[8]. Finally, Ang II stimulates the release of ***aldosterone*** from the zona glomerulosa of the adrenal glands. Aldosterone increases sodium reabsorption and potassium excretion in the distal tubules and cortical collecting ducts,

which is accompanied by water reabsorption through osmosis. Thus, aldosterone increases both sodium and water content in the extracellular fluid[8]. It can therefore alter blood volume and, secondary to Ang II's direct effects on the kidneys, contribute to the long-term elevation of BP[8]. In turn, Ang II is cleaved by ACE2 to yield the vasodilator ***Angiotensin (1-7) (Ang [1-7])***, a 7-amino acid peptide[29]. Ang (1-7) is an important regulator of cardiovascular and renal function; it promotes vasodilation, stimulates nitric oxide synthase, counteracts the detrimental actions of Ang II via the AT_1 receptor and binds to the AT_2 receptor to induce vasodilation mediated by NO and bradykinin[30]. It may also augment bradykinin binding to its B_2 receptor, leading to vasodilatory effects and pressure natriuresis in the kidneys contributing a drop in BP[30]. Ang II can also be degraded by aminopeptidases (*eg.* aminopeptidase A) and endopeptidases (*eg.* prolyl endopeptidase) to functional angiotensin peptides[29].

In an alternate pathway, Ang I is converted by ACE2 to form the nonapeptide Ang (1-9). Ang (1-9) augments the vasodilatory effect of bradykinin via conformational changes to its B_2 receptor but its actions in the heart and kidney are not well understood[30]. ACE then cleaves a peptide from Ang (1-9) to yield the cardio- and renoprotective Ang (1-7)[30]. Ang (1-7) is, in turn, degraded by ACE into inactive angiotensin peptides. To accommodate a rapid yet stabilized response to specific stimuli, ACE and ACE2 have complementary functions. ACE2 balances the actions of ACE by producing a different substrate from Ang I and by degrading Ang II. Similarly, ACE breaks down Ang (1-9) and Ang (1-7) produced by the enzymatic activity of ACE2. Recent studies have demonstrated the importance of ACE2 in maintaining the balance of the RAAS. ACE2 is essential in regulating local levels of Ang II and its physiological antagonist Ang (1-7), which protects against Ang II-mediated injury and dysfunction[29]. Promoting the enzymatic activity or expression of ACE2 has been shown to significantly reduce BP in spontaneously hypertensive rats[31-33]. While some of the actions of ACE2 are local, others influence the central origins of high BP, such as controlling baroreceptor responsiveness. Injection of an ACE2 inhibitor into the nucleus tractus solitarius reduces the reflex bradycardia accompanying baroreceptor stimulation[34]. As a result, a balance between ACE and ACE2 is desirable to achieve effective BP regulation via the RAAS.

In contrast to the effects produced by the RAAS when BP or blood volume is low, the RAAS induces the opposite effects when BP is high and the extracellular fluid is expanded. Such conditions increase the delivery of ions to the kidney and reduce renin secretion by the juxtaglomerular cells. This leads to a decrease in the production of angiotensin, a reduction in vasoconstriction, limited retention of sodium and water in the kidneys and an increase in urinary output. As result, extracellular fluid volume and BP return to normal. Thus, the RAAS is an important homeostatic mechanism that regulates BP and blood volume by controlling water and sodium reabsorption in the kidneys.

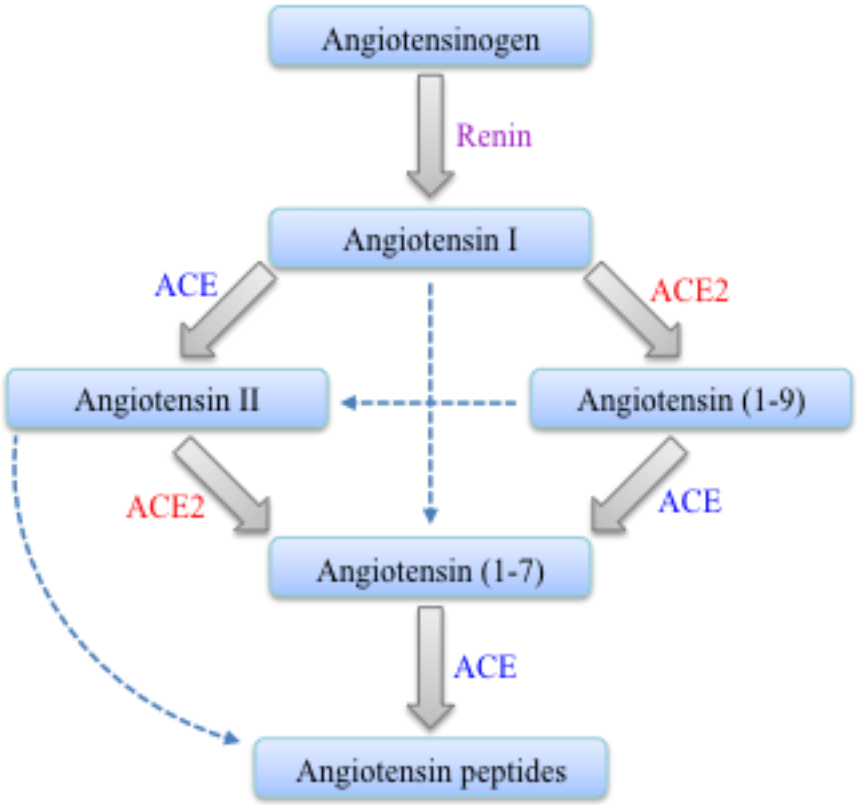

Figure 1.4. Organization of the renin-angiotensin-aldosterone system
Renin converts angiotensinogen into Ang I, which is broken down by ACE to form Ang II, a potent vasoconstrictor that stimulates the release of aldosterone. ACE2 then converts Ang II to Ang (1-7), a vasodilator with cardio- and reno-protective effects. Alternatively, Ang I is broken down by ACE2 into Ang (1-9). ACE converts Ang (1-9) to Ang (1-7), which it degrades into inactive peptides. ACE and ACE2 have complementary functions to balance the regulation of the system. Dashed lines represent additional pathways that break down substrates via various enzymes (*eg.*chymases, endopeptidases and aminopeptidases). Adapted from Tikellis *et al.*, 2011[29] and Danilczyk and Penninger, 2006[30].
ACE = angiotensin converting enzyme, Ang = angiotensin.

1.1.2.4 Oxidative stress, inflammation and endothelial dysfunction

Another physiological component of BP regulation is the endothelium. The endothelium plays a pivotal role in maintaining basal and dynamic vascular tone and function, primarily through the release of vasoactive substances. The endothelium releases vasodilating molecules (*eg.* NO, prostacyclin) on the one hand, and vasoconstricting molecules (*eg.* endothelin-1) on the other hand, thereby controlling local blood flow to tissues. For example, when tissues, such as active muscle, need additional blood, endothelial cells release the vasodilator NO in response to oxygen depletion to increase blood flow[8]. NO is produced by the enzyme endothelial nitric oxide synthase[35]; it confers a protective effect on the vasculature since it possesses antihypertensive and antiatherogenic properties by causing vasodilation and inhibiting leukocyte adhesion, platelet aggregation and vascular smooth muscle cell proliferation[36]. The endothelium can be affected by oxidative stress and inflammation, two pathological processes that not only influence endothelial function but also endothelial structure, resulting in endothelial dysfunction: abnormalities that increase vascular tone, promote thrombosis and alter vascular growth[37]. Epidemiological evidence shows that hypertension is accompanied by increases in oxidative stress, inflammation and endothelial dysfunction, which can already be detected at preclinical stages of the disease[38-40]. Although these processes become more severe with age, they can already be detected in children. For instance, hypertensive children and adolescents exhibit higher levels of oxidative stress than their normotensive counterparts[41].

Oxidative stress results from an imbalance between the production and clearance of oxygen free radicals, or more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS)[37]. ROS and RNS are products of normal cellular metabolism that have both beneficial and deleterious effects[42]. Free radicals possess unpaired electrons, making them highly unstable and reactive[37]; thus, their production and degradation are tightly regulated to maintain redox equilibrium[42]. At low concentrations, ROS function as signalling molecules to maintain vascular integrity by regulating endothelial function and vascular contraction-relaxation; however, in excess, they may damage cellular lipids, membranes,

proteins and DNA, producing vascular damage[42]. An imbalance between prooxidants and antioxidants results in oxidative stress, characterized by the overproduction of ROS/RNS that overwhelm the cellular antioxidant defense system[43]. Antioxidants stabilize or deactivate free radicals to protect the body's cells and organs from oxidative damage[37]. These can be endogenously produced or obtained from one's diet. Enzymatic antioxidants (*eg.* superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic antioxidants (*eg.* Vitamins E and C, carotenoids, flavonoids, lipoic acid) work synergistically to protect against oxidative stress[37]. For example, the superoxide anion radical ($O_2^{\bullet -}$) is generated by several enzymatic reactions, which occur mostly within the mitochondria of cells[44]. The mitochondrial electron transport chain is the principle source of ATP and is, therefore, essential to life; however, during this process, a small number of electrons "leak" to oxygen prematurely, mediated by NADPH oxidases and xanthine oxidase, forming superoxide[42]. The antioxidant superoxide dismutase catalyses superoxide into hydrogen peroxide (H_2O_2), which is then converted into water by glutathione peroxidase, an abundant and key antioxidant enzyme[42].

Superoxide, hydrogen peroxide and other dangerous ROS/RNS, including hydroxyl ($^{\bullet}OH$) and NO radicals ($NO^{\bullet}$), play an important role in the pathophysiology of hypertension[37]. ROS are produced in the vasculature by endothelial, adventitial and vascular smooth muscle cells and are primarily derived from NADPH oxidase, an enzyme regulated by humoral (cytokines, growth factors and vasoactive agents) and physical factors (stretch, pulsetile strain and shear stress)[43]. Oxidative stress contributes to elevated BP through increased Ang II-induced vasoconstriction, decreased NO bioavailability in the vasculature and kidneys and vascular remodeling (Figure 1.5)[43]. Thus, ROS production is tightly linked with the actions of Ang-II, a potent vasoconstrictor[42]. Within vascular cells, Ang II promotes oxidative stress and inflammation via AT_1 receptor-mediated pathways[37]. Ang-II increases the formation of ROS by vascular smooth muscle cells; for example, by activating NADPH oxidase, which leads to enhanced vascular superoxide formation[45]. Superoxide reacts extremely efficiently with NO, decreasing the bioavailability of the major endothelium-derived vasodilating factor[46]. When superoxide is in excess, the ROS will

preferentially react with NO over its antioxidant enzyme, superoxide dismutase[37], leading to a loss of normal NO-mediated signaling. In the kidneys, elevated superoxide production produces long-term BP elevations through NO bioinactivation, which influences afferent arterial tone, feedback responses between the renal tubules and glomeruli and sodium reabsorption[47]. In addition to having direct effects on vascular function (elevated vascular tone and impaired vasodilation), ROS also change vascular structure via several mechanimsms[37]. For example, superoxide promotes cell proliferation while hydrogen peroxide induces apoptosis[42]. Furthermore, vascular smooth muscle cell growth involves peroxisome proliferator-activated receptor-γ (PPARγ), a transcription factor with antioxidant effects that regulates expression of genes that promote or suppress ROS in vascular cells[37]. Decreased expression of PPARγ increases superoxide and impairs vascular function, producing vascular smooth muscle cell hypertrophy and remodelling[48]. Conversely, activation of PPARγ prevents vascular remodeling in hypertension, decreases expression of AT_1 receptors and NADPH oxidase and increases superoxide dismutase expression[49].

Oxidative stress activates circulating inflammatory cells called monocytes, increasing their ability to infiltrate the vascular wall and differentiate into macrophages, which cause further oxidative damage and inflammation[50]. During this process, there is massive production of ROS by activated macrophages via the phagocytic isoform of NADPH oxidase[42]. Macrophages also produce pro-inflammatory cytokines (*eg.* IL-6 and TNF-α) and acute-phase reactants (*eg.* C-reactive protein [CRP] and fibrinogen)[51]. Cytokines stimulate the expression of cell adhesion molecules, vital to cell growth, leading to vascular hypertrophy[52], and produce NO radicals via inducible nitric oxide synthase (iNOS)[42]. NO radicals react with superoxide to give peroxynitrite, a potent oxidizing agent that damages DNA and oxidizes lipids[42]. Furthermore, CRP decreases the expression of endothelial nitric oxide synthase (eNOS), leading to reduced NO production and impaired vasodilatory response[53]. CRP also augments the expression of adhesion molecules, stimulates the expression of clotting factors and induces further oxidative stress and the secretion of other cytokines[53]. In sum, both oxidative stress and inflammation play a major role in producing endothelial dysfunction (Figure 1.5)[37]. Once the endothelium becomes compromised, it enhances endothelin-1 production, a powerful vasoconstricting peptide that increases vascular tone, and

secretes molecules such as platelet-derived growth factor and transforming growth factor β, which enhance endothelial remodeling resulting in defective vasodilation and further increases in BP[54]. Epidemiological evidence supports the link between high BP and oxidative stress. Studies show that children and adolescence with hypertension demonstrate higher levels of oxidative stress, measured by nitrates, lipid peroxidation end-products (*eg.* thiols) and glutathione, than those with normal blood pressure[41].

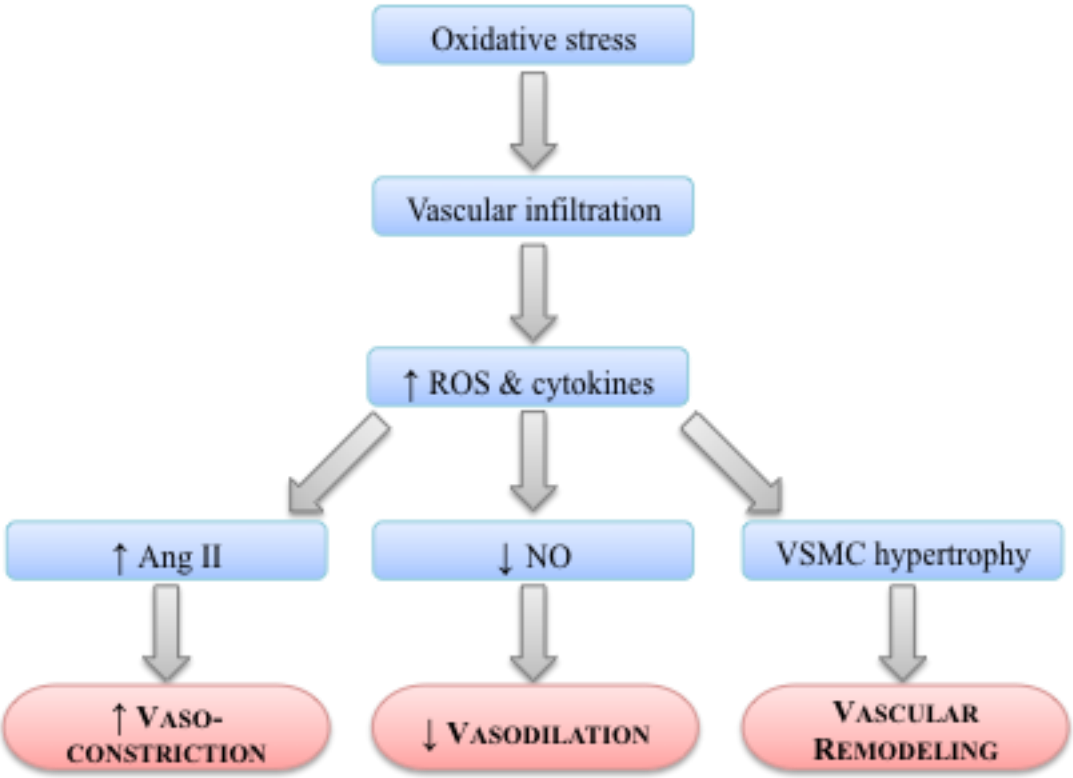

Figure 1.5. The link between oxidative stress, inflammation, endothelial dysfunction and high BP
Oxidative stress leads to increased infiltration by monocytes into the vascular wall. These differentiate into macrophages, which cause further oxidative damage and inflammation via the enhanced production of ROS and cytokines, which in turn (1) increase Ang II-induced vasoconstriction; (2) decrease NO production resulting in reduced vasodilation and blood flow; and (3) cause vascular remodeling through vascular smooth muscle cell growth.
Ang II = angiotensin II, NO = nitric oxide, ROS = reactive oxygen species, VSMC = vascular smooth muscle cell.

1.1.3 ASSESSING CARDIOVASCULAR REACTIVITY

In both laboratory and clinical research, cardiovascular reactivity and recovery can be measured in response to laboratory stressors, during standardized stress protocols, or in response to stressors encountered in every day life, using ambulatory BP monitoring (ABPM). In the laboratory, reactivity can be defined in numerous ways, depending on the number of times a cardiovascular parameter is measured throughout the stress protocol. Consider the case of blood pressure as an example. If three BP measurements are taken (pre-stress, stress and post-stress), then BP reactivity can be defined as the change in BP from a baseline value to that observed during stress (*eg.* $BP_{stress} - BP_{baseline} = 140 - 120 = 20$ mm Hg). In this case, BP reactivity can be an absolute change (*i.e.* $\Delta BP = BP_{final} - BP_{initial}$), a relative change (*i.e.* percent change in BP), or a change value adjusted for baseline BP and/or recovery BP. On the other hand, if multiple BP measurements are taken, then BP reactivity can be defined as the peak BP response attained during stress (*eg.* $BP_{max} = 140$ mm Hg). Alternatively, an average BP response during stress (which varies in duration) can be used to calculate the change in BP (*eg.* $BP_{stress} - BP_{baseline}$, where BP_{stress} = mean BP during 5 min of stress). Additionally, when multiple stressors are used, an aggregate score can be generated which averages BP responses across different challenge (*eg.* $\Delta BP = [\Delta BP_1 + \Delta BP_2 + \Delta BP_3] \div 3$).

Similar to BP reactivity, BP recovery can be quantified as the absolute or relative change in BP (from baseline or from stress), the time to return to baseline BP once the stressor is removed, or the BP value after a given recovery period (*i.e.* 1 min, 5 min, 10 min of recovery). Many studies report only raw recovery data, without correcting for baseline or stressor levels[55]. The benefit of looking at cardiovascular recovery is that it captures both the magnitude and the duration of the stress response. Another method for analyzing repeated measures of BP, which applies to both reactivity and recovery, is to calculate the area under the curve, or to use curve-fitting approaches to compare cardiovascular responses between individuals. For example, latent growth curve modelling establishes a trajectory of change over time for each individual; the characteristics of this curve (*eg.* slope) can be compared to others in the sample[56].

Although BP reactivity is most commonly assessed using laboratory stressors, it can also be measured during 'real-life' with ABPM. In this instance, subjects wear a 24-hour BP monitor and BP, CO and TPR are continuously recorded during day-to-day activities. This data can be supplemented with measures of general activity and self-reported behaviour[57]. Ambulatory cardiovascular reactivity can be assessed in multiple ways: (1) determine the response to a specific stressor (*eg.* an exam, public speaking); (2) derive a measure of cardiovascular variability, correcting for activity and posture if possible (*eg.* day-time and night-time BP variability); (3) relate cardiovascular responses to self-reports of the participants perceived stress, emotion or demands[57]. These approaches are advantageous because they measure cardiovascular responses to actual stressors encountered in everyday life, cardiovascular variability is non selective and a wide range of situations can be sampled; however, some weaknesses of these methods are that cardiovascular variability reflects many processes that are non stress-related (*eg.* postprandial changes in BP) and the self-reported data may be biased[57]. A review of the literature suggests that the cardiovascular response to lab stressors relates to the response in real life when examining the response to an objective or self-reported stressor but the evidence linked to cardiovascular variability is less consistent[57].

There are a wide variety of stressors used to test cardiovascular reactivity and recovery. Regardless of the stressor(s) selected, the intent is to produce a measurable change in the variable being measured. This change is thought to be a stable individual difference and a useful marker of disease risk. Although measured in the laboratory, most stressors are meant to mimic those encountered in every day life. The different types of stressors and the characteristic cardiovascular response for each are described in detail. These can be broadly categorized into two groups: physical challenges, comprising of active and passive stressors, and mental challenges, including cognitive and social stressors. Manipulations involving the social environment are known to alter cardiovascular responses to challenges. For example, harassment can enhance responding while the presence of a friend may reduce the response to an evaluative threat[18].

1.1.3.1 Physical challenges

Active stressors:

Posture test: Participants begin in an initial supine resting condition, assume a standing position and return to supine or sitting for recovery. Active standing is an orthostatic stress causing a transient drop in BP as blood pools in the lower body, followed by a reflex elevation in HR resulting from baroreflex stimulation and a sympathetically mediated BP overshoot[58]. Contraction of leg and abdominal muscles initiates the exercise reflex, causing sympathetic activation and vagal withdrawal[20]. After prolonged standing (>5 min), humoral mechanisms contribute to maintaining BP due to the increase in plasma catecholamines[20]. The posture test is best suited to asses the initial phase of orthostatic challenge[20].

Aerobic exercise: Exercise elicits marked cardiovascular responses, characterized by increases in HR, CO and BP. Exercise protocols vary but can include, for example, a short sprint (typically used in young children), a treadmill test (following a set protocol), cycle exercise and step exercise tasks. The rise in BP during exercise results from sympathetic stimulation to the vasculature while the abrupt rise in HR occurs as result of vagal withdrawal and sympathetic activation to the heart[59].

Isometric handgrip exercise: The maximal handgrip strength of subjects is determined initially. During the test, subjects are told to contract muscles in their forearm at 30% of their maximum handgrip strength for a set amount of time (*eg.* 2 min), with a visual display showing the actual and desired forces[60]. Higher brain regions activate the vasomotor centre, along with afferent feedback from active muscles via the exercise reflex, to induce shifts in autonomic balance. This induces a cardiovascular response characterized by an increase in sympathetic activity and vagal withdrawal, resulting in a rise in BP[60].

Passive stressors:

Head-up tilt: Similar to the posture test, the cardiovascular response to head-up tilt is mediated by baroreceptor function; however, without the confounding effect of the exercise reflex. Subjects begin supine on a tilt table, which is then slowly tilted to the head-up position (usually 60 or 70° tilt)[61]. Tilt activates the baroreflex, which induces a reflex rise in HR, BP and peripheral vascular resistance due to sympathetic activation and vagal withdrawal[20]. Some individuals, particularly women, exhibit orthostatic hypotension[61, 62]. Since the cardiovascular adaptation to tilt is more gradual than the posture test, it is better suited to evaluate the neural control of BP during prolonged orthostatic challenge (>5 min)[20].

Lower body negative pressure (LBNP): In addition to active standing and head-up tilt, LBNP is another method to determine orthostatic tolerance. Subjects lie in a supine position with a special chamber surrounding their legs, which progressively reduces the pressure around the lower body relative to ambient pressure, up to -100 mm Hg[63]. This results in presyncopal symptoms, including a drop in BP, bradycardia and the onset of nausea, sweating or dizziness, as blood in the upper body drains to the lower body where there is less resistance[63]. LBNP causes a reduction in SV and CO, a compensatory increase in HR, and an elevation of BP.

Cold pressor test: Invented by Hines and Brown in 1936, the cold pressor test activates afferent pain and temperature fibers in the skin to assess the function of ANS[60]. The test consists of the brief immersion of a hand or foot in ice water (4°C), invoking HR acceleration, peripheral vasoconstriction and an increase in BP due to sympathetic activation resulting from pain and low temperature[18, 20]. Alternatively, a cold pressor may be applied to the forehead eliciting a diving reflex, characterized by sympathetic activation of the vasculature and vagal stimulation of the heart, inducing peripheral vasoconstriction with a subsequent increase in BP and bradycardia upon facial contact with the cold compress[18, 20]. This task evokes a strong α-adrenergic activation[64].

1.1.3.2 Mental challenges

Cognitive stressors:

Mental arithmetic: Subjects are given math problems to perform in their head. Sometimes subjects are put under additional stress (*eg*. given a time constraint, hassled). Mental arithmetic causes an abrupt rise in BP and HR due to sympathetic and HPA activation, mediated through the central nervous system[65, 66].

Serial subtraction task: Subjects asked to mentally and sequentially subtract backward by a given number, as quickly and accurately as possibly. Answers are given aloud. Serial subtraction produces the same cardiovascular response as mental arithmetic[67].

Mirror tracing: Participants outline a star with a metal stylus while viewing only its reflection in the mirror, as many times and making the least amount of mistakes as possible, during a set amount of time. Deviations are recorded and tallied[68]. This task produces increases in vascular resistance and BP due to increased α-adrenergic activity, and moderate increases in HR[64, 69, 70].

Stroop colour-word test: A series of names of colours written in different coloured fonts is presented to subjects on a computer monitor, and subjects are asked to indicate the colour of the *font*, not read the word aloud. This stressor increases both BP and HR[71].

Video game: Subjects play a video game for a given period (*eg.* Atari breakout; 5 min), and are often presented with the lure of a monetary reward. This challenge elicits increases in BP and HR[64, 72].

Reaction time-shock avoidance: Participants are presented with a signal that they must react to as quickly as possible (*eg.* hear a sound, press a button)[68]. This elicits a mainly cardiac (β-adrenergic) pattern of response, characterized by increases in CO and HR[73]. This task can be paired with shock avoidance: if subjects are too slow, a harmless shock is administered. In recent decades, a shock is never administered – merely the threat of shock is meant to cause duress.

Social stressors (i.e. emotional challenges):

Active or passive speech: To assess active speech, subjects are given a topic, told to prepare a speech and then give a speech while being recorded or watched. In contrast, passive speech would be, for example, reading a passage aloud to an audience. Both HR and BP increase during speech tasks[74]. Given the interpersonal component of public speaking, this task elicits a strong β-adrenergic response[75].

Stress interview: Subjects are interviewed (*eg.* for 10 min) about a recent, stressful, interpersonal situation, resulting in increases in BP and HR[73].

Anger recall interview: Following a preparation period, participants describe a previous interpersonal situation which made them angry, outlining the situation, their responses and their satisfaction with the outcome[68]. Cardiovascular responses are analogous to the stress interview.

Social competence interview (SCI): This type of interview measures physiological changes while participants describe an interpersonal source of emotional distress. Subjects are asked to describe in detail the situation, their thoughts and emotions, the strategies they employed to resolve the situation and how they would have ideally solved the problem[76]. Similar to the other emotionally engaging interviews, the SCI produces a mixed vascular and cardiac response, with increases in BP and HR[70].

Parent-child discussion: This consists of a live conversation between a parent and their child on an agreed upon contentious issue (*eg.* allowance) in the presence of a facilitator[77], eliciting a cardiovascular response.

1.2 BLOOD PRESSURE REACTIVITY AND ITS RELATIONSHIP TO HYPERTENSION

Hypertension, the chronic elevation of BP, is a major problem in human health that has reached epidemic proportions. Normal BP in adults 18 years and older is SBP < 120 mm Hg and DBP < 80 mm Hg (*i.e.* <120/80 mm Hg). Classic hypertension is defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg. In Canada, 27% of the adult population is hypertensive[3]. The prevalence of hypertension in the United States is 65 million, equivalent to about one-third of Americans, according to the National Health and Nutrition Examination Survey (NHANES)[2]. An additional one-quarter is considered prehypertensive, with BP above normal but below the hypertensive range (120 ≤ SBP < 140 mm Hg and 80 ≤ DBP < 90 mm Hg)[2]. Prehypertensive individuals have greater health risks and will very likely become hypertensive, given that BP and the prevalence of hypertension increase with age[2]. Data from the NHANES and Framingham Heart Study show that as age progresses, the rise in SBP is accompanied by an age-related decrease in DBP, and a subsequent elevation in pulse pressure[78, 79]. As a result, isolated systolic hypertension (SBP ≥ 140 mm Hg and normal DBP < 90 mm Hg) is the most common form of hypertension across age groups[80, 81]. As the population ages, the rate of isolated systolic hypertension will continue to increase.

In children and adolescents, the criteria for hypertension are different than in adults. BP status is classified based on age-, sex- and height-specific percentiles, according to the U.S. Centres for Disease Control and Prevention growth charts[82]. Measurements below the 90th percentile are considered normal, while those in the 90 to 95th percentile are categorized as prehypertension. Hypertension is defined as being in the top 95th percentile of sitting SBP or DBP[83]. The prevalence of hypertension in Canadian adolescents (ages 12 to 19 years) is around 2% according to the Canadian Health Measures Survey[84]. Data from NHANES in the United States show a clear upward trend in prevalence of prehypertension and hypertension in youth from 1988 to 2006[85]. In this age group, hypertension is associated with increased left ventricular mass, fatty streaks and fibrous plaques in the coronary arteries and aorta, and arterial wall

thickening[84]. Those with elevated BP at a young age are at increased risk of developing hypertension as adults, given that BP levels track from childhood to adulthood[86].

Some hypertensive individuals may only exhibit elevated BP in the presence of a health care worker but otherwise have normal BP, a phenomenon termed white-coat hypertension[87]. Alternatively, one can have masked hypertension: normal BP in a clinical setting but elevated BP elsewhere (at home, work, school, etc.)[2]. There are various causes of hypertension but many forms of the disease are neurogenic in origin. Neurogenic hypertension arises from an abnormality of the ANS, which can originate in either the central control regions (*eg.* medulla, hypothalamus) or in the afferent arms of the system (*eg.* baroreceptors, renal afferents)[17]. Other types of hypertension manifest as a result of vascular or renal defects.

Hypertension is a major risk factor for coronary artery disease, stroke and renal failure[2]. Despite its high incidence, hypertension remains one of the most modifiable risk factors for cardiovascular disease in Canada and globally[4, 5]. Hypertension is a leading risk factor for cardiovascular disease because it causes structural changes to blood vessels resulting from increases in shear stress and transmural pressure[1, 88]. SBP is a stronger correlate of cardiovascular disease risk than DBP, according to data collected in the Framingham Heart Study[89]. In fact, each mm Hg elevation in SBP corresponds to a 1% increase in mortality during 8 years of follow-up[90]. While high SBP predicts cardiovascular events, low DBP compounds this risk[91]. Isolated systolic hypertension is associated with a greater risk of acute myocardial infarction, stroke and death[92]. Despite the direct relationship observed between BP and cardiovascular disease and mortality, antihypertensive drug therapy is effective at reducing the risk of cardiovascular events in hypertensive individuals[2].

1.2.1 PREDICTING FUTURE BLOOD PRESSURE STATUS – HYPERTENSION

The main usefulness of studies on BP reactivity is their ability to predict the development of hypertension and cardiovascular disease, with follow-up intervals of up to 45 years[93]. Longitudinal studies show that "high" childhood BP traces well to "high" adult BP, suggesting that adult hypertension originates in childhood[94]. Similarly, children and adolescents with enhanced BP reactivity and slower, less efficient BP recovery are more likely to develop hypertension as adults. Studies of borderline hypertensive adolescents and young adults have found that all subjects who progressed to hypertension at follow-up 4 to 5 years later manifested a high BP response to mental stress[95, 96]. Similarly, BP responses to a posture change and video game were significant independent predictors of future resting BP in children with a positive family history of essential hypertension, explaining up to 12% of the variance in resting SBP 5 years later[97]. Even in children as young as six or seven years old, BP responses to various challenges (posture change, forehead cold and treadmill exercise) are predictive of resting BP levels one to two and a half years later[98, 99].

Individual differences in cardiovascular reactivity are believed to be a marker of disease vulnerability, although some critics question the reliability over time and across settings and the usefulness of cardiovascular reactivity and recovery as a disease indicator. However, there is ample evidence suggesting the usefulness of these parameters in predicting the future development of hypertension, notably when these test are done in children and adolescents. Much of the previous research has focused on cardiovascular reactivity, but in recent years, the usefulness of recovery data has been increasingly acknowledged. For instance, BP recovery captures both the magnitude and duration of BP elevation[100]. The ability to return to baseline levels of cardiovascular activity may be an important component for healthy functioning and homeostasis[101]. Several longitudinal studies, with follow-up periods ranging from 3 to 10 years, have reported that slower BP recovery from mental and physical challenges is associated with elevated resting BP and hypertension at follow-up[96, 102-104]. In the

Framingham Heart Study (n>2300), elevated recovery BP following treadmill testing predicted the development of hypertension 8 years later, although this was only observed in men[104].

A growing body of evidence suggests that pre-clinical features of hypertension and cardiovascular disease emerge already during adolescence[6, 105, 106]. Enhanced BP reactivity and delayed BP recovery may be two such features. Both are present in healthy adolescent offspring of hypertensive parents[107, 108] and, in longitudinal studies, they predict future resting and ambulatory BP and left ventricular mass[73, 109-111]. Individuals who are at a high risk for hypertension may exhibit an exaggerated stress-induced cardiovascular response at a young age[109]. In a comprehensive review by Treiber *et al.*, the authors identified twelve studies that reported positive associations between stressor-related BP reactivity and subsequent BP elevations in normotensive children and adolescents[112]. Recently, Matthews *et al.* showed that the magnitude of BP increase in response to acute stressors in >4100 subjects in the CARDIA study (age at entry, 18 to 30 years) predicted the likelihood of hypertension at follow-up 13 years later, where larger responses were associated with earlier onset of hypertension in originally normotensive adults[64]. Similarly, in the Framingham study, greater BP reactivity to exercise predicted new onset hypertension in normotensive men and women[104].

The literature on BP reactivity and recovery provides compelling evidence that BP is elevated in the presence of a stressor; however, the underlying mechanisms by which these BP responses translate into chronic BP elevation remain poorly specified[113]. Both parameters enhance pressure load on the vessels, heart and kidneys, leading to structural and functional changes that in turn contribute to further BP elevations and thus the progression of hypertensions and cardiovascular disease[114]. Acute stress-induced elevations in BP are primarily attributed to enhanced sympathetic activation, but may also include diminished nitric oxide production and vagal withdrawal[115, 116]. Long-term regulatory changes that perpetuate hypertension involve vascular remodelling and endothelial dysfunction. Vascular remodelling involves alterations in vessel architecture, including decreased lumen diameter and rarefaction (reduction in number of microvessels)[113]. Greater BP variability increases stress on the vessel wall, leading to

hypertrophy of large arteries. This is supported by studies showing that hypertensive patients exhibit steeper BP variations which correlate to increased common carotid artery intima-media thickness[117]. Hemodynamic changes in blood flow and pressure and changes in levels of vasoactive substances (*eg.* angiotensin II, NE, nitric oxide) cause vascular remodelling, leading to a chronic increase in vascular resistance[113]. Vascular remodelling may facilitate the transition from the initial high cardiac output stage of hypertension to the high peripheral resistance stage[113]. The endothelium regulates vascular tone through the production of vasoconstrictive and vasodilatory substances that act of vascular smooth muscle. NO is an important endothelium-derived vasodilatory substance; its catabolism may impair endothelial function. Acute BP elevations may impair endothelial-dependent vasodilation, suggesting a mechanism by which short-term increases in BP could lead to hypertension[118]. In the heart, repeated elevations in BP favour pathogenic adaptations, such as left ventricular remodelling, and persistently elevated BP can lead to sustained cardiovascular burden and end organ damage[68]. Additionally, both vascular remodelling and dysfunction can lead to sustained changes in the renal set-point for BP regulation. Given the role of the kidney in regulating sodium and water balance and thus, blood volume, this would be important for the long-determinant of BP level[119]. As such, the magnitude and duration of BP responses[120] may contribute to the subsequent risk for hypertension[73, 109-111].

1.3 SEX AND BLOOD PRESSURE

The incidence of hypertension is higher in males than females during reproductive age, putting males at increased risk of cardiovascular and renal disease. In Canada, the prevalence of hypertension is 31% in men and 24% in women[121]. In the United States, 30% of men and 26% of women are hypertensive[121]. Epidemiological evidence indicates the same trend in various European countries, with a higher prevalence of hypertension in men than women from the ages of 35 to 64 years old[121]. In Canadian children and adolescents (aged 6 to 19 years), 3.1% of boys compared to 2.4% of girls have borderline or

elevated BP, according to the Canadian Health Measure Survey[84]. Given that this sex difference emerges at the onset of puberty, it becomes much more pronounced in pubertal adolescents. One study in French Canadians reported that the prevalence of high-normal or elevated SBP in boys at 16 years of age was nearly twice as high as in age-matched girls (30% versus 17%, respectively)[122]. Despite these findings, women in North America and Europe are more likely to receive hypertension treatment than their male counterparts; this is especially true in Canada, where 63% of women are treated but only 44% of men, and in the United States (45% in women versus 28% in men)[3]. The association between high BP and the incidence of cardiovascular disease does not differ between the sexes[123]. However, cardiovascular disease kills a higher percentage of women (55%) than men (43%), despite being regarded as a predominantly "male" disease[123]. In the presence of diabetes, the risk of cardiovascular death in women is twice as high compared to men when these two risk factors are clustered together (DECODE Study)[124]. Before menopause, diabetes suppresses the cardiovascular protection attributed to female sex hormones, while after menopause, insulin resistance is higher in women than men[125]. Diabetes also causes a critical reduction in the endothelial-dependent vasodilation reserve and this effect is more pronounced in women than men[126]. Moreover, hyperglycemia reduces NO production mediated by estradiol[127].

There is a growing awareness of fundamental gender dissimilarities in cardiovascular anatomy and pathophysiology. These differences start to manifest at the onset of puberty and become more pronounced with age, until menopause[123]. Given this time trajectory, it is postulated that the sex hormones likely play an important role in BP regulation and the observed sex difference in the incidence of higher BP.

1.3.1 ANATOMICAL DIFFERENCES

Females compared to males have smaller hearts and large blood vessels, resulting in lower SV and higher HR[128]. Even after accounting for the larger body size of males (approximated by body surface

area), SV is 10% lower in females[123]. Females exhibit a higher HR than males. This chronotropic difference appears after sexual maturation and continues in adulthood, although the difference diminishes with age. Studies using double blockade of the sympathetic and parasympathetic nervous system have demonstrated that this difference in HR does not depend on cardiac sympatho-vagal balance[123]. Coronary arteries in females also have a smaller diameter, thinner walls and frequently a winding course[123]. As a result, the clinical manifestation of atherosclerosis often differs between males and females, with only a small percentage of females showing a deep plaque ulceration under the thrombus. It is much more frequent to find a thrombus formation on a superficial lesion[123].

1.3.2 SEX AND BLOOD PRESSURE

BP and the prevalence of hypertension is higher in males than females throughout reproductive life[129, 123]. Resting BP is higher in men than women, beginning in adolescence[129, 123]. This sex difference emerges at the onset of puberty[84], widens as puberty progresses[130, 131] and remains relatively constant until after the female menopause when female BP equals or surpasses that of males[129] (Figure 1.6). Adolescence is a key stage in development when adult BP and body composition develop[123, 132]. It is also during this time that high BP and hypertension emerge. Girls' BP increases during prepubescent growth and stabilizes during puberty, while boys experience a gradual increase in BP from pubescence through age eighteen[133]. In a longitudinal adolescent cohort study, Dasgupta *et al.* showed that there is a sex-specific difference in the evolution of risk for high SBP during adolescence, with risk remaining unchanged among girls but increasing 19% annually among boys[134]. In addition, as puberty progresses, and as part of the normal developmental changes that occur, boys increase their lean body mass while girls increase their fat mass and percent body fat[132].

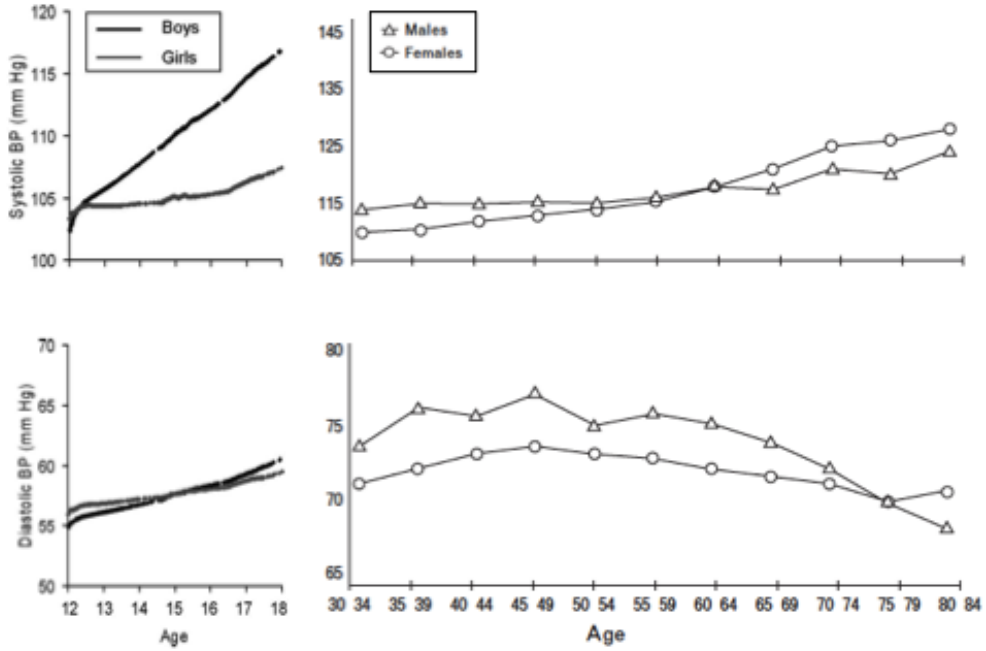

Figure 1.6. BP changes in males and females throughout reproductive life.
Higher BP in males emerges at the onset of puberty during adolescence (right) and lasts throughout reproductive age until female menopause (left). Sex hormones are likely related to the difference in BP observed between the sexes. During childhood, before the production of sex hormones begins in puberty, and after menopause, when the ovaries cease to produce estrogen (a "BP-lowering" female sex hormone), the sex difference in resting BP disappears and BP in females equals or surpasses that in males. Adapted from Maximova et al. 2010[131] and Mercuro et al. 2010[123].

1.3.3 SEX AND BLOOD PRESSURE REACTIVITY

Similar to BP at rest, BP reactivity to both physical and mental challenges is also higher in males than females and shows a similar life course, with sex differences emerging in adolescence[58, 70, 135, 136] (Table 1.1). Males show greater BP reactivity to both physical and mental stressors across a wide variety of challenges[64]; however, this sex difference is often only apparent for systolic, not diastolic, BP[61, 66, 70, 76, 136]. Studies examining BP recovery demonstrate a similar trend with males exhibiting slower recovery than females, irrespective of the type of challenge (Table 1.2). There are no sex differences in BP recovery seen in preadolescents[137, 138].

Table 1.1. Sex differences in SBP and DBP *reactivity to* physical and mental stressors

Reference	*M/F**	*Age†*	*Type of stressor*	*Challenge*	*Sex diff.‡*
SBP Reactivity					
Sallis *et al. J. Dev. Behav. Pediatr.*, 1989	24/39	3 to 4 (3.9±0.8)	Physical	Run-stressor	M=F
Taras & Sallis, *J. Dev. Behav. Pediatr.*, 1992	32/53	3 to 6 (4.4±0.9)	Phys. & mental	Run-stressor, tower-building race, game	M=F
Treiber *et al. Health Psychol.*, 1993	44/43	6 to 8	Physical	Cold pressor, run-stressor	M=F
Allen and Matthews, *Psychophysiology, 1997*	40/39	8 to 10 (8.9)	Phys. & mental	Reaction time, mirror tracing, cold pressor, stress interview	M=F
Murphy *et al. Hypertens.*, 1988	237/242	Gr. 3 (9.2)	Mental	Video game	M=F
Voors *et al. Hypertens.*, 1980	183/185	6 to 13	Physical	Posture change§, cold pressor	M=F
				Isometric handgrip	M>F
Murphy *et al. Health Physchol.*, 1995	142/153	Gr. 3 (9.1) to 9 (15.1)‖	Phys. & mental	Video game (grade 3 to 8), isometric handgrip (grade 9)	M>F
Matthews & Stoney, *Psychosom. Med.*, 1988	96/121	Gr. 2 to 8	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M=F
		Gr. 9 to 12	Phys. & mental	Serial subtraction, mirror tracing	M=F
				Isometric handgrip	M>F
Treiber *et al. Int. J. Psychophys.*, 2001	187/198	(12.7±2.6)	Phys. & mental	Posture change§, video game, social competence interview, parent-child conflict discussion#	M>F
Matthews *et al. Child Dev.*, 1990	56/76	6 to 18 (12.9±2.5)	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M>F
Ewart & Kolodner *Psyochosom., Med.*, 1991	125/131	14 to 15	Mental	Social competence interview, video game, mirror tracing, math	M>F
Tell *et al. Am. J. Epidemiol.*, 1988	500/416	14 to 16	Physical	Posture change§	M>F
Allen and Matthews, *Psychophysiology, 1997*	38/40	15 to 17 (15.6)	Phys. & mental	Reaction time, mirror tracing, cold pressor, stress interview	M>F
Harshfield *et al. Hypertens.*, 2003	151/141	15 to 18 (16±1)	Mental	Video game	M>F
Lai and Linden, *Psychosom. Med.*, 1992	49/56	(19±3)	Mental	Serial subtraction with provocation	M>F
Gerin and Pickering, *J. Hypertens.*, 1995	117/420	17 to 23	Mental	Serial subtraction	M=F
Matthews *et al. Circulation*, 2004	1696/1771	18 to 30 (27.4)	Phys. & mental	Cold pressor	M=F
				Mirror tracing, video game	M>F
Light *et al. Health Psychol.*, 1993	76/79	18 to 49	Phys. & mental	Math	M=F
				Reaction time, active/passive speech, cold pressor	M>F
Matthews & Stoney, *Psychosom. Med.*, 1988	93/125	31 to 62 (42)	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M>F
Barnett *et al. Hypertens.*, 1999	41/48	20 to 83 (49.5)	Physical	Head-up tilt	M>F
DBP Reactivity					
Treiber *et al. Health Psychol.*, 1993	44/43	6 to 8	Physical	Cold pressor, run-stressor	M=F
Allen and Matthews, *Psychophysiology, 1997*	40/39	8 to 10 (8.9)	Phys. & mental	Reaction time, mirror tracing, cold pressor, stress interview	M=F
Murphy *et al. Hypertens.*, 1988	237/242	Gr. 3 (9.2)	Mental	Video game	M=F
Voors *et al. Hypertens.*, 1980	183/185	6 to 13	Physical	Posture change§	M=F
				Isometric handgrip, cold pressor	M>F
Murphy *et al. Health Physchol.*, 1995	142/153	Gr. 3 to 9 (9.1-15.1) ‖	Phys. & mental	Video game (grade 3 to 8), isometric handgrip (grade 9)	M>F
Matthews & Stoney, *Psychosom. Med.*, 1988	96/121	Gr. 2 to 8	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M=F
		Gr. 9 to 12	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M=F
Treiber *et al. Int. J. Psychophys.*, 2001	187/198	(12.7±2.6)	Phys. & mental	Posture change§, video game, social competence interview, parent-child conflict discussion#	M=F

Matthews *et al. Child Dev.*, 1990	56/76	6 to 18 (12.9±2.5)	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M>F
Ewart & Kolodner *Psyochosom., Med.*, 1991	125/131	14 to 15	Mental	Social competence interview, video game, mirror tracing, math	M=F
Tell *et al. Am. J. Epidemiol.*, 1988	500/416	14 to 16	Physical	Posture change§	M>F
Allen and Matthews, *Psychophysiology, 1997*	38/40	15 to 17 (15.6)	Phys. & mental	Reaction time, mirror tracing, cold pressor, stress interview	M=F
Harshfield *et al. Hypertens.*, 2003	151/141	15 to 18 (16±1)	Mental	Video game	M>F
Lai and Linden, *Psychosom. Med.*, 1992	49/56	(19±3)	Mental	Serial subtraction with provocation	M>F
Gerin and Pickering, *J. Hypertens.*, 1995	117/420	17 to 23	Mental	Serial subtraction	M=F
Matthews *et al. Circulation*, 2004	1696/1771	18 to 30 (27.4)	Phys. & mental	Cold pressor, mirror tracing	M>F
				Video game	M=F
Light *et al. Health Psychol.*, 1993	76/79	18 to 49	Phys. & mental	Math, reaction time, active/passive speech	M=F
				Cold pressor	M>F
Matthews & Stoney, *Psychosom. Med.*, 1988	93/125	31 to 62 (42)	Phys. & mental	Serial subtraction, mirror tracing, isometric handgrip	M>F
Barnett *et al. Hypertens.*, 1999	41/48	20 to 83 (49.5)	Physical	Head-up tilt	M=F

* Expressed as n.

† Given as range (mean ± SD).

‡ Sex difference: M=F, males and females demonstrate equivalent BP reactivity; M>F, males exhibit greater BP reactivity (greater increase in BP during stressor).

§ Posture change is the transition from supine to standing.

|| Same individuals re-evaluated every year.

Aggregate score.

Abbreviations: SBP = systolic blood pressure, DBP = diastolic blood pressure, M = males, F = females, Diff. = difference, Phys. = physical, Gr. = grade, SD = standard deviation.

Table 1.2. Sex differences in SBP and DBP *recovery from* physical and mental stressors

Reference	*M/F**	*Age*†	*Type of stressor*	*Challenge*	*Sex diff.*‡
SBP Recovery					
Treiber *et al. Am. J. Dis. Child.,* 1989	37/38	4 to 6 (5.4±0.6)	Physical	Run-stressor	M=F
Treiber *et al. Health Psychol.*, 1993	44/43	6 to 8	Physical	Run-stressor, cold pressor	M=F
Treiber *et al. Int. J. Psychophys.*, 2001	187/198	(12.7±2.6)	Phys. & mental	Posture change§, video game, social competence interview, parent-child conflict discussion‖	M>F
Jackson *et al. Int. J. Psychophys.*, 1999	137/135	(13.5±2.6)	Phys. & mental	Posture change§, video game, social competence interview, parent-child conflict discussion‖	M>F
Harshfield *et al. Hypertens.,* 2003	151/141	15 to 18 (16±1)	Mental	Video game	M<F
Gerin and Pickering *J. Hypertens.*, 1995	117/420	17 to 23	Mental	Serial subtraction	M=F
Jorgensen and Houston, *Motivation Emotion*, 1981	29/32	Undergrad	Mental	Stroop colour-word test, serial subtraction	M>F
				Shock avoidance	M=F
Light *et al. Health Psychol.*, 1993	76/79	18 to 49	Phys. & mental	Math, reaction time, active/passive speech, cold pressor	M>F
Gillin *et al. Int. J. Psychophys.*, 1996	64/21	20 to 52 (35.6)	Mental	Active speech	M>F
				Mirror tracing	M=F
DBP Recovery					
Treiber *et al. Am. J. Dis. Child.,* 1989	37/38	4 to 6 (5.4±0.6)	Physical	Run-stressor	M=F
Treiber *et al. Health Psychol.*, 1993	44/43	6 to 8	Physical	Run-stressor, cold pressor	M=F
Treiber *et al. Int. J. Psychophys.*, 2001	187/198	(12.7±2.6)	Phys. & mental	Posture change§, video game, social competence interview, parent-child conflict discussion‖	M=F
Jackson *et al. Int. J. Psychophys.*, 1999	137/135	(13.5±2.6)	Phys. & mental	Posture change§, video game, social competence interview, parent-child conflict discussion‖	M=F
Harshfield *et al. Hypertens.,* 2003	151/141	15 to 18 (16±1)	Mental	Video game	M<F
Gerin and Pickering *J. Hypertens.*, 1995	117/420	17 to 23	Mental	Serial subtraction	M=F
Jorgensen and Houston, *Motivation Emotion*, 1981	29/32	Undergrad	Mental	Stroop colour-word test, serial subtraction, shock avoidance	M=F
Light *et al. Health Psychol.*, 1993	76/79	18 to 49	Phys. & mental	Math, active/passive speech, cold pressor	M>F
				Reaction time	M=F
Gillin *et al. Int. J. Psychophys.*, 1996	64/21	20 to 52 (35.6)	Mental	Active speech	M>F
				Mirror tracing	M=F

* Expressed as n.

† Given as range (mean ± SD).

‡ Sex difference: M=F, males and females demonstrate equivalent BP recovery; M>F, males exhibit slower BP recovery (smaller decrease in BP after stressor); M<F, females exhibit slower BP recovery.

§ Posture change is the transition from supine to standing.

‖ Aggregate score.

Abbreviations: SBP = systolic blood pressure, DBP = diastolic blood pressure, M = males, F = females, Diff. = difference, Phys. = physical, SD = standard deviation.

1.3.4 ROLE OF SEX HORMONES IN BP REGULATION

Although the incidence of higher BP in males during reproductive age has been well documented, the mechanisms responsible for the increase in BP in this sex remain not well understood; however, sex hormones likely play an important role. Sex steroid hormones include three main classes, estrogens, progestins and androgens, which each have metabolic, vascular and cardiac activities and affect cardiovascular functions both directly, through either genomic or non-genomic pathways, and indirectly[123].

Estradiol and estrogens, female reproductive hormones, cause arterial vasodilation and improve endothelial function[123]. Estradiol interacts with two classes of receptors, estrogen receptor alpha (ERα) and beta (ERβ), and causes both rapid vasodilating effects and long-term effects through the modification of gene and protein expression. Molecular complexes located in the membrane of endothelial and vascular smooth muscle cells mediate the rapid response to estradiol[139, 140]. At the vascular level, the genomic effects of estradiol are mediated by the ERα and ERβ receptors expressed in endothelial and vascular smooth muscle cells[141, 142]. Estradiol administration causes endothelial-dependent vasodilation in both coronary and peripheral arteries in post-menopausal women[143, 144]. However, the endothelial response to estradiol depends on the time of intervention following estradiol deprivation and is only beneficial within the first five years after menopause, after which the ER receptors become inactive due to lack of stimulation causing ER methylation[145]. Estradiol does not produce relaxation in venous smooth muscle cells, although it increases the vasoconstrictive response to endothelin-1[146]. Estrogen is believed to make the large arteries more distensible. Ahimastos *et al.* showed that pre-pubertal females had stiffer large arteries but developed more distensible large arteries post-puberty, with corresponding increases in estradiol and progesterone, where as males developed stiffer large arteries and higher levels of testosterone[147]. Adkisson *et al.* has shown that women exhibit reduced BP and enhanced vascular reactivity during the late follicular phase of the menstrual cycle, which corresponds to peak estrogen

levels[148]. Estradiol also decreases norepinephrine spillover in response to mental stress, leading to an attenuated BP response[149].

The major progestin, progesterone, interacts with progesterone receptors class A and B (PR-A and PR-B). These receptors are expressed in endothelial and vascular smooth muscle cells; however, the vascular effects of progesterone are not well understood. PR-A and PR-B receptors located in cardiomyocytes help regulate cardiac growth[150]. Progesterone also increases plasma volume, although the underlying mechanism remains unknown. This may occur via the direct stimulation of sodium retention through mineralocorticoid receptors, or indirectly through its effects on BP[150]. Acute and prolonged treatment with progesterone lowers BP[151, 152], which might result in stimulation of low-pressure receptors, leading to sodium retention and increased blood volume. Given that the hypotensive effect of progesterone occurs with minutes of administration[152], the hormone likely regulates vascular tone through a non-genomic mechanism.

On the other hand, the main male reproductive hormone, testosterone, has a negative effect on cardiovascular and endothelial function[153-155]. Testosterone is thought to promote BP elevation by compromising renal function and impairing vascular reactivity, which can lead to abnormal sympatho-activation[156]. In response to adrenergic agonists, testosterone enhances vasoconstriction[157]. Levels of plasma norepinephrine, which are primarily derived from sympathetic nerve endings, increase in males as they advance through puberty and as their testosterone levels increase[158]. This may explain, in part, the higher BP seen in males beginning in puberty. In experimental models, testosterone can worsen cardiac function and stimulate cell hypertrophy[154]. Following ischemia-reperfusion injury, castrated male horses (that can no longer produce testosterone in their testes) recover vascular function better than controls with normal testosterone production and females treated with testosterone worsen, suggesting that the hormone impairs endothelial function[159]. Moreover, androgen receptors (AR), expressed in the heart and vasculature, have both genomic and non-genomic effects. AR receptors are involved in cardiac regulation and cardiac hypertrophy, *i.e.* remodelling of the heart in response to an increase in cardiac load[160]. In

spontaneously hypertensive rats, hypertension is worse in males than females; castration attenuates the development of hypertension and AR receptor inhibition reduces BP[161]. While much of the evidence suggests a moderately negative role of androgens and ARs on cardiovascular function, whether endogenous testosterone adversely affects hypertension and cardiovascular disease in men or women remains unclear[123].

Importantly, testosterone can be converted into estrogen by aromatase, an enzyme that is expressed in subcutaneous adipose tissue. Men who are peripherally obese therefore gain some of the cardioprotective effects of estrogen, due to the conversion of testosterone to estrogens by aromatase[162]. Men lacking this enzyme show endothelial dysfunction, early atherosclerosis development and metabolic disturbances[123]. This android or male fat distribution pattern, concentrated around the abdomen, is indicative of testosterone deficiency and male aging, as the production of testosterone declines. In males, testosterone inhibits the accumulation of adipose tissue; this is especially true of visceral fat (VF), which has a higher density of AR receptors[163]. Androgens seem to have the opposite effect in females, causing VF accumulation[164, 165]. By contrast, estrogens promote subcutaneous fat accumulation in the lower body (gluteal and femoral regions), resulting in a gynoid fat distribution[166].

The gender dichotomy can be also connected with genetic factors and genes controlling sex steroids[123]. Sex differences with respect to BP regulation originate from genes on both the sex and autosomal chromosomes. Gene involved in steroidogenesis, resulting in the production of estrogens, progestins and androgens, are also likely key modulators of hypertension and BP stress response. One such gene is *CY17A1*, encoding the enzyme CYP17, which is involved in the production of sex steroids in the adrenal gland and gonads. Its activities lead to the production of dehydroepiandrosterone (DHEA) and androstenedione, precursors to male and female sex hormones. This is discussed more in Section 1.5, which describes the genetics of BP and hypertension in more detail.

1.4 OBESITY AND HYPERTENSION

1.4.1 DEFINITION, PREVALENCE, BODY FAT DISTRIBUTION (FAT DEPOTS)

Overweight and obesity are defined according to body mass index (BMI, weight in kilograms/height^2 in meters). In adults, a BMI of 25 to 29 kg/m^2 is considered overweight and obesity is defined as a BMI greater or equal to 30 kg/m^2. The rise in overweight and obesity is becoming a global epidemic. From 1988 – 1994 to 1999 – 2000, the prevalence of overweight/obesity among adults increased from 56% to 65% while the rise in obesity jumped from 23% to 31%, according to the National Health Examination Survey[167]. Today, two-thirds of the U.S. population is either overweight or obese. In adolescents, the prevalence of obesity has also risen substantially, with obesity rates tripling over the past 25 years. Presently, approximately a quarter of Canadian children and adolescents are overweight or obese[168]. This is an alarming statistic, given that excess adiposity, especially visceral obesity, is an important risk factor for the development of hypertension[94, 169, 170]. It is estimated that, at the population level, 65 to 78% of adult hypertension is attributed to obesity[171]. Hypertension is now emerging in children and adolescents[88], which is partly attributable to a rise in the prevalence of obesity in the young[172]. Today, about a third of overweight adolescents (BMI>95th percentile) are affected by hypertension[173].

The risks associated with obesity are considerably dependent on the distribution of body fat. Subcutaneous adipose tissue, deposited under the skin's surface, is less detrimental than visceral adipose tissue, lying within the innermost aspect of the abdominal cavity. Visceral adipose tissue is considered to be an important depot that links obesity to hypertension, mediated by a cluster of risk factors known as the metabolic syndrome[174]. The metabolic syndrome is defined by the co-occurrence of visceral obesity, elevated BP, dyslipidemia, insulin resistance and/or glucose intolerance, and a proinflammatory state[175].

1.4.2 OBESITY AND BLOOD PRESSURE

Of the known risk factors for cardiovascular disease, obesity tracks most strongly from childhood into adult life[176] and is a leading risk factor for hypertension in both males and females. Across all ages (in children, adolescents and adults), excess body-fat promotes higher BP and an increased likelihood of hypertension[94, 122, 131, 169]. This relationship is stronger for visceral VF than for body fat deposited elsewhere in the body[94, 169, 170], more so in males than in females[177, 178]. The association between visceral obesity and elevated BP has been extensively studied in adults and more recently in young subjects[179]. Excess adiposity has many metabolic and pathophysiological consequences that are pivotal to the development of hypertension and which develop early in the course of obesity[53]. Early obesity is characterized by increased vascular oxidative stress and endothelial dysfunction, before the development of systemic oxidative stress and insulin resistance[36]. All of these processes act on various organs of the body to contribute to the development of cardiovascular disease, including high BP, in childhood.

Several mechanisms explain how excess adiposity, especially excess VF, increases BP (Figure 1.7). Energy imbalance in an obesogenic environment (*eg.* characterized by a diet high in fat) causes an excess of circulating glucose and triglycerides, resulting in hypertrophy (increase in cell size) and hyperplasia (increase in cell number) of adipose tissue[53]. Adipocyte dysfunction occurs when the increase in adipocyte cell size and number become insufficient to absorb excess circulating nutrients. This manifests as the altered secretion of adipokines, mitochondrial dysfunction and oxidative stress, increased inflammation, pro-thrombotic state and insulin resistance at the cellular level[53].

Adipocytes produce multiple hormones, peptides and molecules that affect cardiovascular function by way of endocrine, autocrine and paracrine mechanisms. Adipose tissue releases leptin whose main role is to signal to the hypothalamus that adequate energy stores are present[180]. Leptin likely plays a role in raising BP through SNS hyperactivity, sodium retention and increased vascular resistance[94]. Indeed, serum leptin levels are higher in obese and in hypertensive patients[94]. Second, fat is stored as triacylglyceride which gets broken down to free fatty acids (FFAs) and glycerol. VF is more

metabolically active and lypolytically sensitive than SF, leading to the release of more FFAs into the portal circulation[181]. Elevated FFAs produce SNS activation that affects both cardiac ANS activity and hemodynamic measures[182]. Furthermore, obese people exhibit a hyperactive RAAS system, with elevated levels of angiotensinogen, renin, aldosterone and angiotensin-converting enzyme (ACE), leading to SNS activation and increased BP[35]. Obesity is also associated with increased ROS, inflammation and endothelial dysfunction, all of which contribute to the development of hypertension. Dysfunctional adipocytes increase the release of proinflammatory cytokines (*eg.* IL-1, IL-6 and TNF-α) which maintain inflammation in the vascular wall, resulting in oxidative stress and endothelial dysfunction[35]. Evidence suggests that VF, compared to subcutaneous fat (SF), over-expresses certain factors such as interleukin-6[181]. Visceral fat is characterized by higher macrophage content than subcutaneous fat and therefore makes a more important contribution to systemic inflammation in obesity[51]. Many of these processes work additively in obese individuals to promote hypertension.

Data on the association between inflammation and obesity are emerging in youth. Adipocyte dysfunction is characterized by local inflammation with enhanced infiltration by inflammatory cells and an elevation in proinflammatory cytokines that activate additional inflammatory pathways[53]. For example, activation of mononuclear phagocytes (monocytes), results in the up-regulation of IL-1[183]. IL-1 is an upstream regulator with many downstream effects; in the liver, IL-1 activation leads to an increase in acute-phase reactants such as CRP[183]. Inflammation also leads to oxidative stress, characteristic of obesity, which generates free radicals faster than the body's ability to detoxify them, resulting in vascular damage over time. Moreover, excess lipid accumulation leads to increased endoplasmic reticulum (ER) activity, which synthesizes proteins and lipids. This can overwhelm the capacity of the ER to properly fold proteins, activating the unfolded protein response[53]. If this response cannot compensate for the unfolded proteins, apoptosis results. ER stress and the presence of excess FFAs can lead to oxidative stress in mitochondria, producing ROS[53]. TNF- α production is stimulated by FFAs which acts on enzymes to contribute to cellular insulin resistance[53]. Oxidative stress also induces insulin resistance at the cellular level by reducing the effect of insulin on glucose transporters[53]. Insulin resistance is a

proinflammatory condition that, in turn, increases the production of TNF-α and other cytokines[183]. In the Framingham Heart Study, both visceral and subcutaneous adipose tissue volumes were associated with circulating biomarkers of oxidative stress and inflammation[184]. Thus, inflammation may be an important mechanism linking obesity with cardiovascular disease.

Moreover, endothelial dysfunction is worsened in obesity due to the metabolic complications arising from excess adiposity[35]. Dysfunction of the endothelium can be caused by altered lipid profiles[175]. For example, hypercholesteremia (high cholesterol levels) is associated with a greater response to vasoconstrictors and an impaired endothelial-dependent vasodilation[185]. Oxidative stress contributes to endothelial dysfunction through its effects on the endothelial wall and a reduction in NO. Proinflammatory cytokines (*eg.* IL-1, IL-6, and TNF-α) add to the inflammatory process in the arterial wall and impair endothelial dependent dilation in arteries and veins. However, corticosteroids prevent this impairment. The inflammatory process is crucially involved in endothelial dysfunction. This is supported by many studies that demonstrate an association between inflammatory markers (*eg.* CRP) and endothelial dysfunction[186]. Furthermore, low serum adiponectin levels found in obesity are associated with low NO production, endothelial dysfunction and hypertension[35]. Endothelin-1 released from adipose tissue causes vasoconstriction and may impair the capacity for NO synthesis[35]. A study comparing brachial artery function and stiffness in severely obese children and normal-weight children found that the obese children had lower arterial compliance, lower distensibility, increased wall stress, increased stiffness, impaired endothelial function and increased insulin resistance[187]. Moreover, aerobic exercise improved arterial endothelial function in overweight children and adolescents despite no change in body weight or body composition, suggesting exercise has a beneficial effect on the health of the vasculature and may be a viable strategy to help prevent hypertension [188].

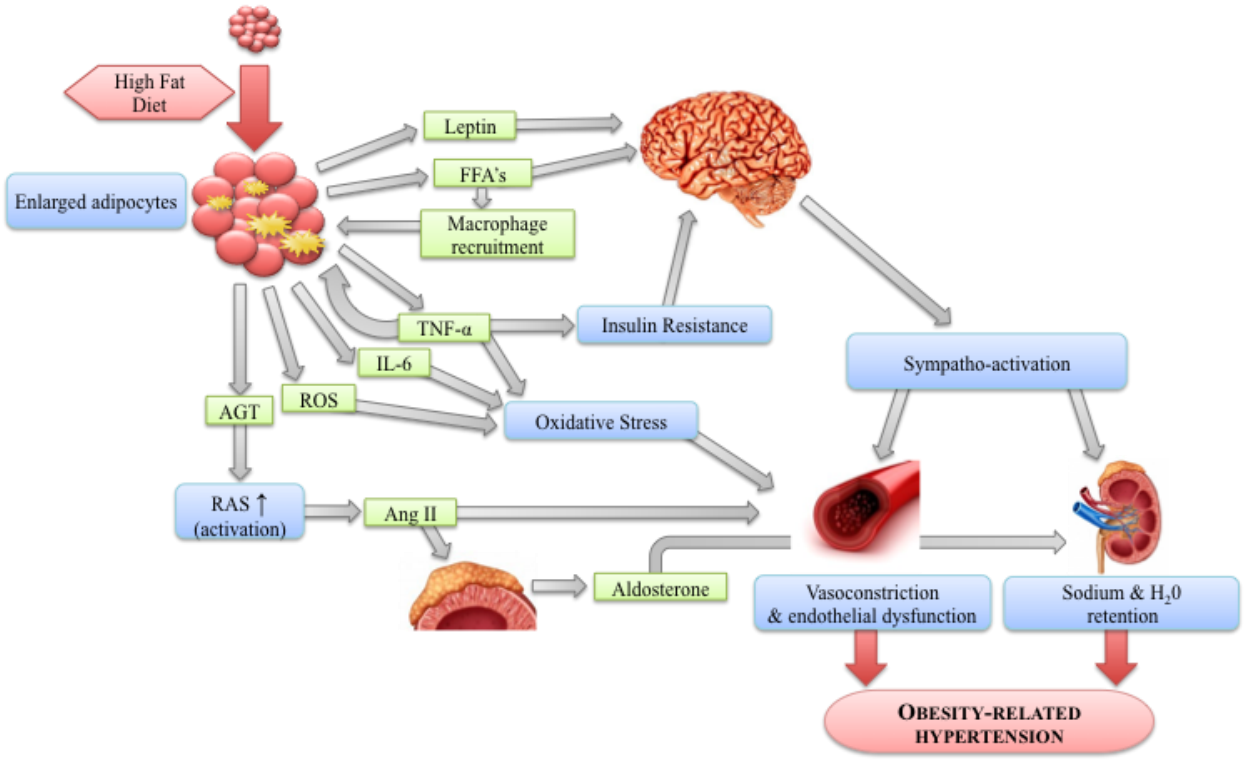

Figure 1.7. Mechanisms linking obesity to hypertension
A diet high in fat leads to enlarged adipocytes, which release a variety of proinflammatory factors, ROS, excess FFAs, and hormones leading to RAS activation, oxidative stress and insulin resistance. This results in sympathetic stimulation to the blood vessels and kidneys. Increased vasoconstriction and endothelial dysfunction in the vasculature, combined with sodium and water retention in the kidneys, results in obesity-related hypertension.
AGT = angiotensinogen, Ang II = angiontensin II, FFA = free fatty acids, IL-6 = interleukin 6, RAS = renin-angiotensin-aldosterone system, ROS = reactive oxygen species, TNF-α = tumor necrosis factor α.

1.4.3 OBESITY AND BLOOD PRESSURE REACTIVITY

Despite what is known about the link between adiposity and hypertension, studies on the relationship between visceral adiposity and BP reactivity to stress are limited and do not measure VF directly[189]. A few studies have examined this relationship indirectly in adults[190, 191] and in adolescents[192, 193]. Each of the above studies found an association between greater central adiposity and exaggerated cardiovascular responses to stress. However, these studies relied on waist circumference and waist-to-hip

ratio as estimates of central obesity, which cannot distinguish between VF and SF. This is essential given the metabolic differences between these two fat depots. None used direct measures, such as magnetic resonance imaging (MRI), to precisely quantify the amount of VF.

Previous research on obesity and BP reactivity has produced mixed results on the association between measures of overall adiposity, such as BMI and percent body fat, and BP reactivity to physical and mental challenges (Table 1.3). Studies have found a positive[135, 194], negative[195] or no[196-198] association between overall adiposity and SBP reactivity; however, they have consistently demonstrated no relationship between overall adiposity and DBP reactivity[135, 195-198]. This is likely explained by the fact that excess adiposity increases BP via sympatho-activation, which affects mainly systolic, not diastolic, BP, although the reasons for this remain unclear. Moreover, studies on the relationship between "abdominal" adiposity and stress reactivity are sparse and measured VF only indirectly with waist circumference or waist-to-hip ratio[189]. These studies found an association between greater abdominal adiposity and exaggerated BP responses to physical and mental challenges, in both adolescents[192, 193] and adults[190, 191, 196, 199]. With respect to adiposity and BP recovery, there is limited evidence in the literature that a higher BMI or greater waist-to-hip ratio is associated with slower BP recovery[135, 196] (Table 1.4). Furthermore, there is an emerging trend that the relationship between total or abdominal adiposity and BP reactivity and recovery is stronger in male versus female adolescents[135, 193].

Table 1.3. Abdominal and total adiposity and BP *reactivity to* physical and mental stressors

Reference	*M/F**	*Age*†	*Type of stressor*	*Challenge*	*Outcome, adiposity*	*Outcome, BP reactivity*	*Adiposity related to BP?*‡	*Sex diff.*§
Abdominal adiposity								
Goldbacher *et al. Health Psychol.*, 2005	104/107	14 to 16 (14.6)	Phys. & mental	Serial subtraction, mirror tracing, interview, cold pressor	WC	SBP	Yes (+)	M>F
						DBP	Yes (+)	M only
Barnes *et al. Int. J. Obes*., 1998	n=95	(14.8±1.4)	Phys. & mental	Posture change‖	WHR	SBP, DBP	Yes (+)	
				Video game	WHR	SBP	Yes (+)	
						DBP	No	
				Cold pressor	WHR	SBP, DBP	Yes (+)	
Davis *et al. Health Psychol.*, 1999	F=24#	(38)	Phys. & mental	Active speech, cold pressor	WHR	SBP	No	N/A
						DBP	Yes (+)	N/A
Steptoe & Wardle, *Int. J. Obes.*, 2005	123/102	47 to 59 (52.3)	Mental	Stroop colour-word test, mirror-image tracing	WHR	SBP	No	None
						DBP	Yes (+)	None
Waldstein *et al. Health Psychol.*, 1999	22	52 to 79 (62.1)	Mental	Stroop colour-word test, math, active speech	WC	SBP, DBP	Yes (+)	None
Total adiposity								
Taras & Sallis, *J. Dev. Behav. Pediatr.*, 1992	32/53	3 to 6 (4.4±0.9)	Phys. & mental	Run-stressor, tower-building race, game	BMI	SBP	No	
				Run-stressor	Skinfolds	SBP	Yes (+)	
				Tower-building race, game	Skinfolds	SBP	No	
Alpert *et al. J. Pediatr.*, 1981	n=405	6 to 15	Physical	Cycle ergometer	BSA	SBP	Yes (+)	
Aldo Ferrara *et al. Int. J. Obes.*, 1989	103/63	11	Phys. & mental	Math, isometric handgrip	BMI	SBP, DBP	No	
Barbeau *et al. Obes. Res.*, 2003	26/58	15 to 18 (16±1)	Mental	Video game	% BF (DXA)	SBP, DBP	No	
Wilson *et al. Am. J. Hypertens.*, 2004	63/64	15 to 18 (16±1)	Mental	Video game	% BF	SBP	Yes (-)	
					% BF, FM	DBP	No	
Harshfield *et al. Hypertens.*, 2003	151/141	15 to 18 (16±1)	Mental	Video game	BMI	SBP	Yes (+)	M only
						DBP	No	
Steptoe & Wardle, *Int. J. Obes.*, 2005	123/102	47 to 59 (52.3)	Mental	Stroop colour-word test, mirror-image tracing	BMI	SBP	No	None
						DBP	No	None

* Expressed as n.
† Given as range (mean ± SD).
‡ Significant relationship between adiposity and BP reactivity is either present (Yes) or absent (No) and the direction of the relationship is given in parentheses (positive or negative).
§ Sex difference: M>F, relationship between adiposity and BP reactivity is stronger in males; M only, relationship is only present in males; N/A, not applicable; None, no relationship found; if blank, not reported.

|| Posture change is the transition from supine to standing.
Female only sample.
Abbreviations: M = males, F = females, BP = blood pressure, BPR = blood pressure reactivity, Diff. = difference, Phys. = physical, SBP = systolic blood pressure, DBP = diastolic blood pressure, WC = waist circumference, WHR = waist-to-hip ratio, BMI = body mass index, BSA = body surface area, %BF = percent body fat, DXA = dual energy x-ray absorptiometry, FM = fat mass, SD = standard deviation.

Table 1.4. Abdominal and total adiposity and BP *recovery from* physical and mental stressors

Reference	*M/F**	*Age*†	*Type of stressor*	*Challenge*	*Outcome, adiposity*	*Outcome, BP recovery*	*Adiposity related to BP?*‡	*Sex diff.*§
Abdominal adiposity								
Steptoe & Wardle, *Int. J. Obes.*, 2005	123/102	47 to 59 (52.3)	Mental	Stroop colour-word test, mirror-image tracing	WHR	SBP, DBP	Yes (+)	
Total adiposity								
Barbeau *et al. Obes. Res.*, 2003	26/58	15 to 18 (16±1)	Mental	Video game	% BF (DXA)	SBP, DBP	No	
Wilson *et al. Am. J. Hypertens.*, 2004	63/64	15 to 18 (16±1)	Mental	Video game	% BF, FM	SBP, DBP	No	
Harshfield *et al. Hypertens.*, 2003	151/141	15 to 18 (16±1)	Mental	Video game	BMI	SBP	Yes (+)	M>F
						DBP	No	None
Steptoe & Wardle, *Int. J. Obes.*, 2005	123/102	47 to 59 (52.3)	Mental	Stroop colour-word test, mirror-image tracing	BMI	SBP, DBP	Yes (+)	

* Expressed as n.
† Given as range (mean ± SD).
‡ Significant relationship between adiposity and BP recovery is either present (Yes) or absent (No) and the direction of the relationship is given in parentheses (positive or negative).
§ Sex difference: M>F, relationship between adiposity and BP recovery is stronger in males; None, no relationship found; if blank, not reported.
Abbreviations: M = males, F = females, BP = blood pressure, Diff. = difference, SBP = systolic blood pressure, DBP = diastolic blood pressure, WHR = waist-to-hip ratio, %BF = percent body fat, DXA = dual energy x-ray absorptiometry, FM = fat mass, BMI = body mass index, SD = standard deviation.

There are several potential mechanisms linking stress reactivity and obesity. Stress leads to sympatho-activation, resulting in stimulation of vascular adrenergic receptors and a rise in vascular resistance. Large individual differences in cardiovascular reactivity reflect differences in adrenergic mobilization. Given that the sympatho-adrenergic system is involved in lipolysis and in stress reaction, lipid levels and stress reactivity may be linked by a common mechanism: sympatho-adrenal activity. Epinephrine, released by the adrenal gland in response to sympathetic or HPA activation, is a potent lipolytic agent, which breaks down fat to release FFAs. Obesity and obesity-induced hypertension are associated with increased plasma FFAs and triacylglycerols[200, 201] and, as indicated above, acute systemic administration of FFAs and triacylglycerols have been shown to increase sympathetic activity and BP in animals and humans[202-206]. Hypercholesteremia, present in obesity, is associated with a greater response to vasoconstrictors and an impaired endothelial-dependent vasodilation, resulting in higher BP responses to stress[185].

1.5 GENTICS OF BLOOD PRESSURE AND HYPERTENSION

1.5.1 GENETIC ARCHITECTURE OF COMPLEX TRAITS: BP AND HYPERTENSION

BP is a continuous, quantitative trait where as hypertension, according to its standard definition, is a dichotomous, qualitative trait. From a genetics perspective, BP constitutes a non-Mendelian complex trait, given its normal unimodal distribution across the population[207]. However, there are rare monogenic forms of hypertension that are subject to classic Mendelian laws of inheritance, demonstrating that at least some forms of hypertension, and possibly some components of BP, are Mendelian traits[207]. Susceptibility to hypertension is determined by both genetics and environmental factors. From twin and family studies, it is estimated that the heritability (h^2) of hypertension, the percent of trait variance accounted for by genetic variance, is in the range of up to approximately 50%[208]. For SBP and DBP, estimated heritabilities

range from 15 to 40% and 15 to 30%, respectively[209-212]. Multiple contributory genes as well as gene-by-gene interactions likely contribute to the development of hypertension. The sibling recurrent risk of hypertension is in the range of 1.2 to 1.5, although these numbers are likely influenced by non-genetic factors such as living in the same household (*i.e.* having a shared environment)[213].

Another fundamental problem in hypertension genetics is how to define the ancestral phenotype[207]. In human history, hypertension is a relatively new phenomenon; it is a modern disease heavily dependent on environmental and dietary factors in today's society[207]. For example, members of an African tribe had lower BP levels in their traditional rural environment, where there urinary sodium concentration was lower and their urinary potassium concentration was higher, compared to in their urban environment[214]. In a sodium-deprived environment, one hypothesis is that the default genotype is one that conserves sodium[207]. Similarly, the RAAS may have initially evolved for sodium conservation; however, now, it may play a central role in the pathogenesis of hypertension, given the high consumption of sodium in modern society[215]. As a result, the risk allele for genes associated with hypertension is not necessarily the minor (rare) allele but could likely be the major allele as well. Whether most of the contributing allelic variation to genetically complex diseases such as hypertension consists of common variants with weak effects or rare variants with stronger effect is unknown[209]. Studies investigating the protective, BP-lowering phenotype can be equally as informative as those on hypertension. These are just some of the complications and challenges faced by researchers trying to understand the genetic basis of hypertension.

1.5.2 MAPPING BP AND HYPERTENSION GENES

A multifaceted approach to hypertension genetics is required to overcome these obstacles, combining human and animal studies and exploiting recent advances in genotyping technologies and statistics. Certain strains of rat (*eg.* the spontaneously hypertensive rat [SHR] strain), which have been selectively bred over many generations to yield a particular phenotype, offer the advantage of being

simpler but remaining under complex control. Genome-wide association studies (GWAS) in rat models have revealed quantitative trait loci (QTL) on nearly every chromosome and some important interactions between loci[216, 217]. QTL mapping is a phenotype-driven approach that does not require *a priori* knowledge of causative genes or their function. Thus, it can lead to the identification of many novel genes involved in hypertension. The identification of QTLs influencing BP is complicated by epistasis, where the effects of one gene are masked by the effects of one or several other genes (so called modifier genes), and by limited statistical power given the large number of hypotheses being tested[207]. Likewise, any single QTL explains only a fraction of the observed phenotypic variation; therefore, the phenotype-genotype correlation is often low. Fine mapping can minimize the number of genes in a QTL interval to facilitate the identification of causal variants; however, loss of the hypertensive phenotype suggests the existence of multiple genes[207].

1.5.3 CANDIDATE GENE STUDIES OF BP AND HYPERTENSION

Candidate gene studies are another useful approach to identifying genes underlying complex diseases. These are selected based on the presumed role of the gene in the pathogenesis and pathophysiology of the disease in question, as well as from knowledge derived from experimental models. For hypertension, plausible candidate genes include those involved in the production of sex hormones, the RAAS system, the regulation of vascular tone (*eg.* the endothelial NO synthase gene, *NOS3*), signal transduction (*eg.* the G-protein subunit-β3 gene, *GNB3*), sodium and water handling and promoting oxidative stress or protecting against it via the antioxidant defense network. Although associations have been reported in small and medium-sized cohorts, substantial evidence of association for any single gene or SNP with hypertension is lacking. This is partly due to small effect sizes, a lack of statistical power and inconsistent phenotyping[207]. Moreover, the selection of candidate genes precludes undiscovered pathways and rare variants and gene-by-gene or gene-by-environment interactions are not taken into account[207].

Nonetheless, recent studies have established the role of RAAS[218-220], G-proteins[221], adducin[222] and oxidative stress in hypertension[223].

1.5.4 GENOME-WIDE LINKAGE STUDIES OF BP AND HYPERTENSION

In addition to gene mapping and candidate gene studies, genome-wide linkage and association studies have seen significant improvements in recent years due to powerful statistical software and new genotyping platforms. Linkage studies examine the transmission of disease loci from parents to offspring; modification of this concept led to studies of complex traits in affected sib-pair studies[207]. Linkage mapping, in families or sib-pairs, has been used to identify chromosomal regions that co-segregate during meiosis with a given phenotype[209]. Although this relies on family-based recruitment, which can be difficult to carry out, the latter is a powerful method of performing linkage studies[207]. One of the best examples of a linkage study into hypertension is the British Genetics Study of Hypertension (BRIGHT)[213]. The consortium genotyped 2,010 sibling pairs affected with hypertension from 1599 families, completed a 10 cM genome-wide scan and identified four loci that achieved genome-wide significance, although the underlying gene variants have yet to be identified. Linkage studies have identified QTLs for BP on virtually all human chromosomes[224]. In a meta-analysis of genome-wide scans of BP or hypertension, Koivukoski found susceptibility loci on chromosomes 2 and 3[225]. Linkage mapping is indirect and has identified only relatively large genomic regions that potentially contain genes of interest; further investigations including fine-mapping and sequencing are required to identify causal variants[209]. Linkage studies are limited in that they can only identify highly penetrant loci; nonetheless, they remain an attractive tool in human genetics. This approach is thus best suited for single-gene Mendelian disorders, in which genes are highly deterministic and the correlation between genotype and phenotype is robust[209]. Indeed, linkage studies have been highly successful at identifying monogenic forms of hypertension that segregate in families with minimal environmental contribution[207].

1.5.5 GENOME-WIDE ASSOCATION STUDIES OF BP AND HYPERTENSION

In contrast to linkage studies, genetic association correlates particular alleles, or diploid genotypes, with a given trait[209]. Although association is statistically more powerful for complex traits, a very large number of markers are required to probe for association on a genome-wide basis[209]. Other advantages of GWAS compared to linkage disequilibrium approaches include the hypothesis-free nature of GWAS and its ability to detect smaller genomic regions harboring hypertension genes, which are more easily dissected by direct sequencing[209]. The International Hap Map Project first quantified genome-wide linkage disequilibrium relationships in several geographically distinct populations, to facilitate the process of minimizing the number of markers that might capture the majority of the signals from the un-typed (functional) markers during GWA[226]. A much denser set of variants than that commonly used for linkage mapping is necessary[209]. In contrast to the polymorphic microsatellite markers used in classic genome-wide linkage studies, the introduction of chip-based genotyping arrays prepared the way for GWAS that genotype for an extremely large number of genetic markers, usually 500,000 SNPs[207, 227]. These SNP mapping array sets are available in platforms developed by Affymetrix <http://www.affymetrix.com>, Illumina <http://www.illumina.com> and Perlegen <http://www.perlegen.com>. Given the extremely large number of relatively independent LD blocks within the genome, the threshold for statistical significance must be adjusted downwards, to account for multiple potential comparisons, to a typical *P* value less than 5×10^{-8} or lower[209]. Replication of findings in an independent population, which takes advantage of joint probability, is useful to provide additional support for one's results. Additionally, it is possible to impute several million SNPs through databases such as ~2.5 million SNPs in HapMap I and II <http://hapmap.ncbi.nlm.nih.gov> and ~8 million SNPs in 1000 Genomes Project <http://www.1000genomes.org> sets.

Despite these advancements in genotyping technology, initial GWAS on hypertension failed to produce significant associations with the disease. For example, in 2007, the Wellcome Trust Case Control Consortium investigated several common diseases in a UK population but did not identify any potential

causal variants of hypertension[228]. Likewise, a genome-wide scan for BP and arterial stiffness in the Framingham Heart Study was negative for hypertension[229]. The failure to identify and reproduce common variants associated with BP and/or hypertension was likely due to insufficient SNP density (later studies in these populations at higher SNP density did identify causal variants)[209]. Another probable explanation relates to the nature of the phenotype; BP is difficult to measure and unstable over time, since it exhibits a circadian rhythm and environmental effects can produce instantaneous changes, compared to a more reliable phenotype, such as BMI.

Despite these shortcomings, GWAS started to yield positive results beginning in 2009, and since then, several reproducible loci of BP and hypertension have been reported. Notably, a GWAS in an Amish founder population discovered an association between BP and *STK39*, a Ser/Thr protein kinase likely involved in the control of ion transport in the distal nephron, that almost reached genome-wide significance ($p=1.6x10^{-7}$)[230]. In another founder population from the Pacific island of Kosrae, 3 additional loci of BP were identified: *CACNB2*, *UBE3C* and *CUBN*[231]. In the first GWAS of BP to reach genome-wide significance, Org *et al.* identified *CDH13* in a German population, which was then confirmed in two other European populations; the locus reached genome-wide significance in a combined analysis ($p=5x10^{-8}$)[232]. *CHD13* encodes for an adhesion glycoprotein T-cadherin, a regulator of vascular remodelling and angiogenesis. In a Korean population, an association between *ATP2B1* and BP reached close to genome-wide significance for SBP ($p=1.3x10^{-7}$) and DBP ($p=3x10^{-3}$)[233]. The product of this gene belongs to a family of P-type primary ion transport ATPases; these enzymes play a critical role in intracellular calcium homeostasis and may function as a modifier locus of phasic contraction.

Following these initial discoveries, two important GWAS studies published their findings back-to-back in *Nature Genetics*: the CHARGE consortium and the Global BPgen consortium[234, 235]. These two well-powered studies documented many novel SNP loci in more than 60,000 people of European ancestry. In the first study, Levy *et al.* investigated a population of European descent (n=29,136), and identified 13 SNPs for SBP, 20 for DBP and 10 for hypertension (at $p<4x10^{-7}$). In a meta-analysis with

the Global BPgen Consortium (n=34,433), several loci attained genome-wide significance: 4 for SBP (***CYP17A1***, *ATP2B1, PLEKHA7, SH2B3*), 6 for DBP (*ATP2B1, CACNB2, CSK-ULK3, TBX3-TBX5, ULK4*) and 1 for hypertension (*ATP2B1*)[234]. One gene was associated with all three phenotypes, *ATP2B1*, which encodes a plasma membrane ATPase expressed in the vascular endothelium and is involved in pumping calcium out of the cell. The Global BPgen Consortium also identified a large number of loci, 11 for SBP and 15 for DBP, in a European population[235]. These were validated in two populations with direct genotyping and by *in silico* comparisons with the CHARGE Consortium. Overall, 8 loci attained genome-wide significance for BP and hypertension: 3 for SBP (***CYP17A1***, *MTHFR, PLCD3*) and 5 for DBP (*FGF5, C10orf107, SH2B3, CYP1A2, ZNF652*)[235].

More recently, in the first GWAS in an African-American population, Adayemo *et al.* identified 5 SNPs for SBP, including 2 candidate genes for BP regulation: *SLC24A4*, coding for a sodium/potassium/calcium exchanger, and *CACANA1H*, a target gene for calcium-channel blockers that codes for a voltage-dependent calcium channel, which may influence BP through its regulation of cardiac contraction[236]. Additional GWAS identified 5 non-synonymous SNPs (*i*.e. SNPs that alter the DNA sequence in a coding region such that the amino acid is changed) associated with BP and hypertension in a South Korean population[237]; in the same cohort (KARE), four loci were replicated from the CHARGE and Global BPgen Consortia, including ***CYP17A1***[238]. The Women's Health Genome Study replicated well-established loci of high BP, also including ***CYP17A1***, using self-reported BP data in women, and identified a new locus at *BLK-GATA4* ($p=3.2x10^{-8}$) in a meta-analysis. An initial GWAS of obesity-induced hypertension in the same French Canadian founder population as the one studied here (the Saguenay Youth Study) revealed three loci associated with obesity as well as high BP: *PAX5, MRPS22* and *FTO*[239]. After adjusting for adiposity, the differences in BP became non-significant for the first two genes but remained unchanged for FTO, suggesting that the gene has an effect of BP independent of its effect on adiposity.

Despite the impressive sample sizes and highly significant *P* values in the above studies, there are several limitations to GWAS. First, the effect sizes of the identified the identified genes were rather modest. For example, the SNP with the strongest association with SBP in Global BPgen [rs11191548, $p=7x10^{-24}$] had an effect size of 1.16 mm Hg per risk allele[235]. Although this may have major importance for hypertension at the population level, its implications at the level of the individual are minor[207]. Second, the cumulative effects of the novel loci explained just 1% of population BP variance. This accounts for only a small fraction of heritable trait variance (given that h^2 is estimated at around 50%)[208], resulting in a large amount of unexplained variation. This missing heritability may be attributed to rare variants, structural variants, low power to detect gene-by-gene interactions (epistatic effects), inadequate accounting for a shared environment, undetected copy number variation effects and so on[240]. Typically, predictive models from GWAS are constructed using a small number of SNPs with low *P* values, under the assumption that a few loci underlie the trait of interest[241]. Not surprisingly, this performs poorly for complex traits such as BP that have many contributory genes. New approaches, such as those employed by Yang[242], Makowski[241] and others, are required to explain this missing trait heritability. Third, the confounding effect of BP-lowering medication has been an important problem for GWAS of hypertension (*eg.* some studies added an arbitrary amount to BP values, such as 10 mm Hg, in individuals taking antihypertensive drugs).

Importantly, environmental factors promoting hypertension have been poorly characterized in GWAS subjects up until this point[209]. The impact of an individual's past and present environmental exposure has been largely overlooked. Studies in humans and rodents indicate that organisms at genetic risk of developing hypertension display exaggerated cardiovascular responses to environmental stressors[243, 244]. Thus, the interaction of genes and the environment likely plays a critical role in the development of hypertension. Despite the large number of GWAS on BP in recent years, GWAS of hypertension have not incorporated environmental triggers such as stress-induced BP responses[209]. The focus has been on clinic (resting) BP measurements, and as a result, BP reactivity to and recovery from stressors and ambulatory BP have remained largely overlooked. However, one study by Tomaszewski *et*

al. identified an association of a novel variant at the *MTHFR/CLNC6* locus with ambulatory DBP, using a custom-based gene-centric array, which permits genotyping of ~50,000 common and low-frequency SNPs in more than 2000 candidate genes[245].

In recent studies on twin pairs, it was shown that BP response to environmental stress is influenced by genetic variation at several points within the adrenergic pathway[86, 246-250]. Twin studies have estimated that the heritability of the systolic and diastolic BP response to a math stress test is 44% and 49%, respectively. These estimates were based on observed correlations of 0.40 for systolic and 0.51 for diastolic BP responses in monozygotic twins and 0.18 and 0.29 in dizygotic twins[210]. Substantial overlap exists between the genes that influence BP measured in a clinic, under laboratory stress and during real life. One study estimated that up to 81% of the heritability of clinic SBP and 71% of clinic DBP were attributed to genes that also influenced stress BP; however some genetic components specific to each BP measurement also exist[251]. In conclusion, GWAS studies have clearly demonstrated BP and hypertension susceptibility genes; however, future studies on stress-induced BP responses are needed. It is hoped these findings will improve our understanding of the underlying pathological mechanisms of hypertension.

1.5.6 SEX-SPECIFIC GENETIC DETERMINANTS OF BP AND HYPERTENSION

Despite what is known about the sexual dimorphism of cardiovascular traits and related diseases such as hypertension, sex-specific genetic determinants of high BP have been largely overlooked. Genes on the sex chromosomes are likely responsible for considerable differences in BP and stress responses in males and females, given that males have higher expression of Y-linked genes and females have elevated levels of X-linked gene expression in the myocardium[252]. Only 5% of the Y chromosome recombines by pairing with the X chromosome; thus, 95% of its expression is male-specific[123]. The Y chromosome can influence BP regulation by increasing SNS activation, reflected by higher plasma NE[253]. Another possibility is that the Y chromosome influences BP through renal function by modifying the excretion of

sodium and potassium[254]. The control of BP homeostasis may also be mediated by interactions between the Y chromosome and androgens, their receptors and the RAAS[255].

On the other hand, the X chromosome is shared between males and females and plays an important role in hypertension and other cardiovascular diseases, such as congenital heart disease, Turner syndrome, dilated cardiomyopathies and renal diseases[256]. Importantly, the gene encoding ACE2, *ACE2*, has been characterized on the X chromosome and is a candidate gene for hypertension. ACE2 competes with ACE for the same substrate (Ang I), leading to the production of the vasodilator Ang (1-7) and converting the vasoconstrictor Ang II to inactive Ang (1-9). A strong association between *ACE2* polymorphism and early onset of hypertension has been found in French Canadians[257]. Moreover, an association between a polymorphism in the Ang II type 2 receptor (*AT2-R*) gene, located on the X chromosome, and hypertension has been found in women but not men and this association was stronger in premenopausal women, suggesting a relationship between sex, RAAS and hypertension[258].

Sex differences in BP control also exist with respect to genes located on autosomal chromosomes. Mapping studies of quantitative trait loci have revealed that specific loci involved in the development of hypertension are located on different chromosomes in men and women and affect different BP regulatory systems[259, 260]. The genomic map of cardiovascular phenotypes of hypertension is in fact distinct in male and female populations. A sex-specific genetic architecture of quantitative traits for BP has been proposed, particularly in regions of linkage on chromosomes 2, 4 and 18[261]. Thus, the sex-specific architecture of traits contributing to high BP is a result of the interaction between sexual and autosomal chromosomes. Whether distinct loci contribute to differences in the same phenotype in males and females deserves further investigation. Such studies should better our understanding of the progression of hypertension and contribute to improved prevention through sex-specific therapies.

1.5.7 *CYP17A1* – A LOCUS OF HYPERTENSION

One of the most well-established loci of hypertension that is also involved in the production of sex steroids is *CYP17A1*, identified in the CHARGE and Global BPgen Constortia[234, 235]. Originally identified in subjects of European ancestry, the gene has been replicated in various Asian populations and in the Women's Genome Health Study[238, 262-264]. *CYP17A1*, located on chromosome 10q24.3[265, 266], is a 6.6-kilobase-pair gene consisting of 8 exons[267]. *CYP17A1* is involved in several pathways integral to BP regulation. The gene encodes the enzyme cytochrome P-450c17, which has both 17α-hydroxylase and 17,20-lyase activities and plays a key role in steroidogenesis in the adrenal gland and gonads[267, 268]. During steroidogenesis, cholesterol is converted to pregnenolone, which is subsequently processed to either mineralocorticoids in the adrenal zona glomerulosa (neither enzyme activity present), to glucocorticoids in the zona fasciculata (17α-hydroxylase activity), or to sex steroids in the zona reticularis and gonads (both enzyme activities present)[267, 269]. Human CYP17 17α-hydroxylates pregnenolone and progesterone in approximately equal efficiency, while the 17,20-lyase activity is around fifty times more efficient for converting 17α-hydroxypregnenolone to dehydroepiandrosterone (DHEA) than for the conversion of 17α-hydroxyprogesterone to androstenedione, which is consistent with the large amounts of DHEA secreted by the adult and fetal human adrenal gland[270, 271]. DHEA, a precursor to testosterone and estrogens, possesses some androgenic activity. For a simplified pathway of steroid hormone biosynthesis and the role of CYP17, see Chapter 4, Figure 4.1 (page 122).

Mineralocorticoids (*eg.* aldosterone) increase sodium and water reabsorption in the kidneys leading to increased blood volume and elevated BP, while glucocorticoids (*eg.* cortisol) controls the body's response to stress, including a rise in BP[8]. Sex steroids are also involved in BP regulation[272]; in general, androgens increase vasoconstriction[155] while estrogens enhance vasodilation[273]. In females, there is some evidence that *CYP17A1* is associated with estrogen levels but results in men are inconclusive with respect to androgen levels[274]; however, interpretation of studies of hormone levels are complicated by methodological issues (*eg.* the variation in hormone levels with age, race, weight, behaviours, menstrual

cycle) and interactions with other genes involved in steroid biosynthesis. Thus, *CYP17A1* may influence BP reactivity by sex-specific pathways.

Missense mutations in *CYP17A1* cause a form of adrenal hyperplasia characterized by hypertension, hypokalemia, reduced plasma renin activity, mineralocorticoid excess, salt retention and sexual infantilism[275-277]. Given that males compared to females exhibit higher resting BP, greater BP reactivity and slower BP recovery from stress, higher hypertension risk and a stronger link between obesity and hypertension, it is important to consider gene-sex interactions in the molecular approach to hypertension genetics and *CYP17A1* is a likely candidate.

1.6 AIMS

The aim of this thesis is to further explore the relationships between BP responses to physical and mental challenges and the contribution of, first, visceral adiposity and, second, *CYP17A1*, a candidate gene for hypertension, in modulating these BP responses in males and females. We hypothesized that both these factors would affect BP regulation in response to stressors, and that differences in the underlying mechanisms between the sexes are probable. Given that visceral obesity is a leading risk factor for hypertension in obese children[94], excess visceral adiposity is likely to enhance BP reactivity and slow down BP recovery. *CYP17A1* is a well-established loci of hypertension[234, 235] and an excellent physiological candidate, given its role in the steroidogenesis pathway leading to the production of glucocorticoids, mineralocorticoids and sex steroids. It therefore likely contributes to BP reactivity to physical and mental challenges and may influence BP regulation in a sex-specific manner.

For the purpose of this study, BP reactivity is defined as the magnitude of elevation of an individual's BP in response to a stressor[278] and BP recovery is the decrease in BP after a stressor is removed. These absolute changes are always corrected for respective initial BP values in subsequent analyses in order to account for the baseline BP value, which can impact the magnitude of change. The

BP responses to both a physical challenge, namely a posture test, and a mental challenge, namely a math-stress test, are assessed. Each challenge is non-invasive and produces a marked BP response, although different BP regulatory mechanisms are involved in each. Active standing involves mainly sympathetic activation while mental arithmetic causes a coordinated response between the SNS and the HPA axis. These responses are described in more detail in Sections 2.2.1.1 and 2.2.1.2.

Two studies are presented in detail. In Chapter 3, the role of visceral fat in modulating the BP responses to physical and mental challenges in adolescents is investigated. For this study, we focused solely on SBP because systolic rather then diastolic hypertension is predominant among obese children[122] and young adults[279], the population variance in SBP vastly exceeds that in DBP[91], and finally, during adolescence, SBP increases markedly in males but not females, while DBP remains similar in both sexes[131]. In Chapter 4, I report the association of *CYP17A1* to BP reactivity to a mental challenge in males but not females. This investigation was carried out in adolescents recruited from a French Canadian founder population in Quebec, in which fewer gene variants contribute to the determination of complex traits, such as BP[280-282]. The following chapter describes the general methods used throughout these studies.

2. GENERAL METHODS

This thesis includes chapters detailing two studies, both of which were conducted in a population-based sample of adolescent males and females, as part of the Saguenay Youth Study (SYS). This section of General Methods includes descriptions of the subjects (detailing the SYS, the region of Saguenay Lac Saint-Jean and study recruitment), quantitative phenotyping (including the cardiovascular protocol, anthropometry and estimation of body-fat quantity and distribution), genotyping and statistical analyses employed in the studies.

2.1 SUBJECTS

2.1.1 SAGUENAY YOUTH STUDY

The studies described in the upcoming chapters, Chapters 3 and 4, were part of the SYS. Adolescents aged 12 to 18 years were recruited as part of this on-going, large-scale community-based study of cardiovascular and metabolic health, and brain and behaviour in adolescence. Its original focus was on exploring gene-environment interactions investigating long-term consequences of prenatal exposure to maternal cigarette smoking (PEMCS). To facilitate the identification of genes that modify an individual's response to an *in utero* environment (*i.e.* PEMCS), the study is family-based (adolescent sibships) and is carried out in a population with a known founder effect in the Saguenay Lac Saint-Jean region of Quebec, Canada. The SYS begins with a pre-screening telephone interview, continues with a *home visit* for detailed questionnaires, a *laboratory visit* for psychological testing, a *hospital visit* for cardiovascular and metabolic phenotyping and concludes with a *school visit*[283]. The *hospital visit* is the main focus of my research and is described in more detail below; other measurements are described as needed.

2.1.2 FRENCH-CANADIAN FOUNDER POPULATION

Currently, approximately 275,000 people live in the Saguenay Lac Saint-Jean region, a moderately geographically isolated area extending eastwards from Lac Saint-Jean to the mouth of the Saguenay River, where it reaches the St. Lawrence River at Tadoussac (Figure 1.8). The Saguenay River spans approximately 165 km. The Saguenay population has a known founder effect[284], a loss of genetic variation that occurs when a new population is established by a small number of individuals from a larger population. This occurred first with the French settlers of New France in the 17th and 18th century, and again when the region was founded in the early 19th century. As a result, up to 70% of the genetic pool comes from about 600 French ancestors who originally migrated to the region[285]. The population grew from 5200 inhabitants in 1852 to 275,000 at present, due to high intrinsic growth and low immigration into the region. As such, it is one of the largest population isolates in North America[280-282].

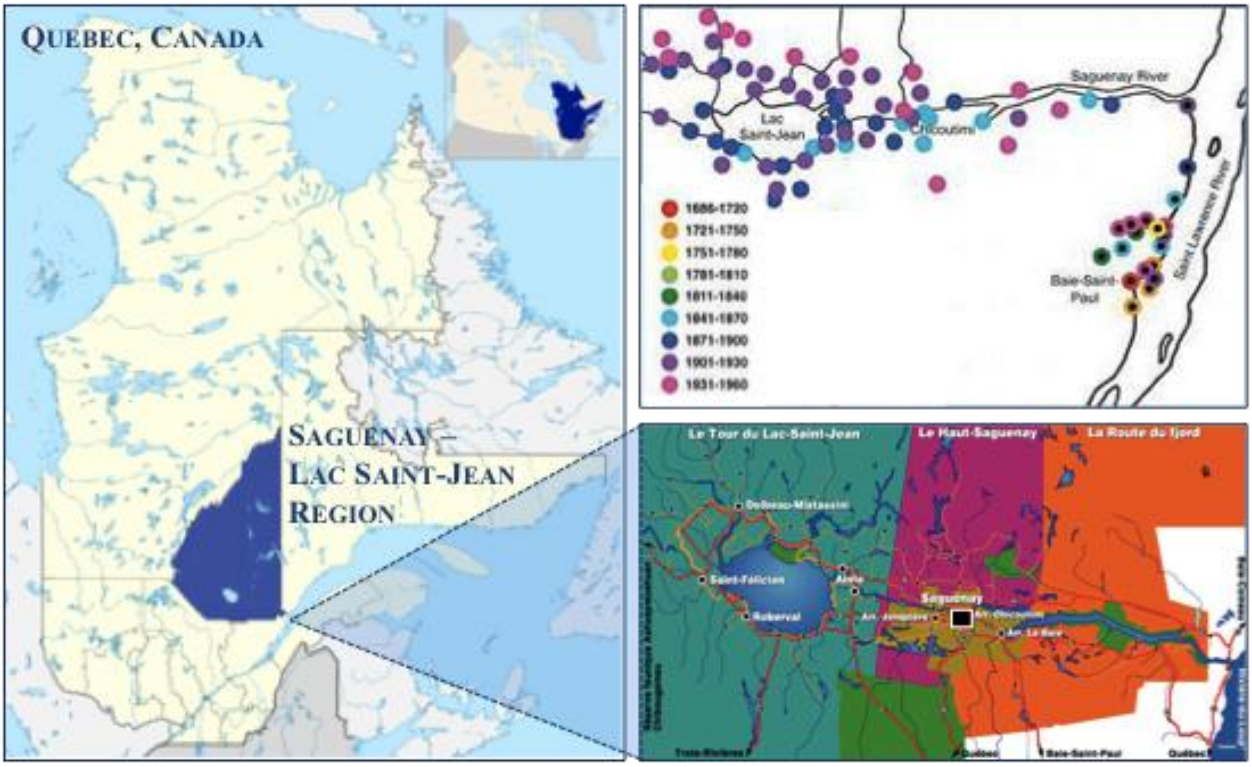

Figure 1.8. Map of the Saguenay Lac Saint-Jean region
The Saguenay-Lac Saint Jean region is located in the middle of Quebec, Canada (left). It spans from Lac Saint-Jean eastward to the mouth of the Saguenay River, where it meets the St. Lawrence River at Tadoussac (bottom right, black square indicates Chicoutimi hospital). French settlers founded the Saguenay region in the early 20th century (top right, showing the range expansion dynamics and wave front at different periods; localities from the Charlevoix region are indicated by a black dot). Image reproduced from Moreau, C. *et al*. Deep human genealogies reveal a selective advantage to be on an expanding wave front. Science 2011; 334:1148-50. Permission granted (http://www.sciencemag.org/site/about/permissions.xhtml).

Given this founder effect, several rare alleles are present at an elevated frequency in the population. There is a higher prevalence of certain recessive disorders in the Saguenay Lac Saint-Jean region compared to other populations, which are thought to have originated from some of its founders[280, 286]. As well, patients with these disorders exhibit limited allelic diversity[281, 282]. These include spastic ataxia, agenesis of the corpus callosum and peripheral neuropathy, oculopharyngeal muscular dystrophy, myotonic dystrophy and cystic fibrosis[280]. Four other diseases with increased incidence in this population, tyrosinemia, familial hypercholesterolemia, familial hyperchylomicronemia and hereditary hemochromatosis, might influence the phenotypes studied here[280]. Tyrosinemia is an autosomal recessive disorder that can cause cirrhosis, hepatocellular carcinoma and other severe consequences; it has a carrier

frequency of 1 out of 22 and affects 1 in 1846 live births. Familial hypercholesterolemia is an autosomal dominant disease of premature atherosclerosis, affecting 1 out of 122 in Saguenay Lac Saint-Jean compared with 1 out of 500 in western populations. Familial hyperchylomicronemia is an autosomal recessive disease resulting in lipoprotein lipase deficiency, with a frequency of 1 in 6000 in the Saguenay region. Hereditary hemocromatosis leads to iron accumulation in tissues, resulting in cirrhosis, diabetes, cardiomyopathy, arthropathy and hypogonadism; its prevalence in the population is 1.4%[287].

The decreased genetic and environmental heterogeneity in this population offers distinct advantages to studying genetic contributions to disease[288]. Given that fewer genes and gene variants contribute to the phenotypic expression of complex genetic traits, such as BP, the potential to identify causal variants in a founder population is higher compared to in the general population. Another major benefit of studying this population is that environmental influences are more homogeneous. The Saguenay Lac Saint-Jean region is relatively geographically isolated and its inhabitants share a similar culture, dietary habits, and certain behaviours, such as physical activity. This founder effect, resulting in reduced genetic variation, combined with more uniform environmental exposures, increases the likelihood of identifying genes of complex genetic traits[281, 282, 289]. Another advantage of founder populations for mapping of complex traits in GWAS is that they have more extensive linkage disequilibrium resulting in more comprehensive coverage of the genome by genotyping platforms[290]. There is also a very low chance of population stratification in population isolates. Population stratification describes the presence of systematic differences in allele frequencies between subpopulations in a given population, possibly due differences in ancestry. Non-random mating (often due to physical separation between groups) and genetic drift (a change in the frequency of an allele in a population due to random sampling) are the root causes of population stratification. On the other hand, caution should be taken when extrapolating findings from a founder population to other populations. Considering the "distinct" genetic makeup of the Saguenay Lac Saint-Jean population, results may not be relevant to other outbred populations[289]. However, given that Melka *et al.* were able to replicate previously identified loci of obesity in a GWAS of obesity and BP in the SYS[239], it seems unlikely that this is a major issue in our sample.

2.1.3 RECRUITMENT

Subjects were recruited from all 24 high schools in the Saguenay Lac Saint-Jean region. Recruitment criteria for subjects exposed to maternal cigarette smoking *in utero* included: (1) age 12 to 18 years; (2) one or more siblings aged 12 to 18 years; (3) maternal and paternal grand-parents of French-Canadian ancestry; and (4) positive history of maternal cigarette smoking during pregnancy (defined as at least one cigarette per day during the second trimester of pregnancy)[283]. Exclusion criteria were: (1) positive history of alcohol abuse during pregnancy (more than 210 ml of alcohol per week, *i.e.* more than 14 bottles of beer, 9 glasses of wine or 7 glasses of whiskey per week); (2) positive medical history of any of the following: type 1 diabetes, systemic rheumatologic disorders, malignant tumors requiring chemotherapy, congenital heart defects, aneurysm, epilepsy, bacterial infection of CNS, brain tumor, head trauma with loss of consciousness for greater than 30 min, muscular dystrophy, myotonic dystrophy, nutritional and metabolic diseases (*eg.* failure to thrive, phenylketonuria), hearing deficit, non-correctable vision problems, schizophrenia, bipolar disorder, IQ less than 70, special education; (3) severe mental illness or mental retardation; (4) contraindications for MRI (*eg.* metal implants, electronic implants, pregnancy or claustrophia); (5) pregnancy duration under 35 weeks; (6) diabetes of the mother during pregnancy (onset before pregnancy, treated by insulin); (7) premature birth, detached placenta, multiple births (*eg.* twins), or hyperbilirubinemia requiring transfusion[283]. Non-exposed subjects (controls) were matched to exposed subjects by the school they attended and maternal education, which are considered good indicators of socioeconomic status. Inclusion and exclusion criteria were identical for controls, except that they must have a negative history of maternal cigarette smoking during pregnancy and during the year preceding pregnancy[283].

Recruitment was conducted by members of the Groupe ECOBES (Groupe d'étude des conditions de vie et des besoins de la population), who presented the study in high school classrooms and sent a letter to parents containing an information pamphlet about the SYS and a consent form for a telephone interview. A nurse contacted interested families in order to verify their eligibility and consent and to

conduct an initial phone interview on demographics, pregnancy history and medical history of the parents and children. A home visit was then conducted with eligible families, in which written consent from the parents and assent from the adolescents were attained before the commencement of data collection. Next, a Saturday morning hospital visit was scheduled during which cardiovascular, body composition and magnetic resonance imaging measurements were performed[283]. The research ethics committee of the Chicoutimi Hospital approved the study protocol.

Based on an initial sample of 408 adolescents, the overall response rate (*i.e.* the number of response cards being returned) was 19% and varied between 8 and 36% depending on the school. Of these, 64% of families agreed to being contacted by the research nurse. Based on the initial telephone interview, 37% of families were excluded for a variety of reasons (cigarette smoking the year before pregnancy [n=104], one of two siblings not interested [n=73], child not of eligible age [n=75], MRI contraindications [braces; n=59], not of French Canadian origin [n=46], medical reasons [n=42], one parent not available [n=30], twins [n=28], premature birth [n=14] and placental detachment/rupture [n=5])[283]. Out of 423 eligible families, there were 229 (26%) exposed and 657 (73%) nonexposed adolescents eligible for the study. From the pool of all eligible families, 199 exposed and 209 nonexposed adolescents were matched, based on maternal education and school attended, coming from a total of 198 families[283].

The current sample consists of subjects recruited and tested between November 2003 and June 2009 (n=596), among whom 490 had complete quality-controlled cardiovascular data sets and were analyzed in the present studies. The prevalence of overweight or obesity (85th age- and sex-specific percentile) in this sample was 29% in boys and 21% in girls, similar to that in the general population of Canadian adolescents[168]. Likewise, the prevalence of hypertension (sitting SBP or DBP 95th age, sex- and height-specific percentile[83]) was 7.1% in boys and 3.3% in girls, also similar to that in the Canadian adolescent population at large[84].

2.2 QUANTITATIVE PHENOTYPING

All subjects underwent a cardiovascular and body composition protocol conducted in the Chicoutimi hospital on Saturdays, commencing between 8:00 and 12:00[283]. The protocol was conducted in an experimental laboratory by a team of trained nurses. All subjects were asked to fast overnight and refrain from vigorous physical activity and drinking caffeine or alcohol for 24 hours before the testing[283]. BP responses to a physical and a mental challenge were derived from continuous BP recordings made throughout the cardiovascular protocol. Weight and height, total body fat and visceral fat were determined by anthropometry, bioimpedance and magnetic resonance imaging, respectively, as part of the body composition protocol.

2.2.1 CARDIOVASCULAR PROTOCOL

The cardiovascular protocol consists of a standardized physical and mental challenge intended to invoke marked BP responses. This protocol lasts 52 minutes and requires subjects to perform simple tasks that occur in every-day life, *i.e.* physical and mental challenges. The physical challenge consisted of a 30-minute ***posture test***, *i.e.* changes in posture (supine, standing, sitting) each lasting ten minutes. During the posture test, each participant, beginning in the supine position, was first supine (10 minutes), then standing (10 minutes), and finally sitting (10 minutes). Throughout the rest of the protocol, subjects remained in the sitting position. The physical challenge was followed by a mental challenge, a ***math-stress test***, which included both an explanation, when subjects were told they would be required to perform a math test, and the test itself. The math-stress test consisted of an initial pre-stress period (4 minutes), an explanation (1 minute), a post-explanation waiting period (4 minutes), a math-stress test (2 minutes), and math-stress test recovery period (10 minutes). The math-stress was a sequence of 23 slides, each presenting two simple arithmetic problems to be solved out loud[291]. The first three slides are presented for 10 seconds and the remaining slides are presented for 5 seconds and separated by a 0.5

second slide change. The level of difficulty increases as the test progresses to ensure some failure in all subjects[291]. This math test is 130 seconds in duration. After the math-stress test, BP parameters continue to be monitored for 15 minutes to capture the recovery from stress. Figure 1.9. illustrates SBP and DBP recordings throughout this protocol, as recorded from one of the subjects.

BP was measured continuously throughout the protocol using a Finometer™ (FMS Finapres, Amsterdam, The Netherlands; described in more detail below), which was set up beside a hospital bed. The Finometer™ is BP cuff that is wrapped around the subject's middle phalanx of the fourth finger on the non-dominant hand. The subject is told to refrain from talking throughout the protocol, except when giving responses during the math test. The protocol begins with a calibration procedure to adjust finger BP to brachial BP levels after 10 minutes of the subject resting in a supine position. Following this initial calibration period, the subjects undergo the posture and math-stress tests as described below.

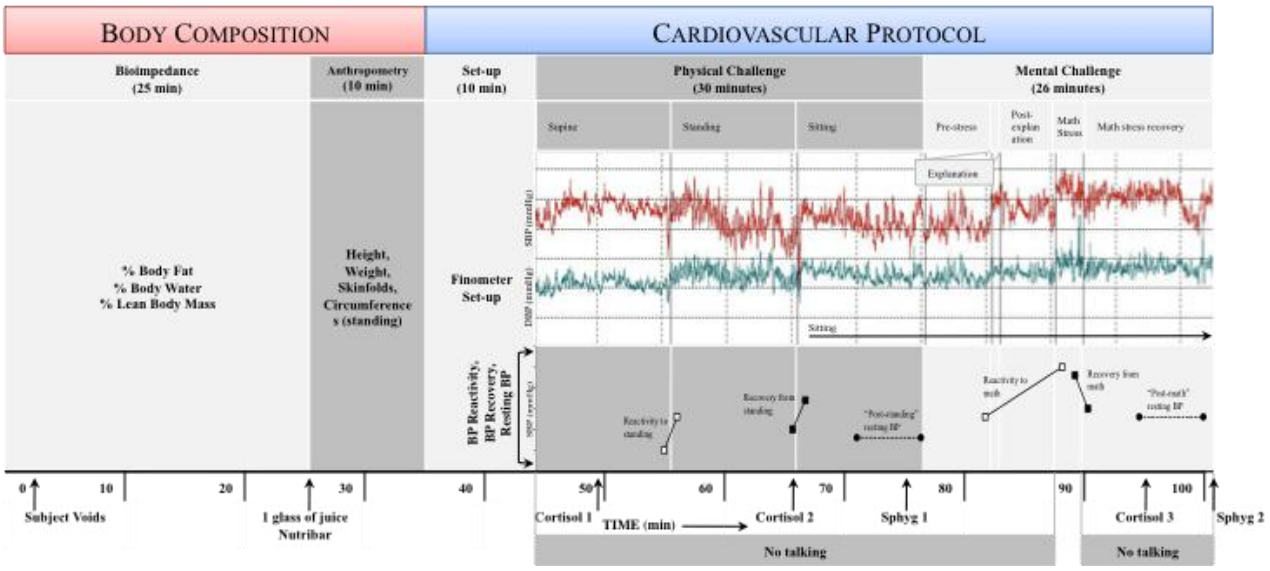

Figure 1.9. The cardiovascular and body composition protocol
Subjects undergo a protocol lasting approximately 100 minutes, which includes both a body composition (bioimpedance and anthropometry) and a cardiovascular protocol (a physical and mental challenge). The physical challenge was a 30-minute posture test and the mental challenge was a 26-minute math-stress test. BP responses to each challenge (BP reactivity and recovery) are derived from beat-to-beat BP recordings using a Finometer™, as well two resting BPs after each challenge after 5 minutes of rest.

2.2.1.1 Blood pressure responses during a posture test

The SNS is mainly involved in controlling BP changes during a change in posture from supine to standing to sitting. While supine, the blood vessels in the body are at relatively the same elevation as the heart, facilitating the movement of blood throughout the circulation. In this position, the low gravitational effects translate into low total peripheral resistance (TPR) compared to other postural positions[292]. However, upon standing, the head and heart become much more elevated than the rest of the body, and blood must be pumped against gravity from the toes up to the heart and head. The cardiovascular system must quickly adapt to these changes to maintain perfusion to the brain and the rest of the body's tissues. Although changes in posture are not usually thought of as "challenging", they do indeed elicit a marked cardiovascular response, which reflects the overall functioning of the cardiovascular system.

Active standing is an orthostatic challenge which causes an early cardiovascular response within the first minute[20]. Gravitational effects of the upright position produce a significant pooling of blood below the level of the heart, causing a drop in BP[58]. Contraction of leg and abdominal muscles compresses blood vessels, causing transient increases in venous return and cardiac; however, this does not fully compensate for the overall decline in total peripheral resistance. Muscle contraction activates the exercise reflex, producing rapid withdrawal of parasympathetic stimulation to the heart and mass sympathetic discharge causing HR to drop within 3 seconds of standing and BP to increase after a delay of a few seconds[20]. HR and BP begin to normalize after 30 seconds of standing. Following this initial response is an early phase of stabilization during 1 to 2 min of standing. Upon standing, the reduction in TPR allows blood to pool in the lower body, decreasing venous return and thus BP. As a result, the drop in BP inhibits the baroreflex system and induces ongoing inhibition of parasympathetic activity and augmentation of sympathetic outflow causing a secondary rise in BP and heart contractility[20, 58]. The posture test is best suited to asses this initial phase of orthostatic challenge[20].

During prolonged standing of more than 5 minutes, humoral mechanisms contribute to maintaining BP and the combined activation of the SNS and RAAS leads to an increase in plasma

catecholamine levels[20]. Circulating E and NE are released from the adrenal medulla, in addition, some NE is released as spillover from nerve endings in the SNS[25]. Activation of RAAS increases further sympathetic activity, vasoconstriction and, over the course of hours, sodium and water retention in the kidneys and higher levels of aldosterone and vasopressin, resulting in chronic BP elevation. However, these long-term BP responses are not the focus of this thesis.

Transitioning from standing to sitting also activates the exercise reflex, given the contraction of leg and postural muscles. This causes a transient spike in BP. Once seated, BP gradually decreases due to the diminished effects of gravity and the lower TPR. Subjects do not need to use their postural muscles as much as when standing and may feel more relaxed. BP in the seated position is very similar to that in the supine position; in fact, BP measurements in these two positions are often used interchangeably in office settings in accordance with current guidelines[2]. However, small but measureable differences in SBP and DBP have been found, with both being slightly higher while seated[292, 293]. Differences in supine and upright BPs vary according to the ability of the cardiovascular system to respond normally to postural changes. Thus, measurement of BP in different positions yields important information on the regulation and control of BP[58]. Moreover, ddelayed BP recovery from stress is believed to be a potential marker of chronic sympathetic activation and low parasympathetic activity.

2.2.1.2 Blood pressure responses during a math-stress test

Like the posture test, math-stress also challenges the cardiovascular system. Acute mental stress elicits a coordinated response between the SNS and the HPA axis, the body's stress-response system, mediated by the CNS. Detection of stressful stimuli involves the thalamus, sensory and prefrontal cortex, which signal to the hypothalamus and brain stem that a stressor is present. This initiates the stress response system, which exerts effects through the sympathetic branch of the nervous system and the HPA axis to modulate the cardiovascular response[25]. Stress causes parasympathetic withdrawal and

sympathetic activation to the heart and vasculature, resulting in elevations in HR, BP and CO. Sympathetic stimulation of the adrenal medulla increases the production of E and NE. These cause vasodilation in skeletal muscle and vasoconstriction in the splanchnic region, redirecting blood flow to the systems needed to respond to stressful stimuli[294]. Simultaneously, activation of the HPA axis causes the release of vasopressin, a potent vasoconstrictor, and CRH from the hypothalamus[25]. These promote the secretion of ACTH from the anterior pituitary, which is carried by the circulation to the adrenal gland, stimulating the secretion of glucocorticoids by the adrenal cortex[25]. The increased secretion of cortisol, which makes up 95% of glucocorticoids secreted, occurs within 3 minutes of the onset of stress[8, 27]. Cortisol acts on its target tissues within milliseconds to minutes[27]. Cortisol increases the sensitivity of vascular smooth muscle cells to NE, increasing their vasoconstrictive response, and suppresses the production of vasodilators such as NO[28]. Together, the sympathetically-mediated rise in catecholamine production and the local effects of cortisol in the vasculature cause an elevation in BP[25].

The autonomic response to stress occurs within seconds (causing vasoconstriction and stimulating NE and E secretion) while the neuroendocrine response via the HPA axis occurs on a slower timescale (within minutes), resulting in cortisol secretion[295]. Within minutes of its initiation, cortisol inhibits the HPA axis in a negative feedback loop, suppressing the release of CRH and ACTH[295]. The acute stress response therefore starts at the onset of stress and lasts for several minutes. Typically, mental arithmetic causes BP, HR and CO to increase as well as renal vasoconstriction and forearm vasodilation[296, 297].

2.2.1.3 Continuous blood pressure monitoring

Throughout the protocol, a non-invasive hemodynamic monitor, Finometer™ (Finapres, Amsterdam, the Netherlands), was used to record continuous finger blood flow. This method, first developed by Penaz, is based on the principle of the unloaded arterial wall[2]. It uses a volume-clamp technique to measure continuously finger blood flow, using an inflatable finger cuff with built-in

photoelectric plethysmograph. The cuff works to keep the diameter of the artery under the cuff constant, such that it is held in a partially open state[2]. A light emitting diode in the cuff on one side of the finger emits a light that is detected by a photodiode located on the opposite side of the finger. A decrease in the amount of light received signals an increase in blood flow and arterial diameter. A fast pressure servo-controller increases pressure in an inflatable air bladder that lines the finger cuff in order to keep the diameter of the finger artery clamped at a constant level. The pressure in the finger cuff equals the intra-arterial pressure provided that the volume-clamp method is active at the proper unloaded diameter of the artery (*i.e.* at zero transmural pressure). The sampling rate is 250 Hz. In order to correct for changes in height between the finger and the level of the heart, a signal from a pressure transducer at heart level, which is connected to the finger cuff by a liquid filled tube, is added to the finger pressure signal. Finger blood flow measurements and published pressure-waveform reconstruction and calibration methods[298, 299] are used to derive brachial artery BP. The Finometer™ uses the brachial pressure waveforms and the Modelflow CO method[300] to derive beat-to-beat values for the following parameters: systolic and diastolic BP (SBP and DBP), inter-beat interval (IBI), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and several other parameters. The bias (less than 5 mm Hg) and the precision (better than 8 mm Hg) of the brachial BP reconstructed from the finger pressure, compared to brachial artery pressure, are within the limits of the Association for the Advancement of Medical Instrumentation (AAMI)[301], according to its manufacturer. More recently, independent validation studies have shown that with supine calibration and reconstructed brachial pressures, the bias and precision are improved, ranging from -1 to -2 mm Hg, and 4.8 to 7.7 mm Hg, respectively[302].

Brachial artery cannulation, connected to a transducer, can be used as an alternative to the Finometer™ (or similar finger-cuff techniques) for continuous, beat-to-beat hemodynamic measurements. This is considered the gold standard in beat-to-beat BP monitoring; however, the method is invasive and unsuitable in healthy adolescents. The Finometer™ has been validated against intra-arterial pressures in several studies[303, 304] and against mercury sphygmomanometer measurements[305]. The mean differences between the mercury sphygmomanometer, the gold standard for clinic BP measurements, and the

Finometer™ readings for SBP and DBP were -1.83 ± 6.8 (SD) and 0.88 ± 7.5 mm Hg, satisfying the validation criteria of AAMI[305]. The Finometer™ is a reliable device for tracking BP in adults and in children over the age of six years[306]. Therefore, given its reliability and non-invasive nature, the Finometer™ can me recommended for both clinic and research purposes. However, regarding its clinical applicability, its relative cost and inconvenience (*eg.* the 10 minute calibration period) compared to other techniques should be taken into account[2]. The Finometer™ is a very effective method of measuring short-term BP changes (such as those described below) and BP variability, making it an extremely valuable tool in research studies. The Finometer™ is ideally suited for the two studies described here, since finger arteries are affected by contraction and dilation in relation to mental and physical (heat, cold, blood loss, orthostasis) stress[307].

2.2.1.4 Blood pressure reactivity and recovery and resting blood pressure

From the continuous BP recordings using the Finometer™, one-minute averages of these data were calculated for the duration of the cardiovascular protocol. These one-minute averages were used to compute BP reactivity and recovery to standing and math-stress, and to estimate two average resting BPs (Figure 1.9). BP reactivity was calculated as a simple change score, that is, the change in BP from the last minute before the onset of stress (*i.e.* initial BP) to the first minute of stress (*i.e.* final BP). Similarly, BP recovery was calculated as the difference in BP between the last minute of stress (*i.e.* initial BP) and the first minute after the stress was terminated (*i.e.* final BP). The four BP responses studied were calculated as follows:

1. *BP reactivity to standing* was a change in BP between the last minute of being supine to the first minute of standing;
2. *BP recovery from standing* was a change in BP between the last minute of standing and the first minute of sitting;

3. *BP reactivity to math-stress* was a change in BP between the last minute before the math-stress test explanation and the first minute of the actual test; and
4. *BP recovery from math-stress* was a change in BP between the last minute of the math-stress test and the first minute after the test, during the recovery period.

In addition, the one-minute SBP and DBP averages across the protocol were used to compute two resting BPs as follows:

1. *"Post-standing" resting BP* was the average BP during the last 5 minutes of sitting during the posture test; and
2. *"Post-math" resting BP* was the average BP during the last 5 minutes of the recovery period after math stress.

These resting BPs were designed to mimic "clinic" BPs, defined by the Canadian Hypertension Society Standards as repeated measures of BP after 5 minutes of rest while seated[308].

2.2.1.5 Data quality-control and processing

For each subject, the time series data were visually inspected and programs were used to facilitate the delineation of each section of the protocol (*i.e.* supine, standing, sitting, and so on), to clean all of the time series data (*i.e.* for each hemodynamic parameter) within each section of the protocol, and to calculate one-minute averages for the protocol. To accomplish this, programs were written in Matlab (Matlab 7.3.0, Mathworks Inc., Natick, United States). Quality control of the data involved the replacement of data points that were clearly signal artefacts with the mean of the previous and subsequent data points. My role in the collection of the SYS data was to calculate systolic and diastolic BP reactivity and recovery from the posture and math stress tests and to calculate mean resting BP values during certain periods of the protocol. BP reactivity and recovery were calculated over different time intervals (one-minute, two-minute and five-minute intervals) and experimenting with different initial and final BP

values. The BP reactivity and recovery calculations described above (section 2.2.1.4) were deemed most appropriate because they accurately capture the acute changes in BP from pre-stress levels to stress levels, occurring within seconds (SNS activation) to minutes (HPA activation) of the respective stressor. Calculations were done in Excel (Excel 12.3.3, Microsoft Excel 2008). All new BP variables were cleaned (mean± 3 SD) and subsequent analyses were carried out in JMP (version 9.0.0, SAS 2012), described in more detail in Section 2.4.

2.2.2 BODY COMPOSITION PROTOCOL

2.2.2.1 Bioimpedance

At the beginning of the body composition protocol, trained nurses estimated total body fat (TBF, *i.e.* fat mass [FM]) and fat free mass (FFM) non-invasively in subjects using multi-frequency bioelectrical impedance (Xitron Hydra Model 4200 Bio-Impedance Spectrum Analyzer, Xitron Technologies, Inc., San Diego, USA). Participants were asked to refrain from caffeine, alcohol and vigorous activity for 24 hours prior to testing[283]. For these whole-body measurements, an electrode is placed on the subject's right wrist and right ankle. This system uses a range of 20 frequencies between 5 KHz and 1 MHz. This technique is a non-invasive way of estimating TBF and FFM based on the principle that electricity can be conducted by dissolved electrolytes in aqueous tissue[309]. It measures the resistance to an electric current passed through the body, consisting of intra-cellular and extracellular branches (two resistors) and the cell membrane acting as a capacitor[309]. At low frequencies, current cannot pass through the membrane and is conducted only through the electrolytes dissolved in extra-cellular water. At infinite frequencies, the current can pass freely through the capacitor (cell membrane). Thus, intracellular resistance can be calculated, which is inversely proportional to the volume of intracellular water[309]. In practice, zero and infinite frequency current cannot be applied to the body; thus, multi-frequency bioimpedance measures resistance and reactance at a range of frequencies. In subjects with normal body water distribution, intracellular and total body water should correlate with FFM[309]. The hydration of FFM in adults is

constant, at approximately 73.2%, while in children under 10 years of age, it varies[310]. In the SYS, age- and sex- appropriate hydration estimates were used (Xitron Technologies, Inc., San Diego, USA). Once TBF and FFM are known, percent body fat (TBF divided by body weight), percent lean body mass (FFM divided by body weight) and % body water (body water divided by body weight) can be derived.

2.2.2.2 Anthropometry

Anthropometric measures were taken following bioimpedance. Nurses measured the height (1-mm precision, Harpenden stadiometer [Holtain, UK]) and weight (0.1-kg precision, Detecto scale [Cardinal scales, USA]) of each subject[177]. From these measurements, BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). In addition, the nurses measured circumferences of the arm, waist, hip, proximal-, mid- and distal-thigh (0.1-cm precision) and subscapular, bicep, tricep, suprailiac and mid thigh skin-folds (1-mm precision) three times[177].

2.2.3 MAGNETIC RESONANCE IMAGING

During the hospital visit, magnetic resonance imaging (MRI) of the abdomen was conducted to assess intra-abdominal fat (visceral fat [VF]) and subcutaneous fat (SF). MRI of the kidney and brain were also performed; this is described elsewhere as it does not relate to my findings[311]. MRI data were collected on a Phillips 1.0-Telsa superconducting magnet (Philips, Eindhoven, the Netherlands)[283]. Ten axial slices of 10-mm thickness, centred on the L4-L5, were acquired with a heavily T1-weighted, single breath-hold, multi-slice spin-echo sequence[283]. T1 relaxation time is a measure of the rate of recovery towards alignment with the scanner's magnetic field[312]; the relaxation time of adipose tissue is much shorter than that of nearly all other tissues, which facilitates its imaging when contrasted against FFM[313]. A single axial slice (10-mm thick) at the level of the umbilicus was selected for quantification of

abdominal fat (Figure 1.10)[177]. These images were first smoothed using an adaptive bilateral filter to remove noise while still preserving edge information[314]. Computational analysis produced an initial fat classification map for each slice using a standard-growing region algorithm. An iterative refinement procedure corrected false positives and false negatives using a battery of morphological operators; these included hysteresis, thresholding over small neighbourhoods and median filtering to remove salt and pepper noise[177, 315]. The resulting classification map was manually segmented into visceral and subcutaneous fat[177]. Visceral fat was defined as adipose tissue within the innermost aspect of the abdominal cavity and not contained within other abdominal organs or muscles, and subcutaneous fat was defined as areas of adipose tissue lying between the skin and the outer aspect of the abdominal cavity[177]. The volumetric measurements of each fat compartment were then computed by a semi-automated method, using a simple histogram counting algorithm to compute the total number of voxels for each type of fat. This semi-automated method was validated against manual segmentation in a random subgroup of 20 subjects (VF: r^2=0.99, SF: r^2=0.97)[177]. The resulting visceral fat volumes were studied in Chapter 3, in relation to BP reactivity and recovery.

The use of MRI for the quantification of body fat is often limited to research but its cost can be prohibitive. Our measurements are restricted to a cross section of the abdomen, although assessment of total body fat by MRI would have offered an ideal depiction of both the quantity of fat and its distribution, an important determinant of cardiovascular risk. However, such assessments are constrained by time and cost. As a more affordable alternative, dual energy x-ray absorptiometry (DXA) can image whole body fat distribution[198], though this method involves ionizing radiation and does not provide three-dimensional images. As a result, DXA cannot distinguish between visceral and subcutaneous adipose tissue.

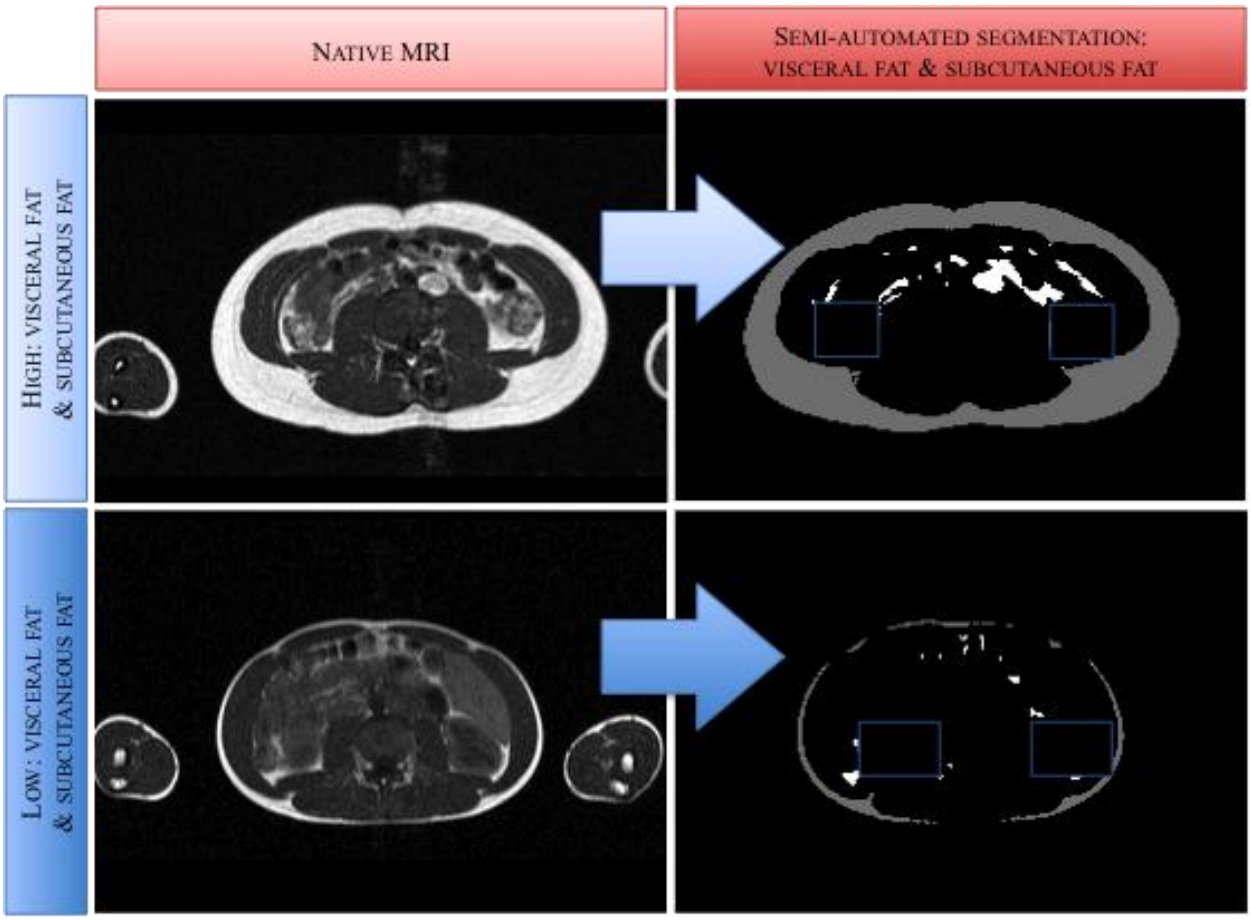

Figure 1.10. Magnetic resonance imaging and quantification of visceral *versus* subcutaneous abdominal fat.
Examples of a native MRI (left) with its corresponding segmented MRI (right) are presented in two subjects with "high" (top) and "low" (bottom) amounts of abdominal fat. In the segmented images, visceral fat is shown in white and subcutaneous fat in grey.
MRI = magnetic resonance imaging.

2.2.4 SEXUAL MATURATION

As part of the Saguenay Youth Study, subjects completed a validated questionnaire to assess their stage of pubertal development. This Puberty Development Scale is an eight-item self-report measure of physical development based on Tanner stages, with separate forms for males and females[316]. In females, data was also collected on the age of menarche, stage of menstrual cycle and oral contraceptive use. In

our sample 13% of girls had not started menstruating and 15% were on oral contraceptives; the stage of the menstrual cycle could not be determined in 24% of girls due to missing data or incorrect reporting[317].

2.2.5 BIOCHEMICAL ANALYSES

A fasting blood sample was taken in subjects between 8:00 and 9:00 am in schools, on a day separate from the hospital visit. This sample was used to evaluate the following: glucose and lipid metabolism (glucose, insulin, total cholesterol, LDL- and HDL-cholesterol, triglycerides); a pro-inflammatory state (C-reactive protein [CRP]); HPA axis activity (cortisol); leptin; and sexual maturation (sex-hormone binding globulin, total testosterone, bio-available testosterone and estradiol)[283]. It should be noted that the levels of estradiol in females, which vary according to the stage of the menstrual cycle but remain consistently higher than in males, could not be accurately determined because of uncertainty in the timing of the menstrual cycle. This sample was also used for deoxyribonucleic acid (DNA) extraction and genotyping, which is described below in Section 2.3.

2.3 GENOTYPING

All adolescents were genotyped with the Illumina Human610-Quad BeadChip (Illumina, San Diego, California). Genotyping was conducted at the Centre National de Génotypage (Paris, France). Quality control of genotyping was conducted, according to the following criteria: (1) SNPs with a call rate <95%, (2) minor allele frequency <0.01 (1%) or (3) not in Hardy-Weinberg equilibrium ($p<1x10^{-4}$) were excluded. Individuals with a discrepancy between reported and X chromosome-derived sex were also excluded. For the study described in Chapter 4, we selected eight single nucleotide polymorphisms (SNPs) covering the entire region of our candidate gene, *CYP17A1*: rs4919683, rs4919682, rs10883782, rs17115100, rs2486758, rs619824, rs6163 and rs4919686.

2.4 STATISTICAL ANALYSES

All statistical analyses described in Chapters 3 and 4 were carried out in JMP (version 9.0.0, SAS 2010) and all genotype-phenotype association tests in Chapter 4 were conducted in Merlin (version 1.1.2, 2007). Prior to any analyses, we excluded statistical outliers, identified as values larger or smaller than mean ± three standard deviations. Variables that were not normally distributed (*i.e.* total body fat and VF) were log transformed.

In Chapter 3, multivariate analysis of co-variance (MANCOVA) was carried out with JMP (version 9.0.0, SAS 2010), using the Fit Model function, to examine: (1) the effect of sex on BP reactivity and recovery to standing and to math, *i.e.* to see if these BP responses differed between males and females. These analyses were performed while adjusting for height, age and initial (baseline) BP (i.e. the last minute prior to respective changes). Using the same model, we also assessed whether: (2) the quantity of VF is associated with enhanced BP reactivity and/or delayed BP recovery to each challenge in males and females separately and (3) whether BP reactivity and recovery to each challenge are associated with "post-standing" and "post-math" resting BPs. These analyses were performed while adjusting for height, age and initial BP and, in further analyses, for total body fat. Sexual maturation, *i.e.* puberty stage (self-reported, Tanner stage 1-5), and prenatal exposure to maternal cigarette smoking were also included as covariates in additional analyses.

This statistical model, MANCOVA, is an extension of analysis of covariance (ANCOVA), in the case where there is more than one dependent variable. It was chosen because it allows for the characterization of differences in group means (*eg.* between males and females) in regards to a linear combination of multiple dependent variables and their interactions, while controlling for covariates. This is achieved by creating a single dependent measure from a combination of all dependent measures that maximizes the between group differences. The underlying assumptions that must be met, in order for MANCOVA to be applied appropriately, include: (1) each dependent variable (or linear combination of

dependent variables) must be normally distributed for each group (reduces type I error); (2) each observation must be independent of other observations, *i.e.* random sampling is employed (reduces type I error); (3) each dependent variable must demonstrate similar levels of variance across each independent variable; and (4) the covariance matrices for each dependent variable must be equal across all levels of the independent variable (reduces type I error and increases statistical power). Removal of outliers and transformation, as done here, help ensure assumption (1) is met.

In Chapter 4, genotype-phenotype association tests were conducted in Merlin (version 1.1.2, 2007)[318, 319] under an additive genetic model for the following phenotypes: (1) SBP and DBP reactivity to math, (2) SBP and DBP reactivity to standing, (3) post-standing resting SBP and DBP, and (4) post-math resting SBP and DBP. Analyses were done in males and females separately, with age, height and, when appropriate, initial (baseline) BP included as covariates. Previously identified and validated loci were considered significant if their $p<0.05$ and they were in linkage disequilibrium (LD) with and showed the same direction of effect as the previously reported SNPs[234, 235, 238, 262-264]. HaploView 4.2[320] was used to examine the LD structure of SNPs in the Saguenay Youth Study; this was done with HapMap (release 22)[321] when comparing our SNPs to previously identified SNPs not genotyped in our study. In addition, associations between our top SNP and SBP/DBP reactivity to standing and to math were examined with MANCOVA carried out in JMP (version 9.0.0, SAS 2010), to determine the difference in BP reactivity between major and minor allele homozygotes. Analyses were done in males and females separately, with age, height and initial BP as covariates as they could be potential confounders. Puberty stage and prenatal exposure to maternal cigarette smoking were also included as covariates in additional analyses.

Genotype-phenotype analyses were conducted with the quantitative trait-association tools implemented in Merlin (version 1.1.2, 2007)[318, 319] software package. Merlin appropriately combines information from related and unrelated individuals (*i.e.* it takes into account relatedness in our sample between sibships. To account for the potentially confounding effect of age, height and, when appropriate, initial BP, these were included in the Merlin analyses as covariates when required. With Merlin, a simple

regression model is fitted to each trait and a variance component approach is used to account for correlation between different observed phenotypes within each family (sibship). Phenotype heritability is assessed by Merlin using a variance components model[322]. Individuals with missing genotype information were excluded.

3. MANUSCRIPT 1: BLOOD PRESSURE RESPONSES TO PHYSICAL AND MENTAL CHALLENGES IN ADOLESCENT MALES AND FEMALES – THE ROLE OF VISCERAL FAT

3.1 ABSTRACT

Enhanced blood pressure (BP) reactivity and delayed BP recovery from physical and mental challenges predict hypertension, a disease more common in men than women. Visceral obesity is a major risk factor for hypertension, more so in men than women. Here, we investigated whether visceral fat (VF) relates to BP reactivity and recovery from physical and mental challenges in adolescent males and females. In a community-based sample of 285 males and 311 females (12-18 years), we measured VF with magnetic resonance imaging and beat-by-beat BP at rest and during physical (10-minute standing) and mental (2-minute math-stress) challenges. For both challenges, males versus females showed greater BP reactivity (by 3.5 mm Hg, $p<0.0001$ and by 2.6 mmHg, $p=0.02$, respectively) and no difference in BP recovery. VF correlated positively with BP reactivity to the physical and not mental challenge and this was seen in males but not females ($r=0.18$, $p=0.007$). No relationship was observed between VF and BP recovery from either challenge and in either sex. In both sexes, BP reactivity and BP recovery for both challenges (adjusted for age, height and initial BP) correlated closely with resting BP (explaining up to 34 % of variance). In summary, adolescent males versus females demonstrate greater BP reactivity to physical and mental challenges and this sex difference may in part be mediated by a sex-specific relationship of VF to BP reactivity.

3.2 INTRODUCTION

Enhanced blood pressure (BP) reactivity, defined as BP increase in response to a physical or mental stressor[278], and delayed BP recovery, defined as BP return to a pre-stress value once the stressor is removed[100], predict future hypertension[64, 96, 107, 108, 112] and cardiovascular disease[114]. Both parameters enhance pressure load on the vessels, heart and kidney, leading to their structural and functional changes that in turn contribute to further BP elevation (also at rest) and thus development of hypertension and cardiovascular disease[114].

A growing body of evidence suggests that pre-clinical features of hypertension and cardiovascular disease emerge already during adolescence[6, 105, 106]. Enhanced BP reactivity and delayed BP recovery may be two such features. Both are present in healthy adolescent offspring of hypertensive parents[107, 108] and, in longitudinal studies, they predict future hypertension[73, 109-111].

Resting BP is higher in males than females, beginning in adolescence[129, 123]. Likewise, BP reactivity and BP recovery are enhanced and delayed, respectively, in males versus females, beginning in adolescence[58, 64, 70, 84, 129-131, 135, 136]. In addition, visceral obesity is a leading risk factor of hypertension[94, 169, 170] and this relationship is stronger in men than in women[171] and exists already in adolescence[133, 177]. But whether visceral adiposity relates to BP reactivity and BP recovery and whether these relationships are different between males and females has not been studied.

Therefore, the aim of the present study was to investigate how visceral adiposity relates to BP reactivity and recovery from physical and mental challenges in a community-based sample of 285 male and 311 female adolescents. In all participants, visceral fat (VF) was measured directly with magnetic resonance imaging and BP at rest and during physical (10-minute standing) and mental (2-minute math-stress) challenges was recorded beat-by-beat.

3.3 METHODS

3.3.1 PARTICIPANTS

Male (n=285) and female (n=311) white Caucasian adolescents, aged 12 to 18 years, were recruited as part of the Saguenay Youth Study (SYS) in the Saguenay Lac Saint-Jean region of Quebec, Canada. The SYS is a community-based study of cardiovascular and metabolic health, and brain and behaviour in adolescence. All participants were recruited via high schools; detailed recruitment and selection criteria are described in Section 2.1[178, 283]. Written consent from the parents and assent from the adolescents were attained before the commencement of data collection. The research ethics committee of the Chicoutimi Hospital approved the study protocol. The current sample consists of participants recruited and tested between November 2003 and June 2009 (n=596), among whom 490 had complete quality-controlled cardiovascular data sets and were analyzed in the present study (Table 3.1**)**. In this sample, the prevalence of overweight or obesity (85th age- and sex-specific percentile) was 29% in boys and 21% in girls, which is similar to that in the Canadian adolescent population at large (2004 Canadian Community Health Survey[168]). The prevalence of hypertension (sitting SBP or DBP 95th age, sex- and height-specific percentile[83]) was 7.1% in boys and 3.3% in girls, which is also similar to that in the Canadian adolescent population at large (Canadian Health Measures Survey[84]).

Table 3.1. Participant characteristics

Characteristic	Males (n=285)	Females (n=311)	Estimated Difference (95% CI)	p-value
Age, years	14.5 ± 1.9	14.6 ± 1.9	0.2 (-0.1 – 0.5)	0.3
Puberty stage, Tanner 1-5	3.4 ± 0.9	4.1 ± 0.7	0.7 (0.6 – 0.9)	<0.0001
Height, cm	167.0 ± 10.7	159.9 ± 6.8	7.5 (6.2 – 8.7)	<0.0001
Body weight, kg	60.8 ± 16.1	55.1 ± 11.1	0.1 (-1.8 – 2.0)	0.9
BMI, kg/m^2	21.6 ± 4.4	21.5 ± 3.9	0.1 (-0.5 – 0.8)	0.7
Total body fat, kg	10.3 ± 7.7	14.0 ± 7.1	4.8 (3.5 – 6.1)	<0.0001
Visceral fat, cm^3	21.3 ± 23.5	18.7 ± 12.5	2.5 (-0.8 – 5.9)	0.1

Non-adjusted means ± standard deviations shown. Differences between boys and girls were assessed adjusting for age and when appropriate height.
Abbreviations: BMI = body mass index, CI = confidence interval.

3.3.2 ASSESSMENTS

All subjects underwent a body-adiposity and cardiovascular protocol conducted in the Chicoutimi hospital on Saturdays, commencing between 8:00 and 12:00[283].

Body-adiposity*:* Multifrequency bioimpedance analysis estimated total body fat (Xitron, Inc, San Diego, California); participants were asked to refrain from caffeine, alcohol and vigorous activity for 24 hours prior to the measurement. Anthropometric measures were taken to determine weight, height and body mass index. Magnetic resonance imaging of the abdomen was performed on a Phillips 1.0-T superconducting magnet[283]. A single axial slice at the level of the umbilicus, 10-mm thick, acquired with a heavily T1-weighted, single breath-hold, spin-echo sequence was selected for quantification of VF, as described previously in Section 2.2.3[283]. VF was defined as adipose tissue lying within the innermost aspect of the abdominal cavity, not contained within other abdominal organs or muscles[177]. The volumetric measurements were computed by a semi-automated method, previously validated against manual segmentation in a subgroup of subjects[177].

Cardiovascular protocol: This lasted 52 minutes and included simple physical and mental challenges intended to invoke cardiovascular responses. The physical challenge was *a posture test* during which each participant was supine for 10 minutes, standing for 10 minutes and sitting for 10 minutes. The mental challenge was *a math-stress test* consisting of an explanation (40 seconds), waiting period (4 minutes), math-stress (2 minutes) and math-stress recovery (10 minutes) (Figure 3.1). The math-stress was a sequence of 46 simple arithmetic problems of increasing difficulty (to ensure some failure in all subjects) to be solved out loud. Throughout the protocol, a noninvasive hemodynamic monitor, Finometer™ (Finapres, Amsterdam, the Netherlands), was used to record continuous finger blood flow. From this data, the Finometer™ derived beat-by-beat brachial SBP. One-minute averages of these data were calculated for the duration of the protocol and were used to compute SBP reactivity and recovery to standing and math stress as follows (Figure 3.1): *SBP reactivity to standing* was a change in SBP between the last minute of being supine to the first minute of standing; *SBP recovery from standing* was a change

in SBP between the last minute of standing and the first minute of sitting; *SBP reactivity to math-stress* was a change in SBP between the last minute before the math-stress test explanation and the first minute of the actual test; and *SBP recovery from math stress* was a change in SBP between the last minute of the math-stress test and the first minute after the test. In addition, the one-minute SBP averages across the protocol were used to compute two resting BPs as follows (Figure 3.1): *"post-standing" resting SBP* was the average of SBP during the last 5 minutes of sitting during the posture test and *"post-math" resting SBP* was the average SBP during the last 5 minutes of the 10-minute period after the math stress. These resting BPs were designed to mimic "clinic" BPs, defined by the Canadian Hypertension Society Standards as repeated measures of BP after 5 minutes of rest while seated[308]. Sex-specific means and standard deviations of these resting BPs and initial BPs for all reactivity and recovery variables are presented in Table 3.2. In the present study, we chose to study SBP for the following reasons: (1) systolic rather then diastolic hypertension is predominant among obese children[122] and young adults[279]; (2) population variance in SBP vastly exceeds that in DBP[91]; and (3) during adolescence, SBP increases markedly in males but not females, while DBP remains similar in both sexes[131]. This corresponds to the evolutions of risk for high SBP in adolescence, which increases 19% annually in males but remains unchanged in females[131, 134].

Table 3.2. Initial and resting SBP in adolescent males and females

	Boys	Girls	Estimated Difference (95% CI)	p-value
Initial SBP, mmHg				
Pre-standing	119.0 ± 12.1	120.0 ± 12.1	1.0 (-1.2 – 3.3)	0.38
Pre-sitting	124.7 ± 15.1	119.0 ± 15.0	5.7 (2.8 – 8.6)	0.0001
Pre-math test	123.2 ± 14.0	118.5 ± 13.9	4.8 (2.2 – 7.4)	0.0003
Pre-math recovery	138.0 ± 16.9	132.4 ± 16.8	5.5 (2.4 – 8.7)	0.0006
Resting SBP[a], mmHg				
Post-standing	122.8 ± 13.1	118.6 ± 13.0	4.2 (1.8 – 6.6)	0.0008
Post-math	127.6 ± 13.1	124.3 ± 13.1	3.4 (0.9 – 5.8)	0.007

Adjusted means ± standard deviations and differences between boys and girls are shown, adjusted for age and height.
[a]Resting SBP is a 5-minute average of SBP measured while seated after 5 minutes at rest.
Abbreviations: CI = confidence interval.

3.3.3 STATISTICAL ANALYSES

For all statistical analyses, we used multivariate analysis of co-variance (MANCOVA) carried out with JMP (version 9.0.0, SAS 2010). Prior to any analyses, we excluded statistical outliers, identified as values larger or smaller than mean ± three standard deviations. In addition, variables that were not normally distributed (i.e. total body fat and VF) were log transformed. *First*, we examined the main effect of sex (males vs. females) on BP reactivity and recovery to each challenge. These analyses were performed while adjusting for height, age and initial BP (i.e. the last minute prior to respective changes) and, in additional analyses, physical activity (number of 20-min strenuous-exercise sessions per week). *Second*, we assessed whether the quantity of VF is associated with enhanced BP reactivity and delayed BP recovery to each challenge in males and females separately. *Third*, we examined whether BP reactivity and recovery to each challenge are associated with "post-standing" and "post-math" resting BPs. These analyses were performed while adjusting for height, age and initial BP and, in further analyses, for total body fat, puberty stage, and prenatal exposure to maternal cigarette smoking, a potential ascertainment bias (due to selection criteria, about 50% of both males and females were exposed prenatally to maternal cigarette smoking).

3.4 RESULTS

3.4.1 BASIC CHARACTERISTICS OF STUDIED MALES AND FEMALES

Males (n=285) compared with females (n=311) did not differ by age, body weight or BMI. They were taller by 7.5 cm, but they showed lower quantity of total body fat (by 4.8 kg) and similar amount of VF (Table 3.1). Their SBP was similar while being supine, but it was higher throughout the rest of the protocol (during and after standing and math stress) by 3.4 to 5.7 mm Hg (Table 3.2).

3.4.2 BP REACTIVITY AND RECOVERY: SEX DIFFERENCES

Males compared with females showed greater *SBP reactivity* to both physical and mental challenges, and this difference was more pronounced for standing (by 3.5 [1.8-5.2] mmHg, $p<0.0001$) than for math stress (by 2.6 [0.4-4.8] mmHg, $p=0.02$). No differences between males and females were observed in *SBP recovery* from either challenge (Figure 3.2). These sex comparisons were made after adjusting for age, height and initial SBP (*i.e.*, SBP prior to the onset/end of respective challenges) and remained virtually unchanged after additional adjusting for either puberty stage ($p=0.002$ and $p=0.007$, respectively) or prenatal exposure to maternal cigarette smoking ($p<0.0001$ and $p=0.02$, respectively).

3.4.3 BP REACTIVITY AND RECOVERY: VISCERAL ADIPOSITY

VF correlated with SBP reactivity to standing only and in males only (Figure 3.3). This correlation was positive ($r=0.18$, $p=0.007$, Figure 3.3) and remained virtually unchanged after additional adjusting for total body fat ($r=0.21$, $p<0.002$), thus, suggesting that the quantity of VF rather than that of other body fat may enhance SBP reactivity to standing. These results remained similar after additional adjusting for puberty stage ($r=0.17$, $p<0.01$ and $r=0.20$, $p=0.002$; respectively) and for prenatal exposure to maternal cigarette smoking ($r=0.18$, $p=0.007$ and $r=0.22$, $p=0.001$; respectively). In females, an association of VF with BP recovery was no longer significant when additionally adjusted for TBF. No relationship was observed between VF adjusted for TBF and BP recovery from either challenge and in either sex (Figure 3.3).

Given that SBP reactivity to standing was greater in males than females and correlated with VF in males only, we tested whether VF could contribute to the sex difference in this hemodynamic response. Towards this end, we examined the effect of sex on SBP reactivity to standing while adjusting not only for age, height and initial SBP (as previously) but also for VF, independently of TBF. This additional adjusting for VF diminished the difference between males and females (from 3.5 [1.8-5.2] mmHg,

p<0.0001 to 2.7 [0.6-4.8] mmHg, p=0.01), suggesting that VF may indeed contribute to the observed sex difference in SBP reactivity to standing.

3.4.4 BP REACTIVITY AND RECOVERY: RELATIONSHIP TO RESTING ("CLINIC") BP

Finally, we examined whether SBP reactivity and recovery from standing and math stress relate to resting SBP measured under conditions mimicking the standard clinical conditions, i.e., after 5 minutes of rest while being seated[308]. For this purpose, we assessed two resting SBPs: one after the first challenge (10-minute standing) and one after the second challenge (2-minute math-stress test, Figure 3.1). The relationships were tested while adjusting for age, height and respective initial SBP. These analyses showed that, similarly in males and females, SBP reactivity and recovery from standing correlated closely with resting SBP after standing (explaining 18% to 34% of variance, Table 3.3), and SBP reactivity and recovery from math stress correlated closely with resting SBP after math stress (explaining 22% to 25% of variance, Table 3.3). In these relationships, every 1-mm Hg increase in SBP reactivity to standing was associated with a 0.4-mm Hg increase in resting SBP after standing, and every 1-mm Hg increase in SBP reactivity to math stress was associated with a 0.7- to 0.8-mm Hg increase in resting SBP after math stress. These results suggest that simple daily-life activities, such as standing and mental challenge, may impact significantly resting SBP even after 5 minutes of rest.

Table 3.3. The relationship between SBP reactivity to and recovery from standing and math stress and resting SBP

	Males			Females		
	p-value	**r[a]**	**Estimate[b]**	**p-value**	**r[a]**	**Estimate[b]**
Post-standing resting SBP[c]						
Reactivity to standing	<0.0001	0.42	0.4 ± 0.9	<0.0001	0.47	0.4 ± 0.8
Recovery from standing	<0.0001	0.58	0.6 ± 0.8	<0.0001	0.54	0.5 ± 0.8
Post-math resting SBP[c]						
Reactivity to math	<0.0001	0.48	0.8 ± 0.2	<0.0001	0.50	0.7 ± 1.3
Recovery from math	<0.0001	0.50	0.3 ± 0.6	<0.0001	0.47	0.3 ± 0.6

[a]Partial r and p-values are given for the association between resting SBP and reactivity/recovery using a multivariate model, adjusting for age, height and initial BP, in boys and girls separately.
[b]Estimates are given ± standard deviation.
[c]Resting blood pressure is an average seated blood pressure measured over 5 minutes following 5 minutes of rest.
Abbreviations: SBP = systolic blood pressure.

3.5 DISCUSSION

The results of the current study suggest that: (1) adolescent males compared with adolescent females exhibit greater BP reactivity but similar recovery from both physical and mental challenges; (2) in males only, excess VF enhances BP reactivity to physical but not mental challenges, and it does not influence BP recovery from either challenge; and (3) in both male and female adolescents, greater BP reactivity and slower BP recovery from either challenge are closely associated with higher resting BP.

The magnitude and duration of BP responses[120] may contribute to the subsequent risk for hypertension[73, 109-111]. In the current study, BP reactivity to both physical (standing) and mental (math stress) challenges was greater in males than females, but BP recovery from either challenge did not differ between the two sexes. These results are consistent with most previous research [67, 70, 76, 136 61, 66 101, 323, 324] and they suggest that BP reactivity but not BP recovery are modulated, at least in part, by sex-specific pathways. One such pathway may be related to excess VF and its impact on BP. Visceral obesity is a leading risk factor of hypertension[94, 169, 170] and this relationship is stronger in men than women[133, 171, 177]. In agreement with this, we found here that, only in males, VF correlates positively with BP reactivity. We also found that this association is seen only for BP reactivity to standing but not math stress. Standing is a

simple physical challenge that requires rapid activation of the sympathetic nervous system to counteract the gravity-induced fall in BP[19, 20]. It has been shown that the quantity of VF rather than that of subcutaneous fat correlates positively with sympathetic nerve activity[174, 325], and that this VF-sympatho-activation relationship is seen in males but not in females[177, 326]. Thus, this sex VF-related sympatho-activation may be involved in mediating the sex-specific association between VF and BP reactivity to standing.

In contrast to BP reactivity to standing, we did not see any significant association between VF and BP reactivity to mental stress. Mental stress elicits a coordinated response between the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. It increases cardiac output and vascular resistance in several vascular beds, except in skeletal muscle[296]; this redistributes blood towards the muscles, which is functionally relevant for the 'fight-or-flight' stress response. Sympathetic nerve activity following mental stress is highly variable among individuals, with some individuals (~50%) exhibiting a reduction in muscle sympathetic nerve activity and an attenuated BP response and others showing no change or an increase in muscle sympathetic nerve activity and an increase in BP[120]. Thus, varying amounts of VF will not necessarily translate into discernable differences in BP reactivity to mental stress, as is seen with standing.

Finally, we saw that, in both sexes, BP reactivity and recovery from standing and math stress were closely related to resting BP measured under standard "clinic" conditions (i.e., being seated at rest for at least 5 minutes)[308]. These results suggest that simple daily-life activities, which include a multitude of arousing stimuli and stressful situations, may have a significant effect on "clinic" BP. It has been shown that individuals with greater sympathetic and BP responses to mental stress had BP that remained higher during succeeding rest periods[120]. Acute stress-induced elevations in BP are primarily attributed to enhanced sympathetic activation, and may also include diminished nitric oxide production and vagal withdrawal[115, 116]. The underlying mechanisms linking enhanced BP reactivity and delayed BP recovery to future hypertension remain not well understood. Both parameters increase the pressure load on the

vessels, heart and kidney, leading to structural and functional changes that in turn contribute to further BP elevations and thus the progression of hypertension. Acute BP elevations increase the shear stress and transmural pressure experienced by the arterial wall[114], leading to greater vessel stiffness and vascular smooth muscle cell hypertrophy, and impair endothelial-dependent vasodilation[118]. These effects contribute to chronic increases in vascular resistance and BP. In the kidneys, vascular remodeling and endothelial dysfunction can cause sustained changes in the renal set-point for BP regulation, leading to persistent elevations in BP[119]. As such, the magnitude and duration of BP responses[120] may contribute to the subsequent risk for hypertension over the long-term[73, 109-111].

In summary, our results suggest that BP reactivity is greater in males than females and this sex difference may in part be mediated by a sex-specific relationship of VF to BP reactivity. In both males and females, BP reactivity and recovery may be sensitive markers of pre-hypertension and, as such, may be useful for identifying adolescents at risk for the disease who should be targeted for early intervention and secondary prevention.

3.6 FIGURES AND FIGURE LEGENDS

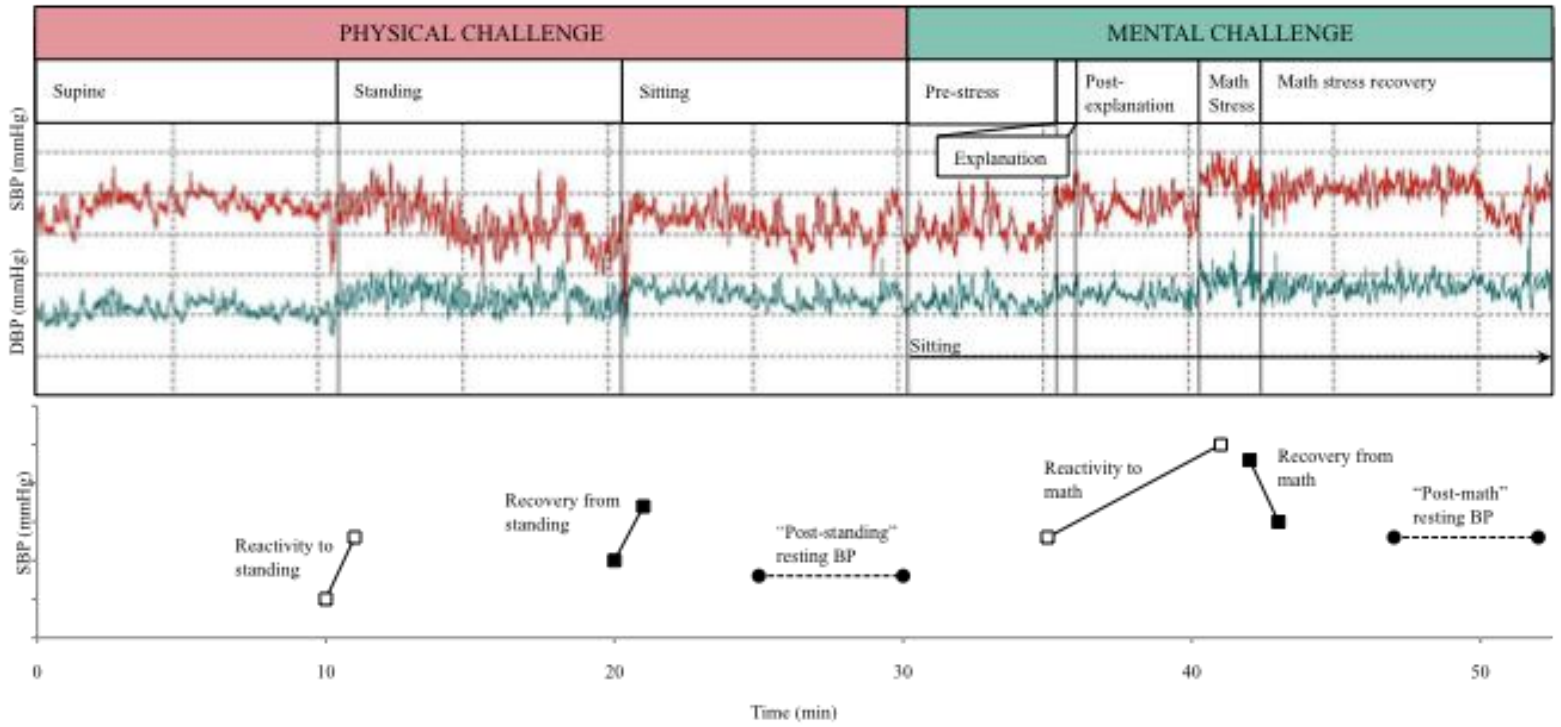

Figure 3.1. Cardiovascular protocol and the determination of BP reactivity and recovery from standing and math stress and post-standing and post-math resting BPs

The top panel shows an example of beat-by-beat blood pressure (BP) recordings during posture and math-stress tests. The posture test consisted of three periods during which the participant was first supine (10 minutes), then standing (10 minutes) and finally sitting (10 minutes). The math-stress test consisted of a pre-stress period (4 minutes), an explanation, when the subject was told he/she was going to be given a math test (40 seconds), a post-explanation waiting period (4 minutes), a math-stress test, when the actual test was administered (2 minutes) and a recovery period following the math-stress (10 minutes). The bottom panel presents BP values used to calculate BP reactivity (white squares) and BP recovery (black squares) from standing and math stress and post-standing and post-math resting BPs (black circles).

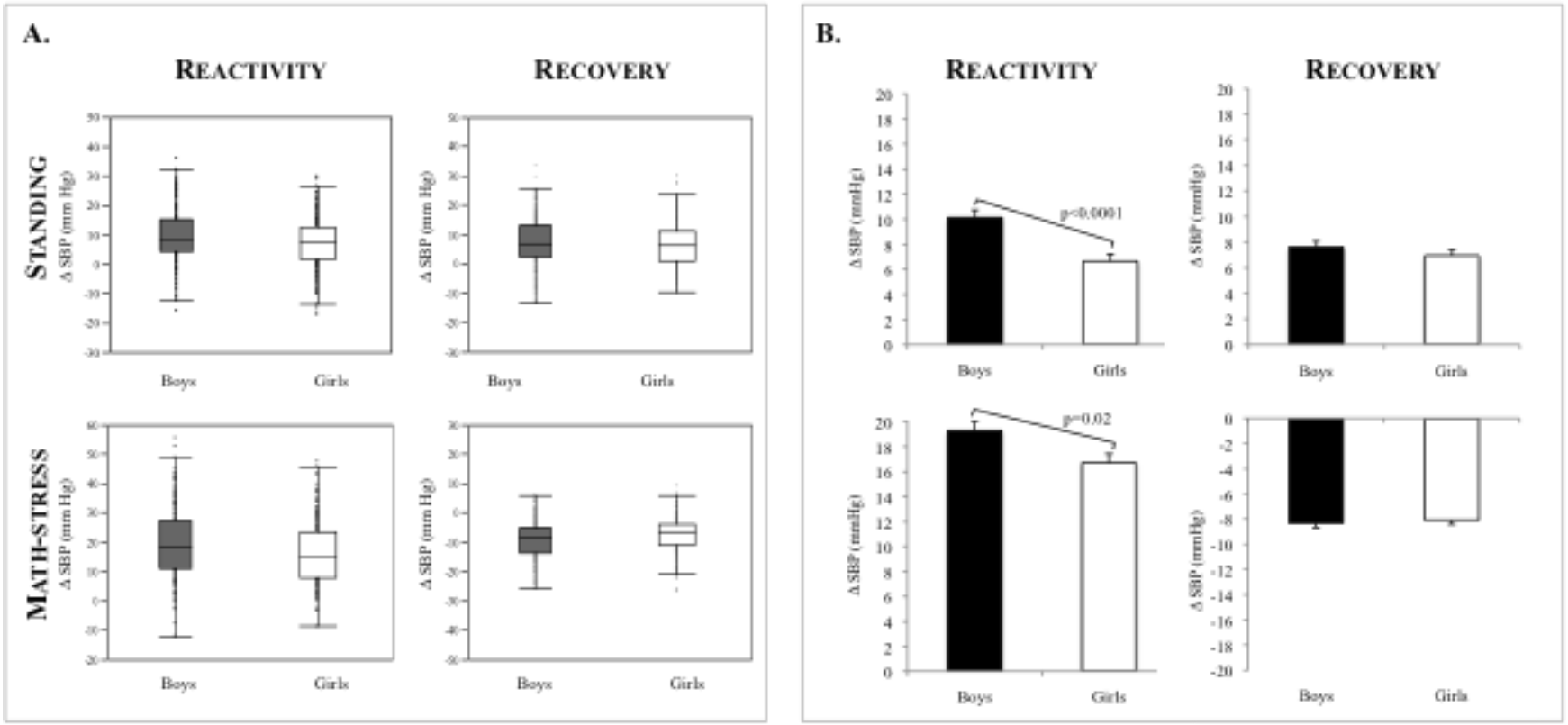

Figure 3.2. BP reactivity and recovery in adolescent males and females

Unadjusted (A) and adjusted (B) means are presented for BP reactivity and recovery from standing and math-stress in males and females. For the box plots in (A), the 25th and 75th percentile are depicted as the top and bottom of the boxes, respectively, with the horizontal line in the box indicating the median value. The whiskers were calculated as the 25th percentile – 1.5*IQR and 75th percentile + 1.5*IQR. Values beyond this range are depicted as black dots. In (B), adjusted means ± standard error are shown (covariates = age, height and initial BP). Note that, as expected, BP recovery from math is reflected by a decrease in BP, while BP recovery from standing is seen as an increase in BP. This is due to the fact that active sitting involves the contraction of leg and abdominal muscles, which causes a compression of the blood vessels and a consequent transient increase in venous return and cardiac output[20, 178].

IQR = interquartile range.

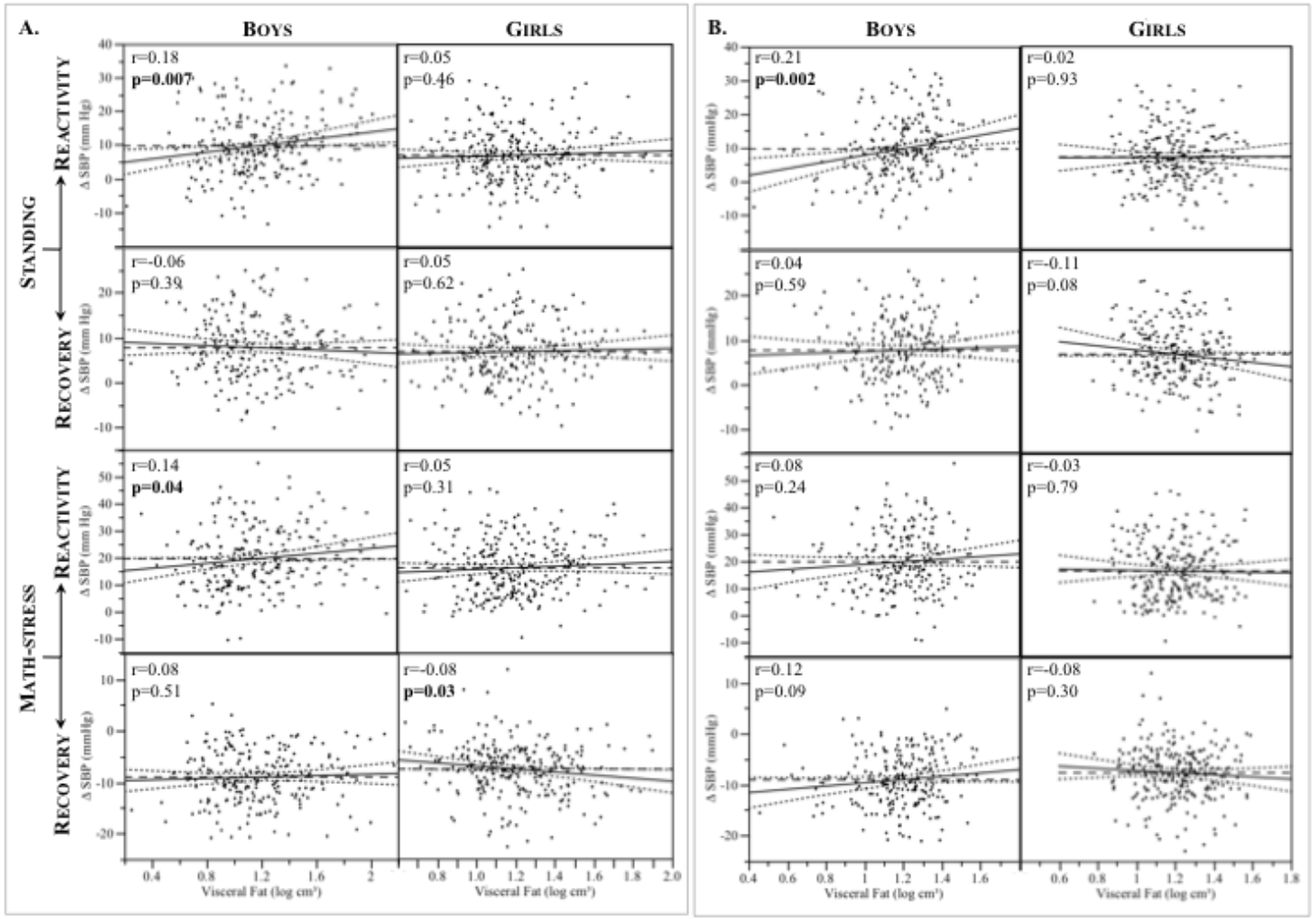

Figure 3.3. Visceral fat and BP reactivity and recovery from physical and mental challenges
The relationships between the quantity of visceral fat (A) and the quantity of visceral fat adjusted for total body fat (B) and BP reactivity and recovery from physical and mental challenges are shown for males and females. The relationships were adjusted for age, height and initial BP (A) and additionally for total body fat (B). The line of mean is indicated by a horizontal dashed line, the line of fit is shown as a solid line and the .05 significance curve is illustrated by a dotted line on either side of the line of fit.

4. *CYP17A1* AND BLOOD PRESSURE REACTIVITY TO MENTAL STRESS IN ADOLESCENT MALES BUT NOT FEMALES

4.1 ABSTRACT

Adolescents who exhibit exaggerated blood pressure (BP) reactivity to physical and mental challenges are at increased risk of developing hypertension in adulthood. BP at rest and in response to challenges is higher in males than females, beginning in early adolescence. *CYP17A1* is one of the best-established gene loci of adult hypertension; it encodes a key enzyme in the steroidogenic pathway that may influence BP through its effects on the production of mineralocorticoids, glucocorticoids and sex hormones. Here, we investigated whether this gene locus is associated with elevated BP at rest and in response to physical (active standing) and mental (math stress) challenges in adolescence. We studied 285 male and 311 female adolescents (age 12-18 years) who were recruited from a genetic founder population. Our results showed that the variant of *CYP17A1* previously associated with hypertension in adults was associated with enhanced BP reactivity to the mental but not physical challenge, and in males but not females. In males, BP increase in response to math stress was higher in major versus minor allele homozygotes by 8.7 mm Hg ($p=3x10^{-4}$), whereas in females it was higher by only 2.0 mm Hg (p=0.31). Resting BP was not associated with the *CYP17A1* variant in either sex. These results suggest that, in adolescent males but not females, *CYP17A1* enhances BP reactivity to mental stress. Whether this effect contributes to the higher prevalence of hypertension in males than females later in life remains to be determined.

4.2 INTRODUCTION

A growing body of evidence suggests that pre-clinical features of hypertension emerge already during adolescence[6, 105, 106], which is a period of human development when adult blood pressure (BP) and body

composition develop[123, 132]. Enhanced BP reactivity to a physical or mental stressor[278] in childhood and adolescence predicts adult hypertension[73, 93, 95]. Marked sex differences exist in resting BP and BP reactivity as well as in the prevalence of hypertension, with all being higher in males than females during reproductive years[58, 70, 76, 123, 129, 135].

Susceptibility to hypertension is determined by both genetic and environmental factors; the estimated heritability of BP and hypertension is around 50%[209]. Similarly, twin studies have estimated that the heritability of the systolic and diastolic BP response to a math stress test is 44% and 49%, respectively. These estimates were based on observed correlations of 0.40 for systolic and 0.51 for diastolic BP responses in monozygotic twins and 0.18 and 0.29 in dizygotic twins[210]. Hypertension is a complex genetic trait with multiple contributory genes and gene-by-gene interactions. One of the most-well-established gene loci of hypertension is *CYP17A1*[234, 235, 238, 262, 263]. The gene encodes the enzyme cytochrome P-450c17 (CYP17) that mediates steroid 17α-hydroxylase and 17,20-lyase activities (Figure 4.1)[268]. The first enzymatic action is key in the steroidogenic pathway that produces mineralocorticoids, which affect sodium handling in the kidney, and glucocorticoids, which control the whole-body response to stress. The second enzyme is involved in sex-steroid biosynthesis. Thus, *CYP17A1* may influence BP reactivity in a sex-specific manner. While *CYP17A1* is associated with resting BP and hypertension in adults, whether the gene influences BP reactivity and whether it plays a role in BP regulation already in adolescence has not been studied.

The aim of this study was to investigate whether *CYP17A1* is associated with BP at rest and in response to physical and mental challenges in adolescence. This primary investigation was carried out in a community-based sample of 596 adolescents recruited from a genetic founder population of Quebec, Canada[280-282].

4.3 METHODS AND PROCEDURES

4.3.1 STUDY POPULATION

White Caucasian adolescents (285 males, 311 females), aged 12 to 18 years, were recruited from a genetic founder population living in the Saguenay-Lac St Jean region of Quebec, Canada, as part of the Saguenay Youth Study[283]. This is a community-based investigation of long-term consequences to prenatal exposure to maternal cigarette smoking on cardio-metabolic and mental health in adolescence. Male and female participants (50% exposed prenatally to maternal cigarette smoking) were recruited via high schools; detailed recruitment and selection criteria are described in Section 2.1[283]. The Saguenay Youth Study is family-based, focusing on collection of sib-pairs.

The Saguenay-Lac St. Jean population is one of the largest founder populations in North America[280-282], originating from French ancestors who migrated to the region in the early 19th century. The population grew from 5,200 inhabitants in 1852 to 285,000 at present, due to high intrinsic growth and little emigration. Because of the founder effect, there is a higher prevalence of certain recessive disorders in the Saguenay-Lac St. Jean region compared to other populations[280], as well as limited allelic diversity among patients with these disorders[281, 282].

The current study sample consists of 596 adolescents recruited and tested between November 2003 and June 2009, including 490 subjects with complete quality-controlled cardiovascular data sets who were analyzed in this study. The prevalence of hypertension (sitting SBP or DBP $\geq$95th age-, sex- and height-specific percentile) in this sample was 7.1% in males and 3.3% in females, which is similar to that in the Canadian adolescent population at large (Canadian Health Measures Survey)[84]. Written consent from the parents and assent from the adolescents were obtained before the commencement of data collection. The research ethics committee of the Chicoutimi hospital approved the study protocols.

4.3.2 ASSESSMENTS

All subjects underwent a 52-minute cardiovascular protocol, conducted in the Chicoutimi hospital on Saturdays, commencing between 8:00 and 12:00[283]. The protocol consisted of physical and mental challenges (Figure 4.2). The physical challenges were changes in posture: each participant was first supine for 10 min, then standing for 10 min and finally sitting for 10 min. The mental challenge was a math-stress test, consisting of an explanation (<1 min), post-explanation waiting period (4 min), math-stress (2 min) and math-stress recovery (10 min). The math stress was a sequence of 46 simple arithmetic problems of increasing difficulty (to ensure some failure in all subjects) to be solved out loud.

Throughout the protocol, a noninvasive hemodynamic monitor, Finometer™ (FNS Finapres, Amsterdam, the Netherlands), was used to record continuous finger blood flow. It derives beat-by-beat brachial systolic BP (SBP) and diastolic BP (DBP) by the reconstruction and level-correction of the finger blood-flow waveform. The Finometer™ has been validated for tracking BP in adults and children over the age of six years[306]. One-minute averages of these data were calculated for the duration of the protocol and used to compute two BP reactivity parameters and 2 resting BPs for each SBP and DBP as follows (Figure 4.2): (1) *BP reactivity to a change in posture from supine to standing*, which was a change in BP from the last minute of supine to the first minute of standing; (2) *BP reactivity to math stress*, which was a change in BP from the last minute of the pre-stress period to the first minute of the math stress (a change in BP over 5 minutes); (3) *"Post-standing" resting BP*, which was the average BP during the last 5 minutes of the 10-min sitting period during the posture test; and (4) *"Post-math" resting BP*, which was the average BP during the last 5 min of the 10-min period following the math stress. Resting BPs were intended to mimic "clinic" BP, defined by the Canadian Hypertension Society as repeated BP measurements taken after 5 minutes of rest while seated[308]. Sex-specific means and standard deviations of all initial and resting BPs for SBP and DBP are found in Table 4.1.

Table 4.1. Pre-challenge and resting BP in adolescent males and females

	Males	Females	Estimated Difference (95% CI)	p-value
A. SBP, mm Hg				
Pre-standing	119.0 ± 12.1	120.0 ± 12.1	1.0 (-1.2 – 3.3)	0.38
Pre-math stress	123.2 ± 14.0	118.5 ± 13.9	4.8 (2.2 – 7.4)	0.0003
Post-standing resting SBP[a]	122.8 ± 13.1	118.6 ± 13.0	4.2 (1.8 – 6.6)	0.0008
Post-math resting SBP[a]	127.6 ± 13.1	124.3 ± 13.1	3.4 (0.9 – 5.8)	0.007
B. DBP, mm Hg				
Pre-standing	70.5 ± 8.0	67.9 ± 8.0	2.6 (1.1 – 4.1)	0.0007
Pre-math stress	79.3 ± 9.7	74.9 ± 9.6	4.4 (2.6 – 6.2)	<0.0001
Post-standing resting DBP[a]	79.1 ± 9.8	74.8 ± 9.7	4.3 (2.5 – 6.1)	<0.0001
Post-math resting DBP[a]	82.8 ± 9.7	78.4 ± 9.7	4.4 (2.6 – 6.2)	<0.0001

Means adjusted for age and height ± standard deviations are shown. The differences between males and females are given, adjusted for age and height.
[a]Resting SBP/DBP is a 5-minute average of SBP/DBP measured while seated after 5 minutes at rest.
Abbreviations: CI = confidence interval.

4.3.3 GENOTYPING

All adolescents were genotyped with the Illumina Human610-Quad BeadChip (Illumina, San Diego, California). Eight single nucleotide polymorphisms (SNPs) covering the entire region of *CYP17A1* were genotyped: rs619824, rs10883782, rs4919682, rs4919683, rs17115100, rs4919686, rs6163, and rs2486758. Genotyping was conducted at the Centre National de Génotypage (Paris, France). All these SNPs past standard quality control: call rate ≥95% and minor allele frequency ≥0.01, and being in Hardy-Weinberg equilibrium ($p>1 \times 10^{-4}$).

4.3.4 STATISTICAL ANALYSES

Genotype-phenotype association tests were conducted in Merlin (version 1.1.2)[318, 319] under an additive model for the following traits: (1) SBP and DBP reactivity to math, (2) SBP and DBP reactivity to standing, (3) post-standing resting SBP and DBP, (4) post-math resting SBP and DBP. With Merlin, a simple regression model is fitted to each trait, and a variance component approach is used to account for correlation between

observed phenotypes within each sibship. Analyses were done in males and females separately, with age, height and, when appropriate, initial BP included as covariates. BP values outside the mean ± three standard deviations (SD) were excluded. Phenotype heritability was assessed by Merlin 1.1.2 using a variance components model[322]. Previously identified and validated loci were considered significant if their $p<0.05$ and they were in linkage disequilibrium (LD) with and showed the same direction of effect as the previously reported SNPs[234, 235, 238, 262-264]. LD structure of these loci was examined in the Saguenay Youth Study with HaploView 4.2[320] and, when comparing our SNPs to previously identified SNPs not genotyped in our study, with HapMap (release 22)[321]. From these loci, the SNP that had the strongest association with BP reactivity was examined in greater detail. Associations between the top SNP and SBP/DBP reactivity to standing and to math were examined with multivariate analysis of covariance carried out in JMP (version 9.0.0, SAS 2010). Analyses were done in males and females separately, with age, height and initial BP as potential confounders. In additional analyses, we also tested puberty stage and prenatal exposure to maternal cigarette smoking as potential confounders.

4.4 RESULTS

4.4.1 BASIC CHARACTERISTICS OF STUDIED MALES AND FEMALES

The average age of males (n=285) and females (n=311) in this study was 14.5 ± 1.9 years (non-adjusted mean ± SD) and 14.6 ± 1.9 years, respectively. Males had a mean body weight of 60.8 ± 16.1 kg and a body mass index (BMI) of 21.6 ± 4.4 kg/m^2 while females weighed 55.1 ± 11.1 kg and had a BMI of 21.5 ± 3.9 kg/m^2. Males compared with females did not differ by age, body weight or BMI (adjusting for age and, when appropriate, height). Males were taller by 7.5 (6.2 – 8.7) cm (estimated difference [95% confidence interval]) ($p<0.0001$) with an average height of 167.0 ± 10.7 cm compared to 159.9 ± 6.8 cm in females. Males were also at an earlier puberty stage (Tanner 1 – 5), differing by 0.7 (0.6 – 0.9) on the Tanner scale ($p<0.0001$), with a mean puberty stage of 3.4 ± 0.9 in males and 4.1 ± 0.7 in females. Their SBP was similar while being supine, but it was higher throughout the rest of the protocol, differing by 4.8 mm Hg before the math stress. DBP in males

was higher than in females throughout the protocol, with a difference of 4.4 mm Hg before the math-stress (Table 4.1). Males and females did not differ by prenatal exposure to maternal cigarette smoking; the proportion of exposed boys was 0.45 ± 0.03 and of exposed girls was 0.50 ± 0.03 (estimated difference=0.05 [-0.03 – 0.13], p=0.21).

4.4.2 CYP17A1 AND SBP AND DBP REACTIVITY TO STANDING AND MATH STRESS

Six of eight *CYP17A1* variants examined in our adolescent population showed significant associations with SBP and DBP reactivity to math stress in males (p=0.0001-0.03, Table 4.2A) but not females (p=0.2-1.0, Table 4.2B). After Bonferroni correction for multiple comparisons, the associations remained significant in males (p=0.0008 to p<0.04). This was adjusted for 8 comparisons: 2 independent phenotypes multiplied by the number of SNPs tested, which was taken as 4 (since the SNPs were in LD, instead of correcting for the total number of SNPs, we corrected for the number of LD blocks [2] plus the number of SNPs not in LD [2], which equals 4). The associations in males were most pronounced for two SNPs (rs619824 and rs6163) that were in good LD in our sample (D'=0.89 in males and D'=0.88 in females, Figure 4.3) and in HapMap CEU (D'=0.92). At rs619824, each risk allele in males was associated with an increase in reactivity to math stress by 3.9 mm Hg of SBP ($p=8.1x10^{-4}$) and by 2.3 mm Hg of DBP ($p=1.1x10^{-4}$, Table 4.2A) so that major versus minor allele homozygotes showed higher pressor responses to math stress by 8.7 mm Hg of SBP ($p=3.0x10^{-4}$) and 4.8 mm Hg of DBP ($p=1.0x10^{-4}$) in males (Figure 4.4). At rs6163, the effects on SBP and DBP reactivity to math stress were similar (Table 4.2A). In females, neither of the SNPs were associated with BP reactivity to math stress. At rs619824, each risk allele increased BP reactivity to math by 1.0 mm Hg of SBP (p=0.3) and 0.3 mm Hg of DBP (p=0.5) so that major versus minor allele homozygotes varied by only 2.0 mm Hg of SBP (p=0.31) and 0.9 mm Hg of DBP (p=0.42) in females (Figure 4.4). The associations at rs6163 were weaker and also did not reach significance. LD analysis showed that both rs6163 and rs619824 were in good LD (D'=1.0, and D'=1.0, respectively, in HapMap CEU) with rs1004467, a variant in *CYP17A1* previously associated with resting

BP/hypertension in adults[234]. Importantly, both SNPs showed a similar direction of effect as the previously reported SNP[234]. There were no observable differences in either sex for SBP or DBP reactivity to standing. None of the variants were associated with SBP or DBP reactivity to standing or resting SBP/DBP (Table 4.2) in either males or females. These results remained virtually unchanged after additional adjusting for either puberty stage (in males, major versus minor allele homozygotes differed by 8.3 mm Hg [p=0.0007] of SBP and by 4.7 mm Hg [p=0.0002] of DBP) or prenatal exposure to maternal cigarette smoking (estimated difference of 8.8 mm Hg [p=0.0003] of SBP and of 4.8 mm Hg [p=0.0001] of DBP, respectively).

In males, all SNPs were in good LD and showed the same direction of effect except rs10883782 and rs2486758 (Table 4.2A). These loci *decreased* SBP and DBP reactivity to math in males, and were in poor LD with each other in our sample (D'=0.05, Figure 4.3A), suggesting that this may be a separate signal.

Table 4.2. *CYP17A1* and BP reactivity and resting BP in male and female adolescents

				Reactivity to math				Reactivity to standing				Post-math resting BP				Post-standing resting BP			
				SBP		DBP		SBP		DBP		SBP		DBP		SBP		DBP	
SNP ID	**Position**	**Alleles (major/ minor)**	**Major allele freq.**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
A. Males																			
rs619824	**104,571,278**	**C/A**	**0.62**	**3.9**	**$8.1x10^{-4}$**	**2.3**	**$1.1x10^{-4}$**	-0.6	0.5	-0.8	0.1	-0.9	0.46	-0.1	1.00	-0.09	0.87	0.06	0.89
rs10883782	**104,573,922**	**A/G**	**0.77**	**-3.8**	**$3.4x10^{-3}$**	**-1.4**	**$3.2x10^{-2}$**	0.3	0.8	0.7	0.2	-2.9	0.03	-1.9	0.06	-1.7	0.26	-1.2	0.29
rs4919682	**104,574,320**	**C/T**	**0.70**	**3.9**	**$1.2x10^{-3}$**	**2.0**	**$1.2x10^{-3}$**	-0.8	0.5	-0.8	0.2	-1.2	0.34	-0.4	0.71	-1.09	0.44	-0.4	0.74
rs4919683	**104,575,115**	**C/A**	**0.61**	**3.7**	**$7.4x10^{-4}$**	**2.1**	**$3.6x10^{-4}$**	-0.8	0.4	-0.8	0.2	-0.9	0.43	-0.4	0.87	-0.7	0.57	-0.6	0.48
rs17115100	104,581,383	G/T	0.95	3.1	0.2	1.4	0.2	-1.1	0.7	-1.0	0.4	1.7	0.65	0.2	1.00	3.03	0.37	-0.5	1.00
rs4919686	**104,582,239**	**A/C**	**0.67**	**3.4**	**$2.5x10^{-3}$**	**1.7**	**$4.7x10^{-3}$**	-0.5	0.6	-0.5	0.4	-0.6	0.62	0.04	0.91	-0.7	0.55	-0.3	0.89
rs6163	**104,586,914**	**C/A**	**0.61**	**3.9**	**$4.7x10^{-4}$**	**2.0**	**$6.5x10^{-4}$**	-0.7	0.4	-0.7	0.2	-0.5	0.69	-0.1	1.00	-0.3	0.80	-0.4	0.65
rs2486758	104,587,470	T/C	0.80	-1.4	0.3	-0.7	0.3	-0.3	0.8	-0.2	1.0	-0.7	0.66	0.2	1.00	-1.02	0.48	0.2	0.78
B. Females																			
rs619824	104,571,278	C/A	0.62	1.0	0.3	0.3	0.5	0.7	0.3	0.2	0.8	1.5	0.19	0.9	0.33	-0.3	0.81	0.4	0.77
rs10883782	104,573,922	A/G	0.79	-0.9	0.4	-0.5	0.4	0.4	0.7	0.3	0.6	-0.2	0.94	-0.8	0.41	0.6	0.63	-0.7	0.51
rs4919682	104,574,320	C/T	0.71	-0.1	0.9	-0.2	0.8	0.5	0.6	0.2	0.8	0.9	0.48	0.3	0.75	-0.5	0.70	-0.1	0.83
rs4919683	104,575,115	C/A	0.61	0.8	0.4	0.4	0.5	0.7	0.3	0.1	0.8	1.8	0.10	1.5	0.08	0.5	0.64	1.1	0.22
rs17115100	104,581,383	G/T	0.95	2.9	0.4	1.0	0.4	2.6	0.1	0.5	0.7	2.1	0.46	2.1	0.25	0.8	0.82	1.9	0.36
rs4919686	104,582,239	A/C	0.68	-0.2	0.8	-0.1	1.0	0.1	1.0	0.2	0.9	1.3	0.30	1.0	0.27	0.3	0.80	0.8	0.51
rs6163	104,586,914	C/A	0.63	0.3	0.7	0.2	0.8	0.7	0.4	0.3	0.7	1.7	0.16	1.3	0.12	0.4	0.68	1.0	0.20
rs2486758	104,587,470	T/C	0.79	-1.2	0.2	-0.6	0.4	-0.4	0.7	0.08	1.0	-1.2	0.38	-0.5	0.53	0.8	0.52	0.7	0.55

SNP-phenotype association was tested separately in males and females, adjusting for age, height and, for reactivity, pre-challenge BP. β is the effect size on BP reactivity or resting BP in *mm Hg* per allele based on the additive genetic model. SNPs in bold face attained p<0.01.

4.5 DISCUSSION

The results of the current study show that one of the best-established gene loci of resting BP/hypertension in adults, *CYP17A1*, may increase BP reactivity to mental stress in adolescent males but not females.

CYP17A1 has been identified as a locus of hypertension in several adult European and Asian populations[234, 235, 238, 262, 263]. Located on chromosome 10q.24.3[265, 266], the 6.6-kilobase-pair gene consists of 8 exons[267]. *CYP17A1* is involved in several pathways integral to BP regulation. The gene encodes the enzyme cytochrome P-450c17, which has both 17α-hydroxylase and 17,20-lyase activities and plays a key role in steroidogenesis in the adrenal gland and sex gonads[267]. During steroidogenesis, cholesterol is converted to pregnenolone, which is subsequently processed to either mineralocorticoids in the adrenal zona glomerulosa (neither enzyme activity present), and glucocorticoids in the zona fasciculata (17α-hydroxylase activity present), or to sex steroids in the zona reticularis and gonads (both enzyme activities present)[267, 269]. Mineralocorticoids (*eg.* aldosterone) increase sodium and water reabsorption in the kidneys leading to increased blood volume and elevated BP, while glucocorticoids (*eg.* cortisol) control the body's response to stress, including a rise in BP[8]. Sex steroids are also involved in BP regulation[272]; in general, androgens increase vasoconstriction[155] while estrogens enhance vasodilation[273]. Consistent with these functions of *CYP17A1*, missense mutations in this gene cause a form of adrenal hyperplasia characterized by hypertension, hypokalemia, reduced plasma renin activity, mineralocorticoid excess, salt retention and sexual infantilism[275-277].

Our study suggests the involvement of *CYP17A1* in regulating BP reactivity to mental stress but not to a simple physical challenge, such as active standing. These results are consistent with the following: (1) mental stress involves the activation of both the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis[327], whereas active standing involves the activation of the

former mainly[19, 20]; and (2) *CYP17A1* is involved in cortisol production, the final effector of the HPA axis, mediating the physiological response to mental stress [327].

The present study shows that *CYP17A1* is associated with BP reactivity to mental stress in males only. Several mechanisms may explain why the gene exerts greater effects on stress-induced BP response in males than females. Given its androgenic activity, *CYP17A1* has been studied as a potential causal factor in metastatic prostate cancer, which is activated by androgens and inhibited by their absence. In a meta-analysis, the rs619824 allele associated in our study with higher BP reactivity to mental stress was associated with higher risk of prostate cancer[328], suggesting a potential role of increased androgen production. Moreover, prostate cancer relies on signaling through the androgen receptor (AR) for maintenance and progression[329]. A previous study in our population found that functional variation in the *AR* gene was associated with higher BP, notably during mental stress, and increased sympathetic modulation of vasomotor tone[317]. Again, this effect was observed in males only. Thus, the risk *CYP17A1* allele may enhance the BP response in male adolescents through increased androgen production, although variations in *AR* may contribute to the sex difference.

A gain of function mutation in *CYP17A1* would increase the production of both androgens, which increase BP and are effective mainly in males, and estrogens, which decrease BP and are effective mainly in females. However, this would also increase cortisol production in both sexes, which in turn suppresses the production of sex hormones due to its anti-reproductive effects. Although previous studies on the relation of *CYP17A1* polymorphisms with hormone levels in men have produced inconsistent and inconclusive results, genotype appears to influence estrogen levels in premenopausal women[330]. However, these studies were limited to adults and focused on polymorphisms in the 5'-UTR region, unlike our study examining the entire gene. Furthermore, the determination of hormone levels is complicated by methodological issues (*eg.* hormone levels vary with age, ethnicity, anthropometric factors such as BMI, and behaviours such as smoking)[330]. Future studies should determine total and free hormone levels, when appropriate, in adolescence. In addition, it is likely that hormone levels, to the extent they are under

genetic control, are determined by a balance in the activities of a range of enzymes controlled by many genes[330]; therefore, the joint effects of *CYP17A1* and other genes in the steroidogenic pathway require further investigation.

CYP17A1 has also been replicated in the Women's Genome Health Study[264]. Although this appears inconsistent with our results, the population consisted of primarily women in post-menopausal age, when the ovaries cease to produce BP-lowering estrogen and the sex difference in BP and hypertension prevalence disappears.

Our study did not observe significant association between *CYP17A1* and resting BP in either males or females. It is likely that, at this stage, the effect of the gene is detectable only on BP reactivity and its effect on resting BP is not yet detectable. If enhanced BP reactivity contributes to long-term changes to the cardiovascular system, then the gene's effect on resting BP may be seen later in development, as aging progresses. Only after the cardiovascular system is exposed to chronically exaggerated reactivity may this translate into more permanent changes to the cardiovascular system leading to sustained high BP. An alternative explanation is that it might relate to an issue of power. If the gene has a small effect size on resting BP, this requires very large sample sizes to detect. For example, the effect sizes per risk allele reported in previous GWAS were minimal (about 1 mm Hg) and the sample sizes were extremely large (over 130,000)[234, 235].

Finally, both the magnitude and duration of BP responses[120] may contribute to the subsequent risk for hypertension[73, 109-111]. Recurring enhanced BP reactivity increases pressure load on the vessels, heart and kidneys, leading to their structural and functional changes, which in turn may further increase BP and thus contribute to the development of hypertension over time. Given that *CYP17A1* may enhance BP reactivity to mental stress, it may be an early marker of hypertension risk. Substantial overlap exists between the genes that influence BP measured in a clinic, under laboratory stress and during real life. One study estimated that up to 81% of the heritability of clinic SBP and 71% of clinic DBP were attributed to genes that also influenced stress BP during a video game challenge and social stress interview; however

some genetic components specific to each BP measurement also exist[251]. Future studies should reveal the mechanisms through which *CYP17A1* influences BP by investigating its effects on the production of: (1) aldosterone (*eg.* serum aldosterone levels), which causes sodium and water retention leading to volume-dependent hypertension, (2) cortisol (*eg.* cortisol reactivity, morning cortisol), which increases BP reactivity and resting BP, and (3) sex hormones in adolescents (*eg.* serum sex hormones, sexual maturation, puberty timing), which have multiple roles in BP regulation. Interactions between the above steroid hormones should be taken into account, since some hormones are inter-related[330].

In summary, our results suggest that, in adolescent males but not females, *CYP17A1* may enhance BP reactivity to mental stress. Whether this effect contributes to the higher prevalence of hypertension in males than females later in life remains to be determined.

4.6 FIGURES AND FIGURE LEGENDS

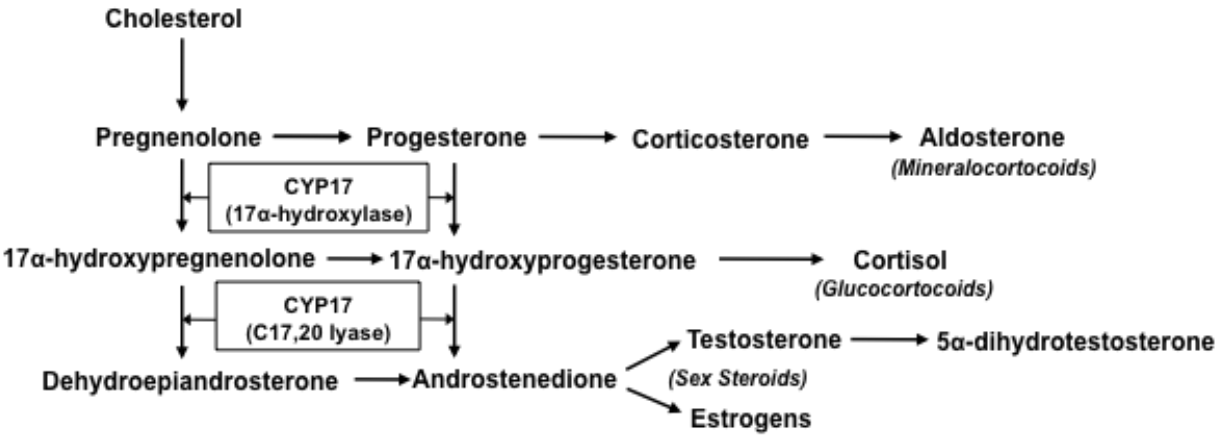

Figure 4.1. Simplified pathway of steroid hormone biosynthesis and the role of CYP17
CYP17A1 encodes the enzyme cytochrome P-450c17α (CYP17) that catalyzes steroid 17α-hydroxylase and 17,20-lyase activities and is hence essential for the synthesis of glucocorticoids (17α-hydroxylase activity) and sex steroids (17,20 lyase activity). Adapted from Molina and Belldegrun 2011[331].

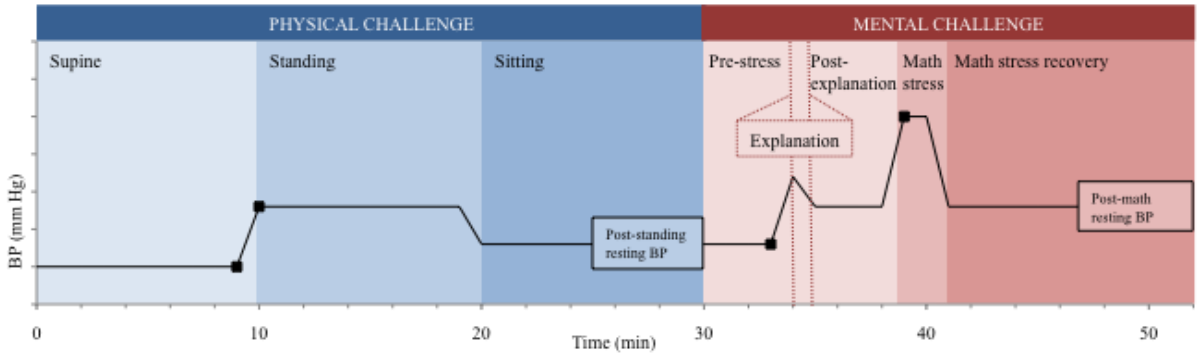

Figure 4.2. The determination of BP reactivity to standing and math stress and post-standing and post-math resting BPs during the cardiovascular protocol
This diagram depicts an example of one-minute BP averages (from continuous beat-to-beat BP recordings) during physical and mental challenges. The physical challenge, active standing, consisted of three periods during which the participant was first supine (10 minutes), then standing (10 minutes) and finally sitting (10 minutes). The math-stress test consisted of a pre-stress period (4 min), an explanation (~1 min), a post-explanation waiting period (4 minutes), math-stress (2 minutes) and math-stress recovery (10 minutes). Black squares indicate the initial and final BP values used to calculate BP reactivity to standing and math stress and the boxes show 5-minute averages of post-standing and post-math resting BPs.

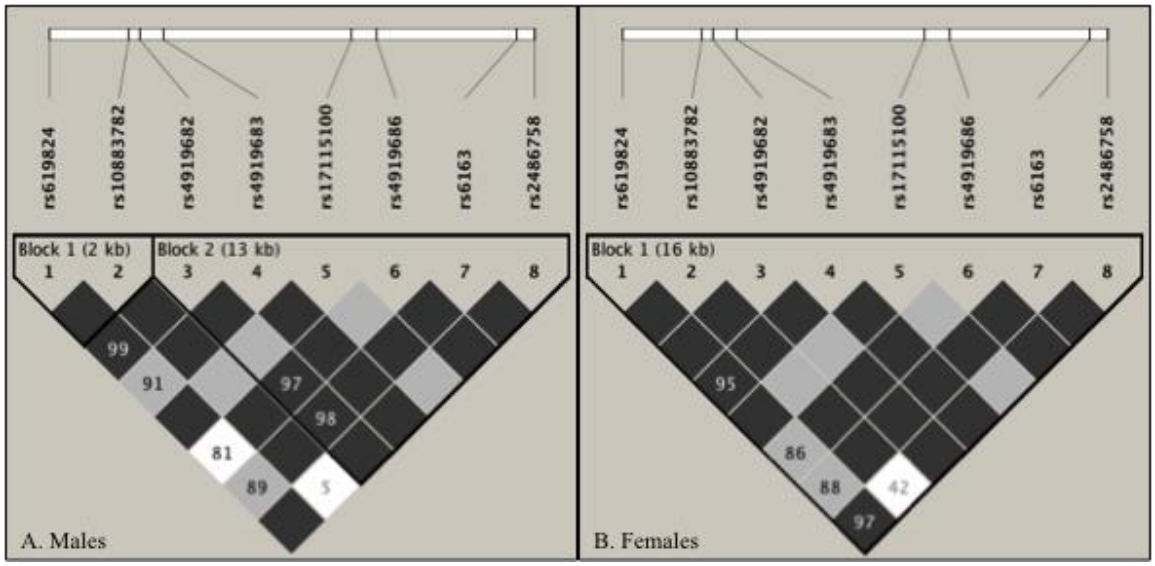

Figure 4.3. The LD structure (D') of eight SNPs in *CYP17A1* associated with BP reactivity to mental stress in males and females

The LD structure (D') of eight SNPs in *CYP17A1* associated with BP reactivity to mental stress in our study population is depicted in males (A) and females (B) (using Haploview 4.2). All SNPs are in good LD, with the exception of rs10883782 and rs2486758, which are in poor LD, especially in males.

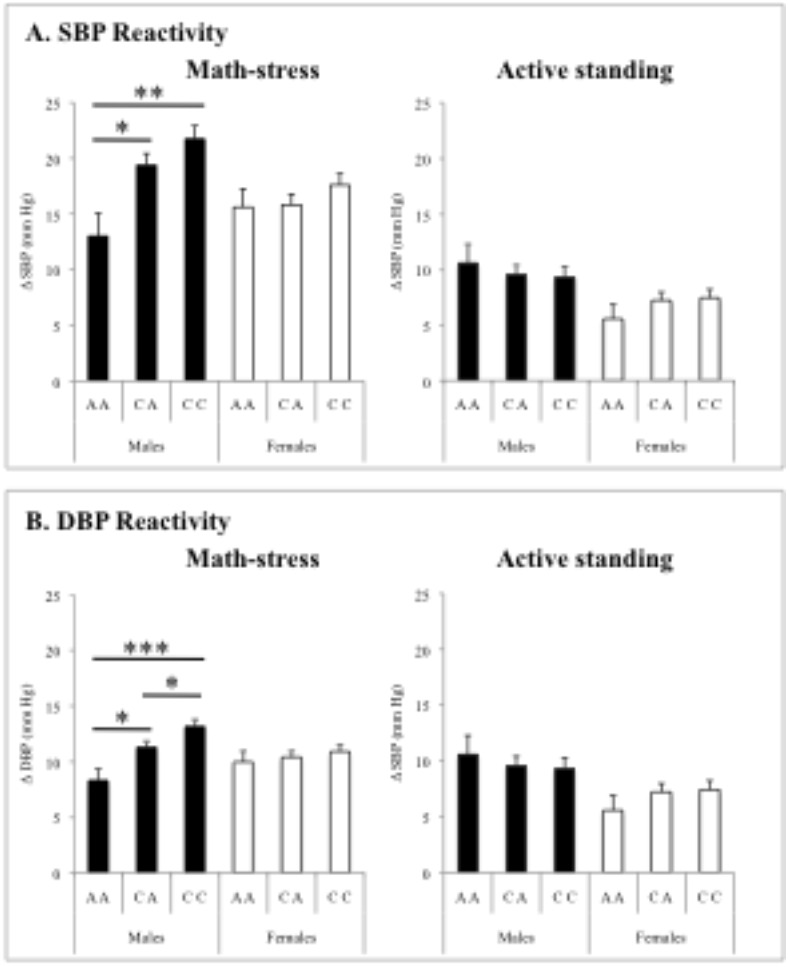

Figure 4.4. ***CYP17A1*** **(rs619824) and BP reactivity to math-stress and active standing in males and females**

Means ± standard error (adjusted for age, height and pre-challenge BP) are presented for SBP (A) and DBP (B). Minor allele homozygotes differed from major allele homozygotes in males but not females, for math but not standing. *p≤0.05, **p≤0.001, ***p≤0.0001

5. GENERAL DISCUSSION AND FUTURE DIRECTIONS

5.1 SUMMARY

In summary, my research has demonstrated that males have greater BP reactivity and slower BP recovery from both active standing and math stress and that, in males only, those with more VF exhibit a greater BP response to standing. Although this sex difference has been previously described in the literature, the relationship between BP reactivity and VF is a novel finding. This is the first to demonstrate that the quantity of VF, independently of the amount of TBF, affects the rise in BP in response to standing. That this relationship is specific to standing, and not math stress, is probably due to different physiological systems involved in regulating responses to these two types of challenges. While a change in posture from supine to standing initially involves mainly SNS activation, mental stress involves a coordinated response between the SNS and HPA axis, resulting in BP increase. Moreover, given that HPA activation is part of the 'fight-or-flight' stress response, about 50% of subjects increase blood flow to their muscles via local vasodilation in the muscle vasculature, to enhance the supply of oxygen and nutrients[120]. This is reflected by a reduction in MSNA and an attenuated BP response to stress because of the reduction in TPR due to local vasodilation.

Alternatively, genetics could help explain the difference in BP responses between males and females. The estimated heritability of hypertension is around 50% and many genes contribute to these complex genetic trait. One such gene is *CYP17A1*, which encodes the enzyme cytochrome P-450c17, which has both 17α-hydroxylase and 17,20-lyase activities and plays a key role in steroidogenesis in the adrenal gland and gonads[267]. Our study demonstrated that this gene, which is involved in synthesis of cortisol, the final effector of the HPA axis, is associated with greater BP reactivity to math stress in boys only. In contrast, it had no effect on BP reactivity to standing (which involves mainly sympathetically-mediated rise in BP). Similarly, it was not associated with resting BP, most likely because its effect size on this variable was too small for our study to detect with our limited sample size. *CYP17A1* is involved

in the steroidogenesis pathway, leading to the production of mineralocorticoids (no CYP17 activity), glucocorticoids (17α-hydroxylase activity) and sex hormones (both 17α-hydroxylase and 17,20-lyase activity). Thus, it is a very likely gene candidate for elevated BP. The end-products of the steroidogenesis pathways, aldosterone, cortisol, estrogen and androgens, are all involved in BP regulation. This is the first study to find an association between *CYP17A1* and BP reactivity to mental stress in an adolescent population. Furthermore, we demonstrated that the gene affects BP in a manner that is sex-specific.

In conclusion, this research on BP reactivity and recovery from physical and mental challenges is useful as it provides additional insight into the mechanisms underlying BP regulation: how these parameters differ between males and females, how they relate to adipose tissue depots and how an important hypertension gene contributes to the observed differences between males and females.

5.2 STUDY LIMITATIONS, STRENGTHS AND FUTURE DIRECTIONS

One of the limitations of the present study is its cross-sectional design, which makes it difficult to determine causality. It would be useful to measure both resting BP and BP reactivity and recovery in the same subjects after a follow-up period, to see how these parameters change over time, and whether the quantity of VF was related to these changes. This would also allow us to determine if enhanced BP reactivity and delayed BP recovery predicted the development of hypertension in subjects after the given follow-up period. Some also question the generalizability of laboratory stress responses to real life settings; however the physical and mental challenges used in this study are meant to mimic those encountered in every day life[57]. Furthermore, there are many factors that are known to influence BP reactivity/recovery so we cannot rule out the possibility that these may have influenced our results. For example, the menstrual cycle affects the hemodynamic response to orthostatic stress in females, and this was not taken into account in our study[332]. Other factors are known to influence cardiovascular reactivity, such as chronic life stress[327], and this was also not taken into account in our study. Similarly, factors that

alter BP recovery include emotion (notably anger), music and exercise[100]. Moreover, measuring circulating levels of catecholamines and corticosteroids would verify the proposed mechanisms underlying BP regulation in response to acute stressors. Finally, the absolute values obtained from beat-to-beat hemodynamics are not as precise as those from 'gold-standard' invasive methods, but they do track fast changes in hemodynamics during experimental protocols, such as the one in this study[21]. Thus, in our study, tracking changes rather that identifying absolute values was more important.

Some argue that using simple change scores (calculating the delta between initial and final BP) to quantify BP reactivity/recovery presents a problem because it assumes that the BP response is linear, when in fact it is dynamic and no linear[56]. Correlations between residualized change scores and simple change scores are known to be high and yield similar findings[68]. However, curve-fitting methods have been proposed as optimal ways of operationalizing BP reactivity and recovery. In the future, we should use curve-fitting approaches to model the beat-to-beat BP data throughout our protocol. This would improve the fit compared to simple change scores. As a result, one could compare the differences in BP responses over the order of seconds, instead of minutes. Additionally, calculating the area under the curve estimates not only the magnitude of the BP response but also the duration of BP elevation. Curve-fitting techniques also improve the ability to capture individual differences reliably[56].

In this thesis, I provided evidence from longitudinal studies that greater BP responses are detrimental given that they may lead to functional and structural changes in the blood vessels, heart and kidney resulting in chronic BP elevation. An alternative interpretation is that too low a BP response is a reflection of non-optimal cardiovascular functioning[333]. It would therefore be interesting to investigate whether non-responders are at increased cardiovascular or metabolic risk and to identify BP-lowering genes in these subjects.

Despite these drawbacks, one of the key strengths of the current study is that is encompasses BP reactivity *and* recovery to both physical *and* mental challenges in adolescent males and females respectively. Moreover, this study relied upon high fidelity phenotyping, notably beat-to-beat BP data

measured continuously throughout the entire cardiovascular protocol and the quantification of visceral fat with MRI data. Given the known differences between males and females, the sex-specific analyses conducted here offer important insights into the mechanisms underlying BP regulation in each sex.

5.3 CLINICAL IMPLICATIONS

Given the links between high BP reactivity in children and adolescents and the development of hypertension in adulthood, strategies to lessen BP responses to stress are imperative. Improved diet and exercise are established ways to lower BP and attenuate BP responses to stressors. These two prevention strategies can lead to weight loss and lower cardiovascular and metabolic risk. While obesity increases sympatho-activation[334], weight loss is associated with a reduction in SNS activity[335]. For overweight and obese individuals, aerobic exercise is better than resistance training at targeting VF and is therefore the most time-efficient and effective exercise mode[336]. Exercise reduces oxidative stress (which causes vasoconstriction and endothelial dysfunction) and improves insulin resistance (which reduces NO production)[337]. One study demonstrated that children who actively commute to school (by walking 1.6 km versus driving) exhibited a dampened BP and stress response to mental stress[71]. This is a viable way for children and adolescents to decrease their BP reactivity to stress. Moreover, exercise is protective against interpersonal stress reactivity in children[338], which is important since social stress is high during adolescence. An alternative prevention method is cognitive restructuring (*eg*. forgiveness). For example, writing about stress can be used as a therapy for patients with both depression/anxiety and cardiovascular disease because it speeds up BP recovery[100]. This also limits rumination, repetitively focusing on stress, which can produce the same sympathetic response as experiencing the stress itself[339]. These are just a few suggested strategies to prevent or reduce exaggerated BP responses to stress in adolescents.

This research highlights key sex differences in BP reactivity and recovery, and how these parameters relate to body-fat distribution and genetic susceptibility to hypertension. In a comprehensive

review by Treiber *et al.*, all 12 studies that assessed associations between stressor-related BP reactivity and subsequent BP elevations in normotensive children and adolescents reported positive results[112]. Given that cardiovascular reactivity and recovery are consistent predictors of future BP status in youth and can be measured in a non-invasive manner, BP reactivity and recovery may be a viable tool for clinicians to identify adolescents at risk for developing hypertension and allow for earlier intervention in the progression of the disease. These results suggest sex differences exist not only in BP adaptation to daily activities, but also in the way visceral and peripheral body fat influence this BP adaptation. Moreover, this research demonstrates that an established hypertension gene (*CYP17A1*) may contribute to the development of hypertension through the regulation of BP in response to mental stress and that, at least in adolescence, this effect may be limited to males. Overall, these results suggest BP reactivity being a more sensitive and earlier marker of enhanced risk for hypertension, particularly in children with excess body fat and "high" risk genetic profile. Also, uncovering genes helps reveal these mechanisms. For example, we still do not know whether *CYP17A1* influences BP through its effect on the mineralocorticoid, glucocorticoid, or sex-hormone pathways. This knowledge would allow for more specific-pathway tailored intervention.

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