The Acute and Chronic Effects of Smoking on Vessel Hemodynamics and Endothelin-1 at Rest and Following Acute Physical Stress

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Submitted: December 15, 2014

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Masters of Science

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ABSTRACT

English

Background: Cigarette smoking has been well established as a major risk factor for cardiovascular disease; however the extent of the underlying vascular dysfunction in young healthy smokers has not been fully established. Arterial stiffening and endothelial dysfunction represent two of the earliest manifestations of smoking-induced cardiovascular damage. Endothelin-1 (ET-1), a potent vasoconstrictor, has been implicated in these processes. While the effect of smoking on arterial stiffness and ET-1 levels has previously been examined at rest, its effect on the ability of the vasculature to respond to increased demands has not yet been investigated. Therefore, the research presented herein aimed to examine the acute and chronic effect of smoking and nicotine exposure alone on arterial stiffness (*Study 1*) and plasma ET-1 levels (*Study 2*) before and after acute physical stress in young healthy individuals.

Methods: Young healthy smokers (n=43) and non-smokers (n=80) underwent the '*arterial stress test*', which involves measurements of blood pressure and arterial stiffness before and after (2, 5, 10, 15 and 20 minutes) an exercise test to exhaustion (maximal oxygen consumption, VO_{2max}) on a treadmill. Cardiorespiratory parameters were obtained throughout exercise using a metabolic cart. Several arterial stiffness and hemodynamic indices were assessed including central and peripheral systolic blood pressure (SBP) and pulse pressure (PP), pulse pressure amplification (PPA), augmentation index corrected for a heart rate (HR) of 75 beats/min (AIx75), subendocardial viability ratio (SEVR), as well as carotid-femoral and carotid-radial pulse wave velocity (cfPWV and crPWV, respectively). Smokers were assessed under 3 conditions (*Study 1 and 2*): a) after 12h smoking abstinence (termed chronic smoking), b) immediately after smoking one cigarette (termed acute smoking), and c) immediately after chewing nicotine gum. Plasma

ET-1 levels were quantified in a subset of 35 smokers and 35 non-smokers pre- and 3 minutes post-exercise using enzyme-linked immunosorbent-assay (*Study 2*).

Results: *Study 1.* Smokers at rest had significantly elevated AIx75, and decreased PPA compared to non-smokers. Smoking a single cigarette dramatically increased central and peripheral SBP, HR, and PPA, as well as significantly lowered SEVR at rest. In response to acute maximal exercise, smokers failed to achieve comparable exercise time and maximal HR as non-smokers, and exhibited a trend for lower VO_{2max}. Furthermore, in response to exercise smokers on all 3 conditions demonstrated lower exercise-induced changes in AIx75 and SEVR compared to non-smokers. After acute smoking, the increase in cfPWV in response to exercise was significantly lower when compared to the chronic condition. Furthermore, chronic smokers exhibited lower area under the curve values during the recovery period for both AIx75 and SEVR. *Study 2.* Post-exercise ET-1 levels were significantly lower than pre-exercise levels in non-smokers and smokers under all 3 conditions. There were no differences in post-exercise ET-1 levels between non-smokers and smokers under all 3 conditions; however the absolute decrease in ET-1 levels was significantly smaller in chronic smokers compared with non-smokers. After acute smoking, smokers had a trend for higher pre-exercise ET-1 levels, as well as a greater decrease in ET-1 post-exercise compared to chronic smoking. The effects of nicotine on the ET-1 response to exercise were intermediate between acute and chronic smoking. The decrease in ET-1 levels observed in non-smokers in response to exercise was significantly associated with exercise induced-changes in inspiratory time, time for a tidal volume cycle, respiratory rate, inspired minute ventilation and peak inspiratory flow.

Conclusions: These findings demonstrate that acute and chronic cigarette smoking leads to an altered endothelial and vessel hemodynamic response even in young healthy smokers. By

incorporating exercise as a vascular stressor in our study, we have taken a novel approach to provide further evidence of dysfunction in smokers, including an altered ET-1 and cardiorespiratory response, as well as a blunted ability of the arteries to respond to increased physical demands. Altogether, we have shown that the *arterial stress test* may serve as a useful tool to identify vascular impairment in young healthy smokers at an early stage before clinical signs of dysfunction become apparent.

French

Introduction: Le tabagisme est un facteur de risque majeur pour les maladies cardiovasculaires; cependant, la dysfonction vasculaire sous-jacente chez les jeunes fumeurs sains n'est pas complètement définie. La rigidité artérielle et la dysfonction endothéliale représentent deux manifestations rapides des dommages cardiovasculaires causés par le tabac. L'endothéline-1 (ET-1), un puissant vasoconstricteur, est impliquée dans ces processus. L'effet du tabagisme sur la rigidité artérielle et les taux d'ET-1 a déjà été examiné au repos, mais son effet sur la capacité des vaisseaux à répondre à des demandes physiologiques accrues n'a pas encore été étudié. Donc, cette recherche vise à examiner les effets chroniques et aigus du tabagisme et de la nicotine sur la rigidité artérielle (étude 1) et les taux plasmatiques d'ET-1 (étude 2) avant et après un effort jusqu'à épuisement chez des jeunes individus sains.

Méthodes: 42 fumeurs et 80 non-fumeurs sains ont subi le '*test de stress artériel*' impliquant des mesures de rigidité artérielle au repos et après (à 2, 5, 10, 15, et 20 minutes) un test à l'effort jusqu'à épuisement (consommation maximale d'oxygène, VO_{2max}). Pendant l'exercice, des paramètres cardiorespiratoires ont été obtenus. Plusieurs indices de rigidité artérielle ont été évalués, incluant la pression artérielle systolique (PAS) centrale et périphérique, la pression pulsée (PP), l'amplification de la PP (APP), l'index d'augmentation ajusté pour une fréquence cardiaque (FC) de 75 battements par minute (ou *augmentation index corrected for a heart rate of 75 beats/minute*, AIx75), le rapport subendocardial de viabilité (ou *subendocardial viability ratio*, SEVR), et la vitesse de propagation de l'onde de pouls carotido-fémorale et carotido-radiale (VPOPcf et VPOPcr, respectivement). Les fumeurs étaient évalués sous 3 conditions distinctes (*étude 1 et 2*): a) après l'abstinence tabagique (12-h, effet chronique), b) immédiatement après avoir fumé une cigarette (effet aigü) et c) immédiatement après avoir

mâché une gomme à la nicotine. Un échantillon de sang prélevé immédiatement avant, et à 3 minutes après l'exercice était évalué pour les concentrations d'ET-1 par immunobuvardage de type Western (*étude 2*).

Résultats: *Étude 1*. Au repos, les fumeurs montraient un AIx75 élevé et une APP diminué comparés aux non-fumeurs. Fumer une seule cigarette aggravaient ces effets en augmentant la FC, la PAS, la APP et en abaissant significativement le SEVR. En réponse à l'exercice maximal aigü, les fumeurs n'atteignaient pas les mêmes durée d'exercice et FC maximale que les non-fumeurs, et présentaient une tendance vers une VO_{2max} inférieure. En réponse à l'exercice suite aux 3 conditions, les fumeurs ont montré une plus petite amplitude des changements d'AIx75 et de SEVR induits par l'exercice comparés aux non-fumeurs. Fumer une cigarette diminuaient significativement l'augmentation de VPOPcf en réponse à l'exercice par rapport à l'abstinence. *Étude 2*. Les taux d'ET-1 après l'exercice étaient significativement plus bas qu'en préexercice chez les non-fumeurs et fumeurs après les 3 conditions. Il n'y avait aucune différence entre les taux préexercice d'ET-1 des non-fumeurs et des fumeurs après l'abstinence, mais après l'exercice ceux-ci étaient supérieurs chez les fumeurs. Les diminutions absolue et relative d'ET-1 étaient significativement plus faibles chez les fumeurs après leur journée d'abstinence que chez les non-fumeurs. De plus, les fumeurs tendaient à avoir des taux d'ET-1 plus élevés au repos, et la diminution absolue d'ET-1 après l'exercice étaient supérieure chez les fumeurs après avoir fumé une cigarette qu'après l'abstinence. La diminution des taux d'ET-1 observés chez les non-fumeurs en réponse à l'exercice était significativement associée avec les changements induits par l'exercice de temps inspiratoire, temps pour un cycle de volume courant, fréquence respiratoire, ventilation minute inspirée et débit maximal inspiratoire.

Conclusion: Ces résultats démontrent que le tabagisme chronique et aigü exercent des effets néfastes sur les artères et l'endothélium même chez les jeunes fumeurs sains. L'intégration de l'exercice comme un facteur de stress vasculaire dans notre étude nous a permis d'adopté une nouvelle approche pour révéler la dysfonction chez les fumeurs, en particulier une réponse modifiée de l'ET-1 et des paramètres cardiorespiratoires, ainsi que d'une capacité inférieure des artères de répondre à des exigences physiques accrues. En conclusion, nous avons également montré que le '*test de stress artériel*' peut servir d'outil à l'identification des altérations vasculaires chez les jeunes fumeurs en bonne santé à un stade précoce avant que les signes cliniques de dysfonctionnement deviennent apparents.

ACKNOWLEDGEMENTS

I would like to acknowledge the help and support from the following individuals, without whom this thesis would not have been possible.

My supervisor, Dr. Stella Daskalopoulou, for her continuous support, and direction throughout the past two years at the Vascular Health Unit. It has been a privilege to be taught and guided by such a knowledgeable and committed supervisor. I am forever grateful for all the opportunities that have been provided for me.

My lab manager, Yessica-Haydee Gomez for her unconditional support over the past two years, and the countless hours spent patiently helping and teaching me.

Robert Doonan and Andrew Mutter, for their involvement in developing many aspects of the SMOKELESS study and recruitment of the majority of subjects included in this study.

All lab volunteers who have helped over the years with subject recruitment, assessment protocols, data input, and blood processing.

Eirini Toli, for her help the ET-1 experiments, analyses and interpretation, and Maxim Gorshkov for his help with the experiments.

Administrative staff, Starr-Ann Coleman, for always keeping me on track.

All the other members of the Vascular Health Unit, as this experience would not have been the same without them.

Our research nurse, Danielle Benoit for her generous help with blood draws for many of our research subjects over the years.

Statisticians, Hassan Behlouli and Susan Scott for their statistical contributions to this project.

My thesis committee advisor Dr. Patricia Tonin, and committee members Dr. Carolyn Baglole and Dr. Simon Rousseau for insight and feedback on my project.

Canadian Institutes of Health Research, for funding the SMOKELESS study.

And importantly, my family, for their unconditional support throughout this degree and all of my endeavors.

ABBREVIATIONS

AIx	Augmentation index
AIx75	Augmentation index corrected for a heart rate of 75 beats per minute
AP	Augmentation Pressure
AUC	Area under the curve
baPWV	Brachial ankle pulse wave velocity
BMI	Body mass index
BP	Blood pressure
$_{C}$DBP	Central diastolic blood pressure
$_{C}$PP	Central pulse pressure
$_{C}$SBP	Central systolic blood pressure
cfPWV	Carotid-fadial pulse wave velocity
CO	Carbon monoxide
CO_2	Carbon dioxide
crPWV	Carotid-radial pulse wave velocity
CVD	Cardiovascular disease
DPTI	Diastolic pressure time index
ECG	Electrocardiogram
ET-1	Endothelin-1
eNOS	Endothelial NO synthase
$FeCO_2$	Fraction of expired CO_2
FeO_2	Fraction of expired O_2
$FiCO_2$	Fraction of inspired CO_2
FiO_2	Fraction of inspired O_2
GEE	Generalized estimating equations
HbCO	Carboxyhemoglobin
HDL	High-density lipoprotein
HR	Heart rate
IPAQ	International physical activity questionnaire
LDL	Low-density lipoprotein
MAP	Mean arterial pressure
Max HR	Maximal heart rate
mRNA	Messenger ribonucleic acid
MUHC	McGill University Health Centre
NO	Nitric oxide
O_2	Oxygen
Peak MET	Peak metabolic equivalent
$_{P}$DBP	Peripheral diastolic blood pressure
$_{P}$PP	Peripheral pulse pressure
$_{P}$SBP	Peripheral systolic blood pressure
$PETCO_2$	Partial pressure of end-tidal CO_2
$PETO_2$	Partial pressure of end-tidal O_2
PEF	Peak expiratory flow
PIF	Peak inspiratory flow
PGI_2	Prostaglandin

PPA	Pulse pressure amplification
PWA	Pulse wave analysis
PWV	Pulse wave velocity
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
RPE	Rating of perceived exhaustion
RR	Respiratory rate
SD	Standard deviation
SE	Standard error
SEVR	Subendocardial viability ratio
Ti	Inspiratory time
TTI	Tension time index
Ttot	Total time for a tidal volume cycle
VDA	Anatomical dead space
VE	Exhaled minute ventilation
VI	Inspired minute ventilation
Vt	Tidal volume
VCO_2	Carbon dioxide production
VO_2	Oxygen production
VO_{2max}	Maximum oxygen consumption
VSMC	Vascular smooth muscle cell
%pred	Percent of age-predicted maximal heart rate

1.0 BACKGROUND

1.1 Cigarette Smoking – A Public Health Concern

Cigarette smoking is the largest preventable cause of death and disability worldwide[1]. Despite substantial declines during the last 30 years, cigarette smoking among adults remains widespread and currently accounts for approximately 440,000 deaths each year in the United States and about 50,000 in Canada[2,3]. The overall mortality among smokers is about 3 times higher than otherwise similar non-smokers, with 50% of deaths caused by smoking-related diseases[1,4]. Life expectancy has been shown to be on average 10 years shorter in smokers than non-smokers[4]. Furthermore, the economic burden of cigarette smoking in Canada includes more than $4.4 billion annually in direct health care costs, as well as loss of productivity[2].

In spite of increased awareness regarding the harmful effects of smoking and the implementation of strict control policies, 16.1%, or approximately 4.6 million Canadians continue to smoke[2]. Young adults comprise the largest proportion of smokers in Canada (20.3% among those aged 20-24, and 21.8% among those aged 25-34)[2] and consume on average 12.7 cigarettes per day[2]. Moreover, the smoking initiation rates remain high at 11.8% among Canadian youth between the ages of 15 and 19[2].

Cigarette smoke is a highly toxic and complex aerosol of over 4,000 identified compounds, including nicotine, carbon monoxide (CO), nitric oxides, reactive oxygen species (ROS), and other harmful metals and oxidants[5]. In addition to its highly carcinogenic effects, smoking leads to a number of pathological consequences within the vascular wall. As a result, extensive research has confirmed a detrimental role for smoking as a modifiable risk factor in the development of atherosclerosis and cardiovascular disease (CVD)[5].

1.2 Cigarette Smoking and Cardiovascular Disease

Smoking increases the risk of all types of CVD, including coronary heart disease, cerebrovascular disease, peripheral artery disease and abdominal aortic aneurysm[6]. Approximately one-third of smoking-related deaths are due to CVD and smoking is the direct cause of 14.8% of all CVD attributable deaths[7,8].

The cardiovascular risks attributed to cigarette smoking increase with the number of cigarettes smoked and with the duration of smoking, and significant risk reduction has been associated with smoking cessation[9-14]. Light smokers are not exempt from increased risk for CVD. The global INTERHEART study established that smoking just five cigarettes per day significantly increased the risk of acute myocardial infarction for both young and older individuals[15].

Despite substantial epidemiological evidence linking smoking with poor cardiovascular outcomes and mortality, the fundamental pathophysiological mechanisms are complex, multifactorial, and still not fully understood. Importantly, many of the smoking-induced cardiovascular manifestations occur very soon after the onset of smoking, and can remain asymptomatic for several decades[5].

The general mechanisms by which smoking leads to cardiovascular damage include alterations in the normal functioning of the endothelium, as well as the structural and dynamic properties of the vascular wall[16]. Therefore, two important early manifestations associated with the underlying pathophysiology of CVD include endothelial dysfunction and arterial stiffening.

1.3 Endothelial Function

The vascular endothelium, through its release of numerous vasoactive substances plays a critical and dynamic role in the continuous modulation of vascular tone[5]. A tightly controlled

balance between endothelium-derived vasodilating and vasoconstricting factors is fundamental in maintaining vascular homeostasis[16]. However, chronic exposure to cigarette smoke compromises the integrity of the vascular endothelium by disrupting this balance, ultimately causing endothelial injury and dysfunction[5]. As such, endothelial dysfunction is most often characterized by an inability to vasodilate in response to vasodilatory stimuli or shear stress[17]. This impaired endothelial state predisposes the vasculature to vasoconstriction, platelet activation, leukocyte adherence, impaired coagulation, thrombosis, and vascular inflammation[5]. Therefore, endothelial dysfunction is considered to be a key initiating event in the pathogenesis of atherosclerosis[5], and has been shown to implicate both nitric oxide (NO) and endothelin-1 (ET-1) in this process[18].

1.3.1 The Role of Nitric Oxide in Endothelial Function

NO is formed in endothelial cells from its precursor L-arginine via the enzymatic action of endothelial NO synthase (eNOS). As a potent vasodilator, NO plays an essential role in the maintenance of vascular tone. NO has also been shown to possess a number of anti-atherogenic properties including the prevention of platelet activation, leukocyte adhesion and migration, oxidation of low-density lipoprotein (LDL), as well as the inhibition of smooth muscle cell proliferation and migration[19]. However, chronic smoking has been shown to disturb this defence mechanism by reducing NO synthesis and bioavailability[5]. The volatile free radicals contained in cigarette smoke lead to the increased generation of ROS, such as superoxide, which can react directly with NO to form peroxynitrite[20]. This not only leads to the inactivation of NO, but peroxynitrite in itself is a highly reactive free radical that contributes to further increases in oxidative stress[21]. Furthermore, smoking is also associated with the uncoupling of eNOS, leading to the endogenous production of ROS, and further reducing NO bioavailability[22]. Overall, this

reduced NO bioavailability impairs vasodilation within the vessel and over time can cause stiffening of the arteries[23].

1.3.2 The Role of Endothelin-1 in Endothelial Function

ET-1 also plays a fundamental role in the regulation of vascular tone, acting as the most potent vasoconstricting peptide[24]. ET-1 is synthesized mainly in vascular endothelial cells through the cleavage of its inactive precursor, big ET-1, resulting in a 21 amino acid peptide that is secreted predominantly abluminally, but also into the circulation[25]. Production of ET-1 is enhanced in response to a number of different stimuli, including hypoxia, tissue injury or stress, and very rapidly exerts its biological effects through the binding to ET_A and ET_B receptors[25]. The vasoconstricting actions of ET-1 are mediated through the ET_A and ET_B receptors found on vascular smooth muscle cells (VSMCs)[25]. However, ET-1 can also mediate vasodilation by binding ET_B receptors found on endothelial cells, triggering the release of NO and prostaglandins (PGI_2)[26]. ET_B receptors also function in the rapid clearance of ET-1 from the circulation mainly within lungs, but also the kidneys[27]. A balance between the ET_A- and ET_B-mediated effects of ET-1 is essential to the overall maintenance of endothelial integrity and vascular homeostasis[28]. Furthermore, NO plays an important role in counterbalancing the vasoconstrictive actions of ET-1 by inhibiting its synthesis in endothelial cells[28].

1.3.3 Acute and Chronic Effects of Smoking on Endothelial Function

Smoking has been shown to cause dysregulation of ET-1 expression and activity[17]. Nicotine and the many other harmful constituents in cigarette smoke, such as tar, CO, metals, and oxidant compounds, have been shown to increase ET-1 mRNA expression in rats[29] as well plasma ET-1 levels in humans, leading to impaired endothelial-dependent vasodilation[30,31]. There is also evidence to suggest that both acute cigarette smoking and nicotine consumption alone both lead

to a rapid elevation of plasma ET-1 in humans[30,32,33]. ET-1 dysregulation has been shown to play a key role in endothelial dysfunction and the development of atherosclerosis[18]. While endothelial dysfunction is typically characterized by reduced NO, there is also accumulating evidence to suggest that ET-1 can also induce endothelial dysfunction by decreasing NO bioavailability[18]. In vivo studies have demonstrated that activation of ET_A and ET_B receptors can promote oxidative stress by increasing ROS production and uncoupling eNOS[18]. Furthermore, ET-1 has been shown to promote vascular inflammation, and induce the expression of adhesion molecules on endothelial cells[34,35]. Clinical evidence further supports a role for ET-1 in this pathological process by demonstrating that dual ET_A and ET_B antagonists can improve NO bioavailability and endothelial dysfunction[18].

Overall, chronic smoking even in young healthy individuals has been associated with a diminished basal vascular tone. Kiowski et al. demonstrated an impaired vasodilator response upon infusion of an NO synthesis inhibitor, as well as low-dose ET-1 in long-term smokers compared to non-smokers[36]. Celermajer et al. observed similar findings whereby cigarette smoking was associated with a dose-related impairment of endothelium-dependent vasodilation in the brachial artery of young healthy adults, suggesting early smoking-induced endothelial damage[17]. Studies have also shown that nicotine exposure alone has also been shown to induce endothelial dysfunction by altering the structural and functional characteristics of VSMCs and endothelial cells[37].

1.4 Arterial Stiffness and Hemodynamics

1.4.1 Pathophysiology of Arterial Stiffness

Arterial stiffness is a general term that is used to reflect the viscoelastic properties or "rigidity" of the vascular wall. The viscoelastic properties of arteries largely depend on the

relationship between elastin, collagen, and VSMCs within the arterial wall[16].

Within the extracellular matrix (ECM), a dynamic and continuous process of production and degradation normally regulates the relative abundance of elastin and collagen within the arterial wall[38]. The elasticity of the large central arteries is the result of a high elastin to collagen ratio within the wall, however this ratio gradually declines towards the periphery as the arteries become more muscular[16]. However, the elasticity of a given arterial segment is never constant, but is dependent on the pressure within the artery[16]. At low pressures elastin fibers absorb the pressure, however at higher pressures the load is transferred towards the inelastic collagen fibers, thereby increasing arterial stiffness[38]. This shift can occur temporarily in response to healthy increases in blood pressure (BP) such as with exercise, however chronic increases in BP can lead to a more permanent decrease in the elastin to collagen ratio[16].

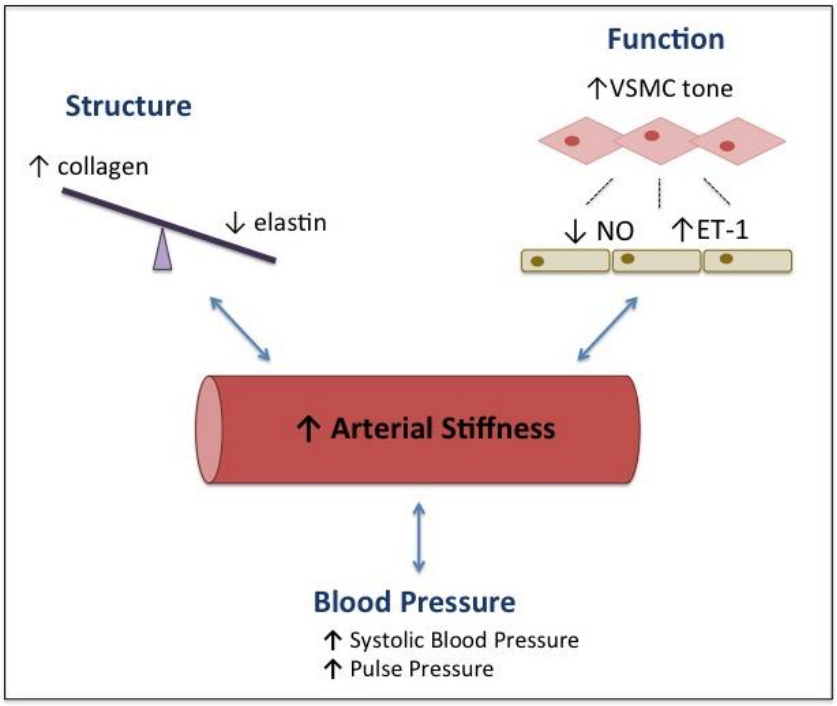
With increasing age, the healthy balance between elastin and collagen becomes disrupted due to a reduction in elastin synthesis and increased degradation, as well as an abnormal overproduction of collagen due to irreversible cross-linking[38]. This leads to a thinning and breakage of elastin fibers, and more than a doubling of collagen content between 20 to 70 years[39] As a result, carotid-femoral PWV (cfPWV), a measure of central arterial stiffness, has been shown to increase several-fold; an average of 0.07 m/s per year from 45 to 65 years, and rising to 0.2 m/s per year after 65 years[40]. These changes occur independently of alterations attributed to atherosclerosis, however various risk factors including hypertension, diabetes, obesity, sedentary lifestyle, high salt intake and smoking can accelerate age-related arterial stiffening[41].

In addition to structural changes within the ECM, arterial stiffness is also influenced by VSMC tone[16]. Smooth muscle tone is regulated extrinsically by the sympathetic nervous system and locally through various paracrine mediators such as angiotensin II, as well as endothelium-

derived NO, PGI_2, and ET-1[16]. As previously discussed, endothelial dysfunction has been implicated in arterial stiffness. However, there is also evidence that arterial stiffness itself can alter endothelial function, and accelerate the stiffening process[42].

Arterial stiffening in itself increases cyclical stresses within the vessel further damaging the elastin fibers, as well as promotes an atherogeneric hemodynamic environment[16]. These combined effects further increase arterial stiffness, creating a self-perpetuating vicious cycle[42].

Figure A. Important Determinants of Arterial Stiffness



Adapted from: Wilkinson and McEniery (2004)[43].

1.4.2 Principles of Wave Reflection

As the left ventricle contracts, it generates a pressure wave within the aorta that propagates forward through the arterial tree toward the periphery. When the forward wave reaches arterial reflection sites, such as bifurcations and high resistance peripheral arterial beds, it is reflected backwards towards the heart. Thus, the observed arterial pressure waveform is a composite of forward wave and the reflected wave[39].

The speed of the forward travelling wave is inversely related to the viscoelastic properties of the wall itself; the more elastic the wall, the slower the velocity. Therefore, in elastic arteries, the velocity of the forward travelling wave is slow, and the reflected wave tends to arrive back at the heart during diastole, increasing central diastolic blood pressure ($_C$DBP) and facilitating myocardial perfusion. However, as the arteries become less compliant, the amplitude and the speed of the forward wave increase, causing the reflected wave to arrive back at the heart earlier, during systole. As a result of this disrupted timing, the reflected wave becomes superimposed on the systolic part of the forward wave, leading to elevated central systolic blood pressure ($_C$SBP), a widened pulse pressure ($_C$PP), as well as increased ventricular load. Furthermore, the favourable coronary artery perfusion during diastole becomes reduced. The clinical consequences of this build up include left ventricular hypertrophy, higher risk of myocardial ischemia and stroke, and increased arterial wall stress leading to the development of atherosclerosis[39].

A characteristic feature of the pressure waveform is its change in shape and amplitude as it travels from the heart to the periphery. While the DBP and mean arterial pressure (MAP) change only slightly throughout the arterial tree, the systolic pressure is greatly amplified as the pressure wave travels from the highly expandable aorta towards less elastic muscular arteries in the periphery. Therefore, both SBP and PP are greater in the arm and leg than they are centrally. This phenomenon was well demonstrated in the Anglo-Cardiff Collaborative Trial II, which showed that central BP could not be reliably inferred from peripheral BP due to the significant gradient between central and peripheral pressures[44]. Therefore, conventional peripheral (brachial) BP is not always a reliable measure of central BP, and thus not as accurate a predictor of cardiovascular health. Moreover, the beneficial reduction in $_C$SBP and $_C$PP with drug

interventions is often underestimated by cuff measurements of peripheral SBP ($_P$SBP) and PP ($_P$PP). For example, the well-known Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) study demonstrated a greater reduction in cardiovascular events in patients treated with calcium channel blockers and angiotensin-converting enzyme (ACE) inhibitors compared with patients treated with a β-blocker plus a thiazide-type diuretic, but they did not observe any differences in the reduction of peripheral BP between the two groups[45]. Subsequently, the Conduit Artery Functional Endpoint (CAFE) study, a branch of the ASCOT study, showed that the decrease in $_C$SBP and $_C$PP was greater in subjects given vasodilator-drugs with respect to those treated with non-vasodilators, despite similar $_P$SBP[46]. Therefore, critical information may be overlooked by only measuring peripheral BP. Furthermore, central BP can offer better prediction for CV risk than peripheral BP alone, and therefore, should be included in the interpretation of vessel hemodynamics and CV risk classification[16].

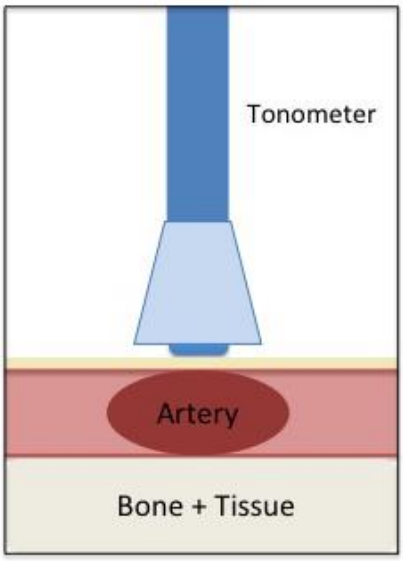
1.4.3 Assessment of Arterial Stiffness

While arterial stiffness has been recognized as a hallmark of the natural aging process for many years, the quantification and evaluation of arterial stiffness as a prognostic indicator of cardiovascular health has only emerged in recent decades. Arterial stiffness is increasingly being recognized as an important cardiovascular risk factor and an independent predictor of all-cause mortality and cardiovascular events[41]. As a result, a large number of measurement techniques and devices have since been developed to measure arterial stiffness non-invasively, including echo-tracking, diastolic pulse contour analysis, magnetic resonance imaging, oscillometric pressure wave detection and applanation tonometry[40,47,48].

1.4.3.1 Applanation Tonometry

Currently, the most simple, reproducible non-invasive technique to measure arterial stiffness is applanation tonometry[49]. It involves placing a small pen-like sensor on the pulse at the wrist (radial artery), neck (carotid artery) and upper leg (femoral artery) to gently compress the artery on an underlying solid structure such as a bone (**Figure B**). A high-fidelity waveform can then be captured allowing for measurement of pulse wave velocity (PWV) and pulse wave analysis (PWA).

Figure B – Applanation Tonometry Technique



Adapted from: Mackenzie et al. (2002)[50]

Pulse Wave Velocity

The measurement of PWV is widely accepted as the most simple, non-invasive, robust, and reproducible method to quantify arterial stiffness[49]. PWV is defined as the speed of the pressure wave travelling along an artery, where higher values of PWV represent increased stiffness. PWV can be calculated by measuring the pulse transit time and the distance traveled between two different sites in the arterial tree: a proximal site and a peripheral, distal site.

Since velocity is equal to distance/time, PWV can be calculated using the following formula:

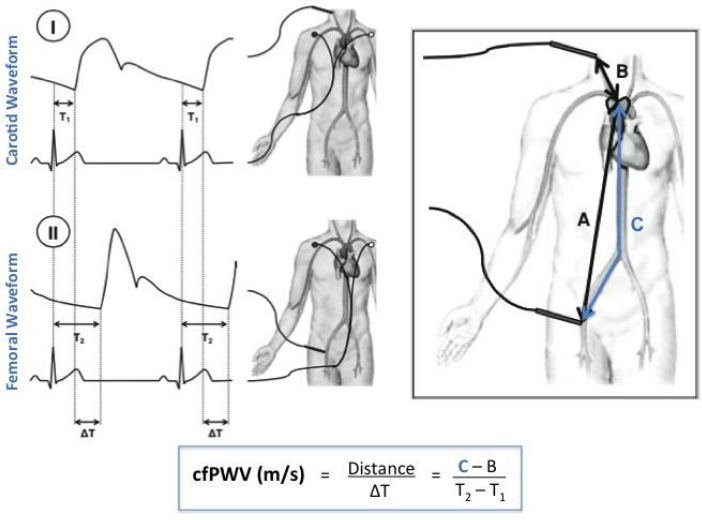
$$\textbf{PWV (m/s)} = \frac{\textbf{Distance between the proximal and distal site}}{\textbf{}\Delta\textbf{T (pulse transit time)}}$$

Measurements of PWV can be obtained at one time where two transducers are used simultaneously on the proximal and distal site, or more commonly, by taking two sequential recordings, and synchronizing them using the R wave of a simultaneously recorded electrocardiogram (ECG). The Sphygmocor system (Sphygmocor, AtCor Medical, Sydney, Australia) (used herein) is based on the latter method, where the transit time between the two sites is calculated as: (time between the R wave and the foot of the proximal pulse) – (time between R wave and the foot of the distal pulse). This method is known as the "foot to foot" method.

The distance between the points is measured over the skin using a tape measure. The distance can either be measured using the "direct" method, by measuring the straight-line distance between the two points, or the "subtraction" method, which involves subtracting the carotid-to-sternal notch distance from the sternal notch-to-femoral (or radial) measurement site distance. The latter method (used herein) has been shown to provide a better approximation of path length since it takes into account the additional distance travelled by the pulse wave in the ascending aorta. This method has been shown to estimate values of path length that are closest to the true MRI-measured arterial path length[51]. As displayed in **Figure C** below, the sternal notch-to-femoral distance can be calculated by way of the umbilicus, as opposed to a direct line, as it more closely follows the path of the descending aorta and the branching of the common iliac artery[52]. Therefore, although different methods exist, standardization within a study is necessary since differences in path length alone can lead to differences in PWV values of up to 30%[49].

PWV can be calculated between a number of arterial sites, but two of the more common measurements include cfPWV, and carotid-radial PWV (crPWV). cfPWV reflects the viscoelastic properties of the aorta and can be considered a measure of central stiffness. cfPWV is considered the "gold standard" measurement, as well as a more clinically relevant measure since it represents the arterial segment that is most prone to stiffening with age and other cardiovascular risk factors[49]. Furthermore, substantial epidemiological evidence has demonstrated cfPWV to be a reliable independent predictor for all-cause and CV mortality in a wide range of patient populations[41,53]. Most notably, the Framingham Heart Study demonstrated that for every one standard deviation (SD) increase in cfPWV, there was a 48% increased CVD risk, independently of other traditional risk factors[41]. crPWV, on the other hand, captures regional artery stiffness in the peripheral muscular arteries (subclavian, brachial and radial arteries). Although it is not considered to have the same predictive value as cfPWV, it has recently been proposed as an alternate and less operator-dependent tool compared to flow-mediated dilation (FMD) for the non-invasive assessment of endothelial dysfunction[54,55].

Figure C – Measurement of Carotid-Femoral Pulse Wave Velocity (cfPWV)



Modified from: Salvi, P (2012)[40].

Pulse Wave Analysis

Applanation tonometry also allows for non-invasive analysis of the central pressure waveform, which can provide a wealth of information related to the timing and magnitude of wave reflection. The most widely used approach involves capturing the arterial waveform at the radial site, and then applying a generalized transfer function to derive the corresponding central pressure waveform. Through this method, accurate estimates of central pressures can be obtained, as well as other indices of central hemodynamics, including the augmentation pressure (AP), augmentation index (AIx), and subendocardial viability ratio (SEVR).

Central pulse pressure ($_{C}$PP) is defined as the difference between the $_{C}$SBP and $_{C}$DBP. $_{C}$PP is dependent on the cardiac output, the mechanical properties of the arteries, and wave reflection. Therefore, $_{C}$PP has widely been acknowledged as a surrogate measure of arterial stiffness[50]. Increased stiffness and earlier wave reflections within the aorta increase the $_{C}$PP due to an increase in $_{C}$SBP and a decrease in $_{C}$DBP. As such, a widened $_{C}$PP is a significant independent predictor of cardiovascular and all-cause mortality[56]. Furthermore, data from the Strong Heart Study found that $_{C}$PP calculated using radial tonometry was more strongly associated with cardiovascular events than $_{P}$PP after adjustment for traditional risk factors[57].

As previously discussed (section 1.4.2), when the forward travelling wave is reflected back towards the heart, it adds to the systolic part of the next outgoing forward wave. The meeting point of the forward travelling wave and the reflected wave is termed the 'inflection point', and the amount of overlap is quantified as the augmentation pressure (AP). Therefore, AP measured in mmHg, corresponds to the part of the $_{C}$PP that is the result of the reflected wave. Since the timing and magnitude of the wave reflection is dependent on arterial stiffness, AP can also be considered an indirect measure of systemic arterial stiffness[40]. The augmentation index (AIx)

provides additional information about the augmentation pressure by taking the $_{C}$PP into account, and is expressed as the ratio between the AP and the $_{C}$PP:

$$\mathbf{AIx\ (\%) = (AP / {}_{C}PP) \times 100}$$

This convention generates a ‘‘continuum’’ of AIx values, ranging from negative to positive, that reflects the timing of the reflected waves[40]. For example, AIx will be represented by a negative value when inflection point occurs after the peak systolic pressure, indicating that the central systolic pressure is not augmented upon the return of the reflected wave. However, if the inflection point occurs before the peak systolic pressure, due to an earlier superimposition of the reflected wave, then AIx will be positive, leading to elevated systolic pressure and ventricular load, as well as decreased coronary artery perfusion during diastole (as demonstrated in **Figure D** below, where P_1 represents the inflection point).

AIx is affected by a number of factors including PWV, the magnitude and timing of wave reflection, arterial tone, the structure of peripheral reflecting sites, as well as age, gender, and height[40]. Heart rate (HR) is also one of the principle factors affecting AIx values. For instance, a 10-beats per minute increase in HR has been shown to cause a decrease in AIx of 3.9%[58]. Therefore, to eliminate the effect of HR, the Sphygmocor system adjusts AIx for a HR of 75 beats per minute, by applying the following formula:

$$\mathbf{AIx75 = AIx - 0.39\ (75 - HR)}$$

Several studies have demonstrated that AIx can significantly predict cardiovascular events and all-cause mortality in a wide range of populations[9-14]. A recent systematic review by Vlachopoulos et al. that compiled the results from many of these studies to include over 5500 patients has demonstrated that for a 10% increase in AIx, the risk for cardiovascular events increased by 31.8% and the risk for all cause mortality increased by 38.4%[59].

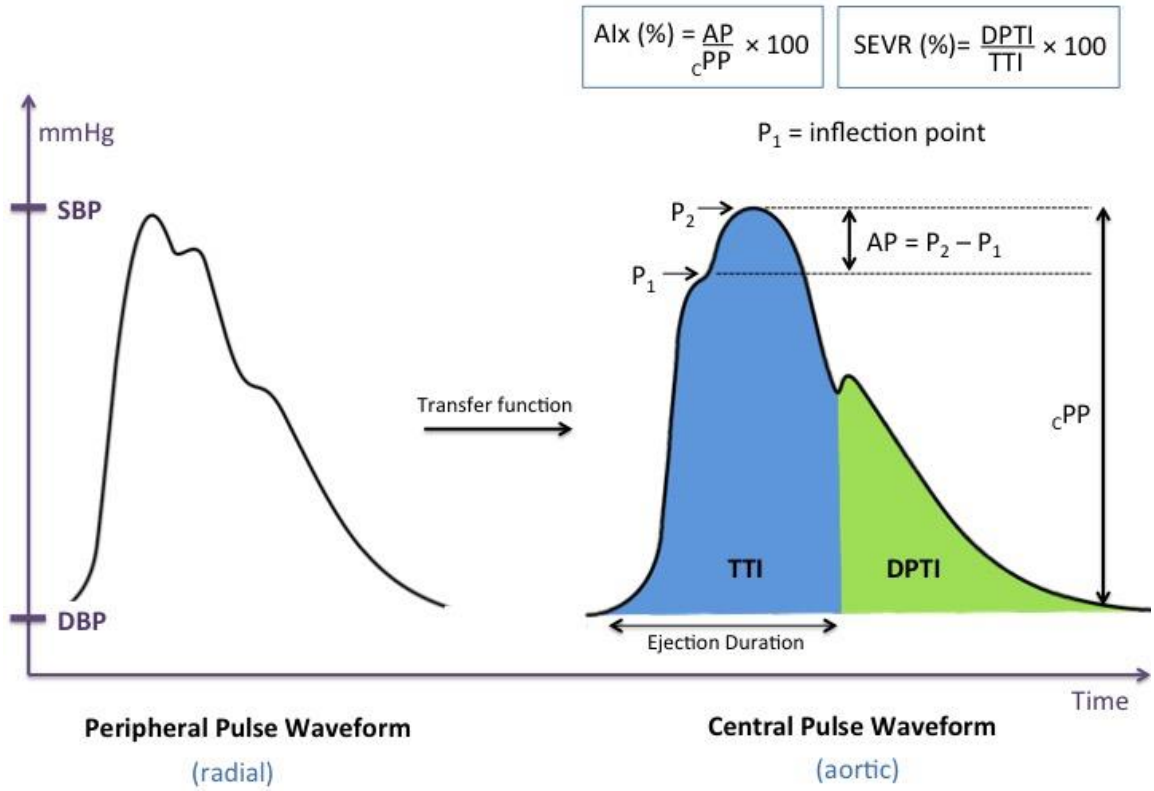
The subendocardial viability ratio (SEVR), also known as the Buckberg index provides a useful index of myocardial oxygen (O_2) supply and demand[60]. It is calculated as the ratio of myocardial O_2 supply represented by the area under the diastolic portion of the central pressure wave (diastolic pressure time index), and O_2 demand, represented by the area under the systolic portion (tension time index)[60], and expressed as a percentage:

$$\textbf{SEVR (\%)} = \frac{\textbf{diastolic pressure time index (DPTI)}}{\textbf{tension time index (TTI)}} \times \textbf{100}$$

SEVR is dependent on the diastolic pressure within the coronary arteries, the left ventricular pressure, as well as the overall duration of diastole[40]. Resting values of SEVR in a young healthy population are normally well over 100%, indicating more than adequate O_2 supply to the heart. However, it is normal to see this ratio fall below 100% with increasing demands during exercise. SEVR indicates under perfusion when below 50%[61].

Lower values of SEVR have been associated with increased arterial stiffness. For example, Guelen et al. demonstrated that cfPWV was associated with estimates of increased O_2 demand as well as decreased cardiac O_2 supply[62]. This could be attributed to the fact that with increased arterial stiffness the reflected wave arrives back earlier during systole, thereby adding to the left ventricular load (increased demand), while no longer contributing adequately to the $_C$DBP (decreased supply)[40].

Figure D – Peripheral and Central Pressure Waveforms and Derived PWA Indices



Pulse Pressure Amplification

$_{P}$PP and $_{C}$PP are independent measures of CV risk, however pulse pressure amplification (PPA), expressed as the ratio of these two measures ($_{P}$PP/$_{C}$PP) offers greater prognostic value[63]. The Partage Study has recently demonstrated that a 10% increase in PPA was associated with a 17% decrease in major cardiovascular events, and a 24% decrease in total mortality[64]. Due to a natural amplification of the pressure wave as it moves towards the periphery $_{C}$PP is lower than the $_{P}$PP for the same mean BP. Therefore, lower values of PPA, due to an increase in $_{C}$PP can indicate unfavorable hemodynamic effects within the central arteries as well higher left ventricular afterload.

Summary Of Arterial Stiffness Indices

PULSE WAVE VELOCITY			
Carotid-femoral pulse wave velocity	cfPWV	Distance/time	Velocity of the pulse wave in the central elastic arteries
Carotid-radial pulse wave velocity	crPWV	Distance/time	Velocity of the pulse wave in the peripheral muscular arteries
PULSE WAVE ANALYSIS			
Central Systolic Blood Pressure	$_{C}$SBP		The maximum BP value in systole
Central Diastolic Blood Pressure	$_{C}$DBP		The BP value in end-diastole
Mean Arterial Pressure	MAP		Mean of the central BP values
Central Pulse Pressure	$_{C}$PP	$_{C}$SBP – $_{C}$DBP	The difference between central systolic and diastolic BP
Augmentation Pressure	AP	$_{C}$SBP – P_1 where P_1 =inflection point	The increase in BP due to the earlier arrival of the reflected wave
Augmentation Index	AIx	(AP/$_{C}$PP) × 100	The percentage increase in BP due to the earlier return of the reflected wave with regard to the PP
Augmentation Index corrected for a HR of 75 beats/min	AIx75	AIx – 0.39 (75 – HR)	The percentage increase in BP due to the earlier return of the reflected wave with regard to the PP adjusted for a HR of 75 beats/min
Subendocardial Viability Ratio	SEVR	DPTI/TTI	Index of myocardial O_2 supply vs. demand
Pulse Pressure Amplification	PPA	$_{P}$PP/$_{C}$PP	Percentage increase in the peripheral PP value with respect to the central PP

Adapted from: Salvi, P. (2012)[40].

1.4.3.2 Validity, Reliability, and Reproducibility

The validity of the Sphygmocor generalized transfer function (used herein) and the consistency of the derived central waveform have been confirmed by direct arterial measurements obtained via catheterization in a wide range of patient groups[65-67]. These studies have all reported excellent correspondence between the non-invasive recordings and directly measured central pressures[65-67].

Furthermore, a high level of inter- and intra- observer reproducibility and repeatability has been demonstrated for PWV and PWA indices of arterial stiffness. These findings have been consistent among various different study populations, of all ages, including healthy and diseased[68-71]. Studies have also demonstrated high reproducibility and repeatability even when relatively inexperienced operators performed the measurements[71].

Other groups have also examined the techniques validity and reproducibility in the context of exercise. For example, Sharman et al. observed no significant differences between non-invasively recorded central waveforms (derived from the radial artery) with invasively recorded central waveforms before, during, or after supine cycling exercise in patients undergoing diagnostic coronary angiography[72]. Furthermore, a study by Holland et al. performed measurements of PWA at rest, during submaximal cycling exercise, and immediately after maximal treadmill exercise on two separate occasions in a healthy population. In spite of significant exercise-induced increases in BP and HR, they reported good reproducibility between visits for all parameters (central BP, AIx, and PPA) at both intensities of exercise[73]. Therefore, these findings support the use of applanation tonometry as a reliable measure of central BP and arterial stiffness both at rest, during, and immediately after exercise.

1.4.4 Acute and Chronic Effects of Smoking on Arterial Stiffness and Hemodynamics

1.4.4.1. Acute Smoking

The effect of acute smoking on arterial stiffness and hemodynamics has been investigated through a number of interventional studies, most of which have been performed in healthy individuals. Apart from transiently increasing both BP and HR, acute smoking has been shown to increase arterial stiffness as measured by AIx, cfPWV, crPWV, and brachial-ankle PWV (baPWV), a measurement that includes both central and peripheral stiffness[74-77]. This acute effect of smoking has been demonstrated in habitual smokers, as well as non-smokers[74,76]. For the most part, non-smokers were found to exhibit a similar increase in arterial stiffness, however Kim et al. found that the acute change in SBP and baPWV in response to smoking two cigarettes was more prominent in smokers than in non-smokers[74]. The increase in arterial stiffness is short in duration (<30 minutes), and most studies have reported the greatest increase within the first 5-10 minutes of smoking[74-78]. Lemogoum et al. demonstrated a significantly increased cfPWV, crPWV, AIx and $_{C}$PP 10 minutes after smoking one cigarette in young healthy current smokers[75]. Mahmud et al. observed similar findings in young healthy smokers demonstrating that smoking one cigarette significantly increased both peripheral and central BP, AIx, and cfPWV within 5 minutes of smoking, compared to age-matched non-smokers[76].

A study by Stefanadis et al. took a more invasive approach in examining the acute effects of smoking through cardiac catheterization in middle-aged smokers. They quantified the aortic pressure-diameter relation, aortic distensibility, pressure-strain elastic modulus, and radial pulsation and found that smoking one cigarette led to an overall decrease in aortic elastic properties within 20 minutes of smoking, further highlighting the significant acute effects of smoking[79].

1.4.4.2 Chronic Smoking

Several studies have also documented the chronic effect of smoking on several indices of arterial stiffness. The effect of chronic smoking on various measures of PWV has been variable, however, appears to be dependent on the arterial segment measured. For example, Levenson et al. and Nguyen et al. demonstrated significantly higher brachial-radial PWV[80] and aorta-femoral PWV[81], respectively in healthy smokers compared to non-smokers. A study by Binder et al. examined the effect of chronic smoking on the stiffness index (SI), a comparable measure to PWV, and observed a higher index in young healthy smokers who had only been smoking for 5-10 years[82]. However, other studies examining cfPWV reported no differences between chronic smokers and non-smokers[83-85]. Chronic smoking has also been associated with increased resting AIx in several studies that compared healthy young smokers and non-smokers[76,83,84,86]. Rehill et al. observed significantly higher AIx in smokers, compared to age and sex matched non-smokers[84]. They also found AIx to be independently correlated with smoking status, as defined by daily cigarette consumption and the number of pack-years (the number of packs smoked per day over the total number of years the person has smoked). Van Trijp et al. observed a similar positive association between AIx and pack-years[87]. Following this trend, Mahmud et al. observed significantly higher AIx in young healthy chronic smokers, compared to non-smokers[76]. They also observed significantly elevated $_{C}$SBP and reduced PPA, despite no differences in $_{P}$SBP in both groups. Altogether, these findings provide evidence that chronic smoking negatively impacts resting vessel hemodynamics, even in younger healthy individuals.

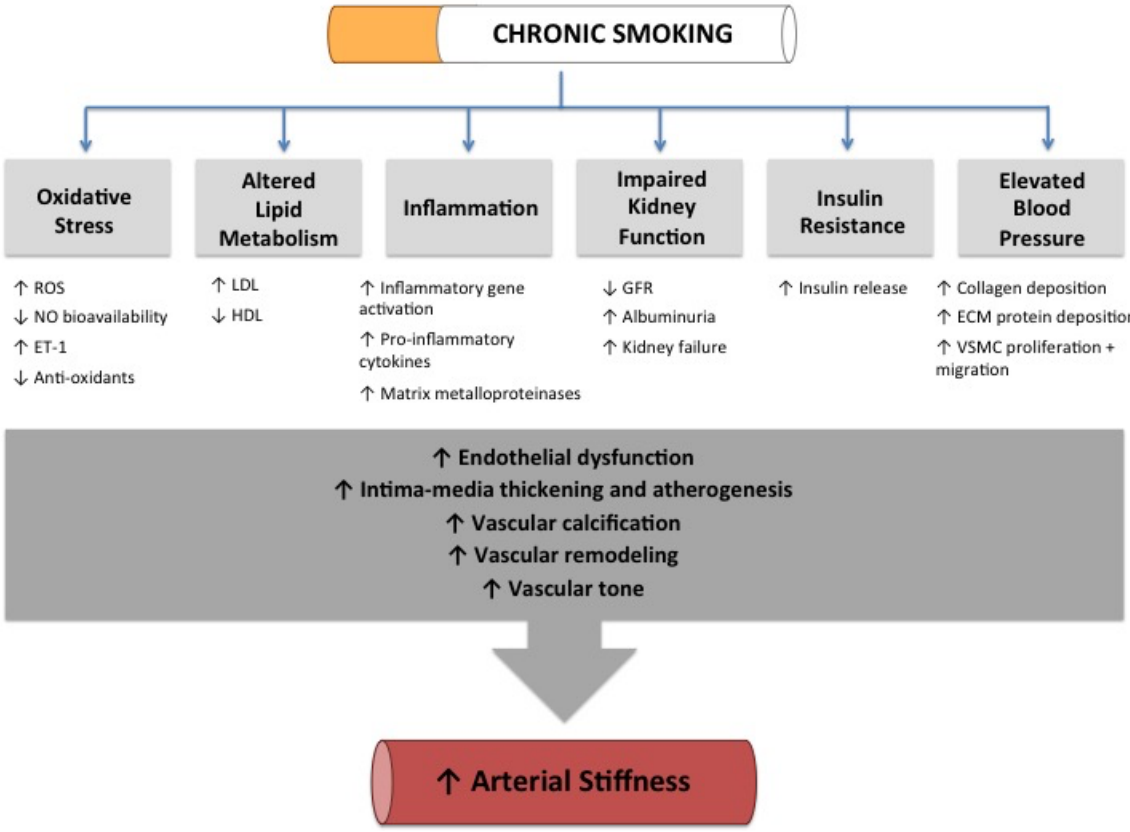
The increase of arterial stiffness and hemodynamics in response to smoking is multifactorial, however these alterations are largely attributed to the atherogenic effects of cigarette smoke.

Smoking can alter *lipid metabolism* both acutely and chronically through its effects on cholesterol transport, and has been shown to alter the lipid profile, increasing LDL and lowering high-density lipoprotein (HDL) levels[6]. These changes contribute to structural modifications within the arterial wall including intima–media thickening and the development of atherogenesis, leading to increased arterial stiffness. Acute and chronic smoking has also been shown to induce *oxidative stress* which also contributes to arterial stiffening[88]. As previously discussed (1.3.1), the free radicals contained within the cigarette smoke increase the production of ROS, which inactivates NO as well inhibits its production through the decreased activity of eNOS within the endothelium. These effects, in addition to reduced anti-oxidant levels, lead to the development of endothelial dysfunction, and correspondingly increased arterial stiffness[88]. This increased oxidative stress has also been shown to increase inflammatory gene activation promoting a chronic *inflammatory state* within the vessel[89]. This leads to increased pro-inflammatory cytokines and activation of monocytes, as well as enhances recruitment and adhesion of leukocytes to the vessel wall[89]. Furthermore, oxidized LDL can further induce the expression of pro-inflammatory cytokines, and other inflammatory mediators in the vascular wall[90]. Numerous studies have demonstrated a strong association between PWV and inflammatory markers, such as C-reactive protein, interleukin 6, interleukin 1-β, tumor necrosis factor-α, and von Willebrand factor[91]. Inflammation also causes vascular calcification and release of matrix metalloproteinases, which degrade important components of the ECM and lead to vascular remodeling[92]. The effects of nicotine alone contribute to this smoking-induced inflammation by increasing neutrophil migration, leukocyte adhesion, and activating dendritic cells with the vascular wall[93]. Other known mechanisms for increased arterial stiffness in chronic smokers

include *insulin resistance*, and diminished *kidney function* (decreased glomerular filtration rate), as result of increased collagen accumulation and calcification of the central arteries (**Figure E**)[88].

Smoking can also induce acute and chronic alterations in HR and BP that adversely affect arterial stiffness. These effects are mediated primarily by nicotine. For example, nicotine increases the levels of circulating catecholamines, augments sympathetic outflow, and causes a long-term reduction in vagal drive[94,95]. Even in young smokers this sympathetic overdrive can lead to chronic elevations of HR and BP[95]. This generates increased tension within the vessels that leads to increased collagen deposition, an altered ECM protein deposition, as well as the proliferation and migration of VSCM cells[16]. Such remodeling of the vessels has been shown to directly increase arterial stiffness (**Figure E**)[16].

Figure E. Mechanisms of Chronic Smoking-Induced Increases in Arterial Stiffness



Adapted from: Doonan et al. (2011)[88] from our group

1.5 Acute Aerobic Exercise as a Vascular Stressor

1.5.1 Cardiovascular Response to Acute Exercise

The cardiovascular system has remarkable capacity to accommodate increased demands during acute physical stress. At rest the skeletal muscle receives less than 20% of total cardiac output, however, during maximal exercise more than 80% of cardiac output can be directed towards the skeletal muscle to meet the increased metabolic requirements[96]. This significant increase in blood flow to working muscles is part of a highly integrated process that involves a dramatic increase in cardiac output, as well as a significant redistribution of blood flow[96].

In general, cardiac output increases with O_2 uptake[96,97]. In the case of incremental exercise to exhaustion (used herein) the cardiac output increases linearly with exercise intensity, but then eventually reaches a plateau upon exhaustion[96]. This increase in cardiac output occurs through changes in both HR and stroke volume[96]. In young healthy individuals maximal exercise can results in a 5-fold increase in cardiac output, a 3-fold increase in HR, and a 2-fold increase in stroke volume[97].

The central nervous system responds early in exercise to decrease vagal tone and increase sympathetic activation of the heart[97]. This causes an immediate and rapid increase in HR. Thereafter, the HR continues to increase linearly in proportion to the exercise intensity, enabling further increases in cardiac output[98]. The HR typically begins to plateau as maximal exercise is approached. By definition, the maximal HR (max HR) should occur at the peak of exercise. In general, the max HR decreases with age (Max HR = 220 − Age)[97]. The HR response is influenced by several other factors, including exercise modality, baseline physical activity, blood volume, medications, as well as smoking status[99]. Stroke volume increases during the early stages of exercise and during incremental exercise, reaches a plateau at workloads equal to approximately 40% of maximal exercise capacity in healthy untrained athletes[96]. The increase in

stroke volume is due to the combined effects of increased venous return due to pumping action of the skeletal muscle, as well as sympathetic stimulation of the heart muscle causing more forceful ejections of blood[97].

During exercise there is a significant redistribution of the cardiac output, which is accomplished by vasoconstriction of inactive tissue circulation and simultaneous vasodilation in the active muscle beds[100]. As a result, relative blood flow is reduced by half to the kidney and splanchnic circulations, and is increased 4-fold to the heart and 10-fold to exercising skeletal muscles, while maintaining a constant relative blood flow to the brain[97].

The rise in muscle blood flow produced by exercise is called exercise hyperemia[96]. One of the main mechanisms for hyperemia includes local vasodilation, which is mediated through vasoactive substances in the local tissues, but also in the endothelium of the surrounding vessels. For example, shear stress, resulting from a rapid increase in blood flow during exercise, stimulates the release of NO and PGI_2 from the endothelium[98]. This causes relaxation of the VSMCs and dilatation of the vessel. The elevation in shear stress not only stimulates NO release, but also activates NO production[101]. Therefore, both increased NO bioavailability and increased NO release contribute to significant vasodilation during exercise. This leads to an overall decrease in total peripheral resistance. Interestingly, the higher the intensity of exercise, the greater the vasodilatory stimulus and thus a greater drop in peripheral resistance[98].

Similarly, vasoconstriction also plays a very important role in the redistribution of blood flow by increasing resistance in the vessels feeding the splanchnic circulation and other non-active internal organs. This mechanism not only allows for the shunting of blood to the exercising muscles, but also functions to maintain adequate MAP despite significantly decreased peripheral resistance[98]. Circulating ET-1 has been shown to play a key role in this regulation, by

causing vasoconstriction through its action on VSMCs. The release of ET-1 during exercise is driven by changes in shear stress within the vessel, hypoxia, and the levels of endogenous factors, such as angiotensin II, norepinephrin and vasopressin[102]. Maeda et al. demonstrated that acute exercise causes tissue-specific changes in the expression of precursor ET-1 mRNA in rats, and that these alterations may function to increase cardiac output and allow for redistribution of blood in response to exercise[103]. Further confirming a role for ET-1 in the exercise-induced redistribution, this same group demonstrated that the natural decrease in blood flow to internal organs, and corresponding increase to exercising muscles was abolished by the administration of an ET_A-receptor antagonist[100]. ET-1 can also trigger vasodilation in response to exercise through its action on the ET_B receptors in the lungs, causing the release of NO and PGI_2. However, the levels of ET-1 in the vasculature during exercise remain intricately counterbalanced by NO, in order to maintain the necessary balance between vasoconstriction and vasodilation throughout the vasculature for adequate blood flow distribution during exercise[103].

Overall, BP changes during exercise are a reflection of cardiac output and change in vascular resistance. For example, SBP increases dramatically with exercise as a function of increased cardiac output and an increase in vascular resistance in the non-exercising vasculature (renal and splanchnic circulations), while DBP is typically slightly lower, reflecting decreased peripheral resistance in response to significant vasodilation in the exercising muscle beds[98].

1.5.1.1 Arterial Stiffness and Acute Exercise

Acute exercise also elicits a number of structural alterations within the arterial wall, leading to overall changes in arterial stiffness during exercise. While several studies have investigated the effect of acute exercise on both central and peripheral arterial stiffness, many of the results appear conflicting. However, this may be attributed to the fact that many of these studies have

applied different exercise modalities, different methodologies for assessing arterial stiffness in varying locations throughout the arterial tree, as well as different measurement time points.

An earlier study at the Vascular Health Unit has demonstrated a significant increase in cfPWV, AIx75 and $_{C}$PP, as well as a significant decrease in SEVR immediately after exercise, measured by 2 or 5 minutes[104]. However, these levels subsequently decreased (or increased in the case of SEVR) towards their baseline values by 10 and 15 minutes. Naka et al. observed the time course of acute changes over a longer period of time (60 minutes) following maximum treadmill exercise in healthy subjects by simultaneously measuring upper and lower limb PWV using an oscillometric method at 1-minute intervals[105]. Immediately after exercise (3 minutes) upper limb PWV was 35% higher than the pre-exercise level, however dropped rapidly thereafter, approaching resting levels by 60 minutes. However, lower limb PWV did not demonstrate this same acute elevation post-exercise, but rather decreased below baseline, only returning towards resting levels towards 60 minutes. Similarly, Rakobowchuk et al. examined central and peripheral stiffness in response to sprint interval exercise (single sprint vs. repeated sprints), as early as 2 minutes post-exercise, and then continuously over a 60-minute recovery period[106]. Central PWV was increased immediately following both the single and repeated sprint interval sessions and returned to baseline resting levels by 20 minutes. In contrast, lower limb (femoral-dorsalis pedis) PWV was reduced immediately following both exercise sessions and returned to resting levels by 44 minutes of recovery. While these studies have all examined arterial stiffness in response to exercise modalities involving both lower and upper limbs, others have also examined the response of arterial stiffness to exercise involving only the lower limbs such as cycling. For example, Tordi et al. demonstrated a differential arterial response in the upper and lower limbs after acute cycling, with significantly decreased PWV in the exercising lower limbs,

while non-significant differences were found in the non-exercising upper limbs[107]. Similarly, Sugawara et al. performed a study where they only exercised one leg on cycle ergometer and found significantly lower femoral-ankle PWV in the exercising limb 2 minutes post-exercise, but not in its non-exercised counterpart[108]. Altogether, these findings suggest that arterial stiffness is differentially affected by acute exercise in the upper vs. lower limbs, and is also dependent on the exercising limbs and modality involved (cycling vs. treadmill).

Other studies that have only measured indices of arterial stiffness at later time points post-exercise have contributed to our understanding of the changes in arterial stiffness that occur during the recovery period after an acute bout of exercise. For example, Kingwell et al. observed significantly lower central (aortic-femoral PWV), and lower limb (femoral-dorsalis pedis) PWV 30 minutes after a single bout of moderate intensity cycling[109]. They did not study any earlier time points, but were able to show that values returned to resting levels by 1 hour post-exercise. Comparable to these findings, Hefferman et al. measured central and peripheral arterial stiffness at 20 minutes after a similar protocol of cycling exercise, and observed decreased central (cfPWV) and peripheral (femoral-dorsalis pedis) PWV[110]. Therefore, during the recovery period post-exercise arterial stiffness values tend to decrease towards (or below) their resting values.

The changes in arterial stiffness after acute exercise have been shown to occur independently of MAP, suggesting that these changes occur as a result of intrinsic changes in the properties of the arterial wall[109,110]. The most likely mechanism relates to alterations in the loading of elastin and collagen within the arterial wall. In response to changes in VSCM tone, the load within the arterial wall may shift from the more extensible elastin fibers to the collagen fibers, thereby making the arterial wall less compliant[109,110]. This could explain the increased central arterial stiffness that has been observed in response to acute exercise. In turn, these changes within the

arterial wall may revert back during the recovery phase leading to a decrease in arterial stiffness. Other possible mechanisms for increased arterial stiffness post-exercise include an acute impairment in endothelial function, vasoconstriction in response to elevated ET-1 levels, and increased circulating catecholamines[111,112]. On the contrary, the decreased arterial stiffness observed in the exercising limbs immediately after exercise may still reflect the vasodilatory state of the arteries feeding the exercising muscle beds.

1.5.1.3 Respiratory Response to Acute Exercise

As with the cardiovascular system, the pulmonary system also responds in a highly coordinated manner during exercise to meet the greatly increased metabolic demands. In response to increased O_2 uptake and carbon dioxide (CO_2) output by the exercising muscles, minute ventilation (VE) responds immediately to maintain an optimal composition of alveolar gas[96]. Impressively, the partial pressures of O_2 and CO_2 in the blood leaving the lung are kept at almost resting levels during maximal exercise[96]. VE increases as a function of the number of breaths per minute (respiratory rate, RR), and the volume of air moved in and out of the lungs during each breath (tidal volume, Vt) (VE = RR × Vt). Once the Vt increases to approximately 50% to 60% of the individual's vital capacity, the Vt reaches a plateau, and further increases in minute ventilation occur as a function of increased RR. VE is very much dependent on the intensity of exercise. During maximal exercise, healthy individuals can increase VE over 10- to 15-fold[97].

During exercise, the relationship between exercise intensity and O_2 consumption is linear until the point at which the ability to continue exercising becomes limited by the body's ability to deliver and utilize O_2 in the exercising tissues[99]. This point of limitation is defined as the maximal O_2 consumption (VO_{2max}). Factors influencing VO_{2max} can be represented by the Fick

equation where VO_{2max} = maximal cardiac output × [arterial O_2 content – venous O_2 content][96]. Therefore, VO_{2max} becomes physiologically limited by the ability of the cardiopulmonary system to deliver the O_2, but also the ability of the exercising muscles to extract O_2. As a result, VO_{2max} is widely recognized as a useful and reproducible measure for assessing the functional limitations of the cardiopulmonary system, as well as aerobic capacity[113]. The higher the VO_{2max} the more efficient the cardiopulmonary system is at meeting the increased demands of the working muscles, and therefore the greater one's aerobic capacity. The most widely used criterion for confirming attainment of VO_{2max} during exercise is a leveling off or plateau in O_2 consumption, despite an increasing rate of exercise[113]. VO_{2max} can be expressed in absolute terms (L/min) or as relative to the person's weight (mL/kg per min).

Exercise also leads to significant changes in the respiratory exchange ratio (RER), which is defined as the ratio of CO_2 production to O_2 consumption (VCO_2/VO_2). This ratio is metabolically significant as it can be used to determine the types of fuel substrates being used for the production of energy (fat vs. carbohydrates). Resting RER values typically range between 0.7 and 1 suggesting primarily fats are being utilized. However during exercise, carbohydrate utilization increases leading to far greater CO_2 elimination. As a result RER rises above 1 and can reach values as high as 1.1-1.3 during maximal exercise[114].

With exercise, there are also significant alterations in the end-tidal partial pressures of O_2 and CO_2, the fraction of expired and inspired O_2 and CO_2, as well as changes in the inspiratory time and the peak inspiratory and expiratory flow rates. Methods exist that allow for the quantification of these changes during cardiopulmonary stress testing and will be discussed further in Methodology (4.4.2).

1.5.2 Acute and Chronic Effects of Smoking on Exercise Capacity

The adverse effects of smoking on the cardiopulmonary response to acute exercise have been well documented. Numerous studies are in agreement that chronic smoking leads to overall impairments in exercise tolerance, aerobic capacity and an altered HR response[115-117]. Large studies comparing chronic smokers to non-smokers have demonstrated significantly worse exercise performance, revealed by slower running times during incremental stress testing[118] or increased time to complete a set distance[119,120]. It was also demonstrated that the exercise performance was directly influenced by smoking status (ie. the daily cigarette consumption and number of years smoked). This reduced exercise performance has also been shown in smokers as young as 22 years of age[121]. Additionally, several studies have demonstrated lower VO_{2max} in smokers in their chronic state (abstinence) after exhaustive exercise when compared to non-smokers[115-117,122]. Interestingly, Sandvik et al. performed a longitudinal study over 7 years and demonstrated a significant decline in physical fitness and lung function in smokers over time, compared to non-smokers[123].

Smoking is also known to affect the ability to increase HR in response to exercise[124]. This attenuated response has been termed chronotropic incompetence, which is defined as the failure to achieve 85% of an age-predicted target HR during a maximal exercise test[124]. Chronotropic incompetence is associated with a loss of autonomic balance, and has been shown to have meaningful prognostic value for cardiovascular health[95]. The Framingham Heart Study observed a strong and dose-related association between smoking status and chronotropic incompetence, and showed that smokers who manifested chronotropic incompetence were at higher risk for cardiovascular events and mortality[124].

Acute smoking can further impair the cardiorespiratory response. A study by Hirsch et al.

evaluated exercise capacity in smokers after an abstinence period from smoking, as well as after acute smoking (3 cigarettes/hour for 5 hours)[122]. They observed significantly lower VO_{2max}, anaerobic threshold, and O_2 pulse after acute smoking, compared to their abstinence condition. The additional impairments in response to acute smoking have mainly been attributed to the CO and nicotine within the cigarette. Even at very low concentrations, CO binds hemoglobin with very high affinity (250 times greater than O_2) leading to the formation of carboxyhemoglobin (HbCO). This not only reduces the capacity of O_2 to bind hemoglobin, but it also results in a significant leftward shift of the oxyhemoglobin dissociation curve thus reducing the unloading of O_2 in the tissues[114]. This decline in O_2 availability has been shown to have negative implications in the cardiorespiratory response to acute exercise. For instance, a study examining the effects of CO exposure alone have demonstrated that CO itself contributes to significant impairments in VO_{2max}[116]. However, this study also found that exercise duration after acute smoking was significantly lower than CO exposure alone, suggesting that damaging compounds in cigarette smoke other than CO might influence exercise capacity[116]. In contrast to these studies, others have reported no significant differences in physical fitness of VO_{2max} between smokers and non-smokers in populations of highly active individuals[125,126], suggesting that physically active individuals are less susceptible to the detrimental effects of smoking on exercise capacity.

1.5.3 Assessment of Cardiopulmonary Capacity

Cardiopulmonary exercise testing is an important tool that allows for the assessment of the cardiovascular and pulmonary response to acute exercise. This assessment can be used to evaluate the functional capacity of the cardiopulmonary system, but importantly can also be used to identify physiological limitations of either system by examining alterations in the normal physiological response to exercise[98]. Acute exercise using a ramped incremental protocol to

exhaustion on a treadmill is the most widely used and clinically applicable method for the assessment of cardiopulmonary capacity[96]. The Bruce Protocol is one of the most widely adopted and validated protocol for exercise testing[96], and involves a progressive increase in workload in stages of 3 minutes through changes in both speed and incline. Modified versions of this protocol exist for individuals with lower exercise tolerance, as well as more active young healthy individuals (used herein)[127].

Using a metabolic cart, "breath-by-breath" measures of several respiratory variables can be obtained. This method samples gas flow and concentration over each breath to obtain inspired and expired volumes of O_2 and CO_2. These quantities can then be combined with the timing of each breath to determine several important respiratory parameters including VO_2, VCO_2, VE, Vt, and RR among others (described in Methods 4.4.2). Continuous measurements of HR can also be obtained using a 3- or 12-lead ECG in conjunction with the metabolic cart.

1.5.4 Arterial Stress Test

In young healthy individuals, smoking is less likely to hinder functioning at rest, however the harmful effects of smoking may become more apparent during physical stress. Acute maximal exercise has wide-ranging effects on the cardiovascular and pulmonary systems and therefore has the potential to elicit abnormalities that would otherwise not be present at rest. This has been best demonstrated with the cardiac stress test, which is commonly used to assess the 'cardiac reserve' and has the potential to detect cardiac abnormalities that are not clinically obvious at rest. In a similar manner, assessing the 'vascular reserve' or the ability of the arteries to respond to increased demands during acute physical stress could also capture critical information about vascular health.

In order to assess the 'vascular reserve', the Vascular Health Unit has developed an arterial

equivalent to the cardiac stress test termed the '*arterial stress test*' (used herein). This involves measuring arterial stiffness before and at several time points immediately after acute maximal treadmill exercise. As such, the *arterial stress test* provides a large amount of quantifiable information such as PWA and PWV, and allows for comparisons between and within subjects under highly controlled and reproducible conditions. The implementation of the *arterial stress test* in our studies provides a useful model to identify underlying limitations due to smoking that would otherwise not be present at rest in young healthy individuals.

2.0 OBJECTIVES

The overall aim of this thesis is to contribute new evidence towards our understanding of the process through which smoking affects vascular function, both at rest and following acute physical stress. This has been carried out through two studies.

The specific objectives are:

Study 1

1) To estimate differences in vessel hemodynamics at rest among young healthy non-smokers and current smokers under the following 3 conditions:
    a. After 12 hours abstinence from smoking (to estimate the effects of chronic smoking)
    b. Immediately after smoking one cigarette (to estimate the effects of acute smoking)
    c. Immediately after chewing nicotine gum (to estimate the effects of nicotine alone)
2) To estimate differences in exercise capacity and the 'vascular reserve' after acute maximal exercise between non-smokers and current smokers under the above 3 conditions

Study 2

1) To estimate differences in resting plasma ET-1 levels between non-smokers and current smokers under the above 3 conditions
2) To estimate differences in the response of plasma ET-1 levels to acute maximal exercise between non-smokers and current smokers under the above 3 conditions

3) To estimate the extent to which exercise-induced changes in ET-1 correlate with cardiorespiratory changes during exercise between non-smokers and current smokers under the above 3 conditions

3.0 HYPOTHESES

Study 1

1) Resting vessel hemodynamics will be more compromised in chronic smokers compared to non-smokers. Among smokers, vessel hemodynamics will be most compromised after acute smoking, while exposure to nicotine gum will be intermediate between acute and chronic smoking
2) Smokers will have diminished exercise capacity and 'vascular reserve' in response to acute maximal exercise

Study 2

1) ET-1 levels will be higher in smokers than non-smokers. Among smokers, ET-1 levels will be highest after acute smoking, while the response to nicotine will be intermediate between acute and chronic smoking
2) After acute maximal exercise, the increase in ET-1 levels will be higher in smokers compared to non-smokers. Among smokers, the increase in ET-1 will be the highest after acute smoking, while the response to nicotine will be intermediate between acute and chronic smoking
3) Exercise-induced changes in ET-1 will be highly correlated with changes in cardiorespiratory parameters in response to exercise

4.0 METHODS

Both studies included in this thesis are components of the larger Canadian Institute of Health Research (CIHR)-funded SMOKELESS Study (Quantification of the effect of SMOKing on artEriaL stiffnESS) (Principle Investigator: Dr. Stella Daskalopoulou, MOP#102626). The study includes a cross-sectional and longitudinal component (follow up assessments at 6-month intervals for 2 years). This thesis comprises the cross-sectional component of the study.

The study was approved by the ethics and scientific reviews boards of the McGill University Health Centre (MUHC) (*Appendix A*) and conforms to the standards set by the previous and most current version of the Declaration of Helsinki[128]. The study was conducted entirely at the Vascular Health Unit (Montreal General Hospital, MUHC).

4.1 Research Subjects

4.1.1 Recruitment

Recruitment for the study was done through notices on local McGill University and Montreal area websites in both French and English (*Appendix B*). Interested subjects were given a short eligibility questionnaire to fill out and return to the research staff for determination of eligibility (*Appendix C*).

4.1.2 Inclusion/Exclusion Criteria

Included in the study were young healthy smokers and non-smokers between the ages of 18 and 45. Exclusion criteria were previously diagnosed cardiovascular disease, congenital heart diseases, traditional cardiovascular risk factors (diabetes mellitus, hypertension, dyslipidemia), metabolic syndrome, renal disease, respiratory diseases, inflammatory/autoimmune diseases, obesity [body mass index (BMI) $\geq$30 kg$\times$m^{-2}], pregnancy, and use of recreational drugs or

history of ever-smoking. Furthermore, subjects who were acutely ill, on cardioprotective medications or taking nicotine replacement or smoking cessation medication were excluded. Also excluded from the study were smokers whose pack-year smoking history was ≤ 1 calculated as (number of packs smoked per day) × (years as a smoker) where 1 pack is considered as 20 cigarettes.

4.2 Study Design

Non-smokers were assessed once while smokers were assessed in 3 different sessions, which were conducted: 1) after 12-h abstinence from smoking (termed chronic smoking); 2) immediately after smoking one cigarette (termed acute smoking); and 3) immediately after chewing a piece of nicotine gum. The 3 sessions were performed in a randomized order, with at least a 48-h interval to allow for full recovery of exercise capacity, and at the same time of the day to reduce circadian variations. On each of these days, smokers were asked to abstain from smoking for 12-h to prevent any potential acute effects of smoking. On the smoking session, smokers were asked to smoke one full, standardized cigarette (nicotine content: 1.1-2.4mg) within 5 minutes under supervision in a designated area outside of the Montreal General Hospital. On the nicotine session, they were asked to chew one piece of nicotine gum (2mg) using the "chew and park" method[129]. They were asked to repeat the process of chewing and parking between the gums and cheek (approximately 1 minute each) for a total of 30 minutes.

4.3 Procedures

4.3.1 Written Consent

All study subjects voluntarily signed a document of informed consent, which disclosed the purpose, assessment protocols and associated risks (*Appendix D).* All documents of written

consent were securely stored to maintain confidentiality, and each subject was assigned a unique subject identification number.

4.3.2 Baseline Assessment

Prior to the assessment, all subjects were asked to abstain from: i) caffeine and alcohol intake for at least 12-h, ii) any strenuous exercise for 24-h, and in the case of smokers iii) smoking for at least 12-h. Upon arrival, research staff confirmed subjects had followed all the instructions, and the time since the last intake of caffeine or alcohol, last cigarette smoked, and most recent bout of physical activity was recorded on the assessment datasheet (*Appendix E).*

Assessments were all performed in the same temperature (22±1°C)- and humidity (60±5%)-controlled environment. Upon their first visit, subjects completed an extensive questionnaire, including sociodemographics, past medical history, current medication use, and lifestyle habits, such as current smoking status and smoking history (*Appendix F*). To assess baseline physical activity levels all subjects also completed the short International Physical Activity Questionnaire (IPAQ), which has been validated in young adults (*Appendix G*). Height and weight were measured, as well as waist and hip circumferences. BMI was then calculated as weight (kg) × height $(m)^{-2}$, and the waist:hip ratio as waist (cm) divided by hip (cm).

4.3.3 Exercise Protocol

To induce physical stress, subjects underwent a supervised incremental treadmill exercise protocol to volitional exhaustion on a treadmill (Trackmaster, FullVision Inc., Newton, KS, USA). All subjects followed a modified version of the Bruce protocol[127] that has been validated for use in young healthy individuals (*Appendix H*). After a 3-minute warm up stage, the speed and incline changed in stages of 3 minutes, progressively becoming more difficult until maximal effort was achieved.

All subjects were equipped with a mask positioned over the nose and mouth in order to measure the volume and gas concentrations (O_2 and CO_2) of inspired and expired air during exercise (discussed further in section 4.4.2). As a result, subjects were asked to refrain from talking during exercise, and instead, communicate non-verbally with hand signals.

The Borg Rating of Perceived Exertion (RPE) Scale (*Appendix I*) was used to subjectively gauge each subject's feelings of effort, strain, discomfort, and/or fatigue experienced during the exercise test[113]. At each 3-minute stage of exercise, subjects were presented with the scale and asked to point to a rating (6 through 20) of their perceived exertion separately in their legs and lungs.

Subjects were deemed to reach maximal exercise capacity when i) they could no longer continue the exercise protocol, ii) they reached at least 90% of their age-predicted maximal HR, and iii) RER >1.1 was reached[113]. If the following criteria were not met, the assessment was excluded from the final analysis. Max HR, time to exercise completion, VO_{2max}, and max RER was recorded. Immediately post-exercise, subjects rested in a supine position.

4.4 Measurements

4.4.1 Arterial Stiffness and Hemodynamic Measurements

All measurements of arterial stiffness and hemodynamics were performed in a resting supine position. Subjects were instructed to remain still and refrain from talking, or falling asleep during the recordings.

Peripheral (brachial) BP was measured manually using cuff sphygmonometry (HEM-705CP, Omron Corp., St. Charles, IL, USA) according to the Canadian Hypertension Education Program guidelines[130] on the opposite arm used for arterial stiffness measurements. The first measure was

discarded to reduce the 'white coat effect', and the average of the two subsequent measures was recorded.

Arterial stiffness measures and central hemodynamics were obtained non-invasively through applanation tonometry using the Sphygmocor system. A high-fidelity micromanometer (Millar Instruments, Houston, TX, USA) placed on the tip of handheld pen-like tonometer was gently applied perpendicularly on the surface of the skin overlying either the radial, carotid, or femoral pulse so as not to occlude the artery. Data was collected directly into a dedicated laptop computer, and assessed visually to ensure the best possible recordings had been obtained. Data was also checked to make sure the recordings were in accordance with the device's internal quality control system. The arterial stiffness testing was performed by well-trained operators at the Vascular Health Unit who had received specialized training from a Sphygmocor representative. Arterial stiffness measurements were obtained before (resting) and at several time points post-exercise (2, 5, 10, 15 and 20 minutes).

Pulse Wave Velocity

PWV measurements, including cfPWV and crPWV were obtained as measures of stiffness of the central elastic arteries, and the peripheral elastic arteries, respectively. Using the Sphygmocor device, a sequence of pulse waves are recorded at a proximal and distal site within the arterial tree e.g. radial and carotid arteries for crPWV, and the femoral and carotid arteries for cfPWV. Since the pulse waves are recorded separately at each site, they are synchronized with the R-wave of the ECG recording. Using an intersecting tangent algorithm to identify the "foot" of the waveform, the Sphygmocor software calculates the transit time between the two sites is calculated as: (time between the R wave and the foot of the proximal pulse) – (time between R-wave and the foot of the distal pulse). Then the "foot-to-foot" distance is calculated between a

minimum of 10 simultaneously recorded waveforms and averaged to obtain the pulse time delay between the two recording sites. The distance travelled by the pulse was measured manually over the skin using a tape measure and approximated using the 'subtraction method' which subtracts the distance between the *sternal notch and the carotid site* from the distance between the *femoral site and sternal notch* (cfPWV) or the *radial site and sternal notch* (cfPWV). PWV was then calculated using the measured distance between the two sites and the calculated time delay between the foot of the proximal and distal waveforms as previously described in Background 2.4.2.2.

Resting measurements of crPWV and cfPWV were performed until two acceptable PWV readings (SD<10%) were within 0.5 m/s of each other. The points were marked with a pen to ensure the same points were used for the measurements post-exercise. Due to time constraints post-exercise, only one reading was obtained for each measure of cfPWV and crPWV. At 2 minutes post-exercise, only cfPWV was assessed, however both cfPWV and crPWV were assessed at all other time points post-exercise (5, 10, 15 and 20 minutes) (**Figure F**). At the same time points, peripheral BP was measured in the contralateral arm.

Pulse Wave Analysis

Obtaining PWA measurements requires only the radial pulse where 10 seconds of sequential waveforms were acquired to generate a single "averaged" peripheral waveform. This waveform was calibrated using the previously obtained peripheral BP measurement. Using a generalized transfer function, the system generates an ascending central pressure waveform that is used for the estimation of several parameters including $_{C}$SBP and $_{C}$DBP, $_{C}$PP, MAP, AP, AIx, AIx75, and SEVR. Resting measurements of PWA were taken until there were two readings with values of

AIx, AIx75 and AP that were within 4% of each other with an operator index >85. Single PWA measurements were then performed post-exercise at 5, 10, 15, and 20 minutes (**Figure F**).

4.4.2 Cardiorespiratory Measurements

A metabolic cart (Ergocard, Medisoft, Sorinne, Belgium) was used during the exercise protocol to obtain breath-by-breath measures of several respiratory parameters including:

Inspiratory time	**Ti**	Duration of the inspiratory phase (seconds)
Total cycle duration	**Ttot**	Total time for a cycle (seconds)
Respiratory rate	**RR**	Number of breaths per minute of breathing (breath/min)
Exhaled minute ventilation	**VE**	Volume of gas exhaled in one minute of breathing (L/min)
Inhaled minute ventilation	**VI**	Volume of gas inhaled in one minute of breathing (L/min)
Tidal volume	**Vt**	Volume of air inhaled or exhaled in a single breath (L)
Fraction of expired O_2	**FeO_2**	Fraction of exhaled air that is O_2 (%)
Fraction of expired CO_2	**$FeCO_2$**	Fraction of exhaled air that is CO_2 (%)
Fraction of inspired O_2	**FiO_2**	Fraction of inhaled air that is O_2 (%)
Fraction of inspired CO_2	**$FiCO_2$**	Fraction of inhaled air that is CO_2 (%)
Oxygen consumption	**VO_2**	Amount of O_2 consumed by the lung in one minute (L/min)
Carbon dioxide production	**VCO_2**	Amount of CO_2 exhaled by the lung in one minute (L/min)
Peak Inspiratory Flow	**PIF**	Maximum speed of inspiration (L/s)
Peak Expiratory Flow	**PEF**	Maximum speed of expiration (L/s)
Partial pressure of end-tidal O_2	**$PETO_2$**	Partial pressure of O_2 at the end of an exhaled breath and a surrogate for alveolar O_2 tensions (PaO_2) (mmHg)
Partial pressure of end-tidal CO_2	**$PETCO_2$**	Partial pressure of CO_2 at the end of an exhaled breath and a surrogate for alveolar CO_2 tensions ($PaCO_2$) (mmHg)
Anatomical dead space	**VDA**	Volume of inspired air that does not mix with the gases already present in the alveoli (mL)
Respiratory Exchange Rate	**RER**	Ratio between the amount of CO_2 produced and O_2 consumed in one breath (VCO_2/VO_2) (unitless)

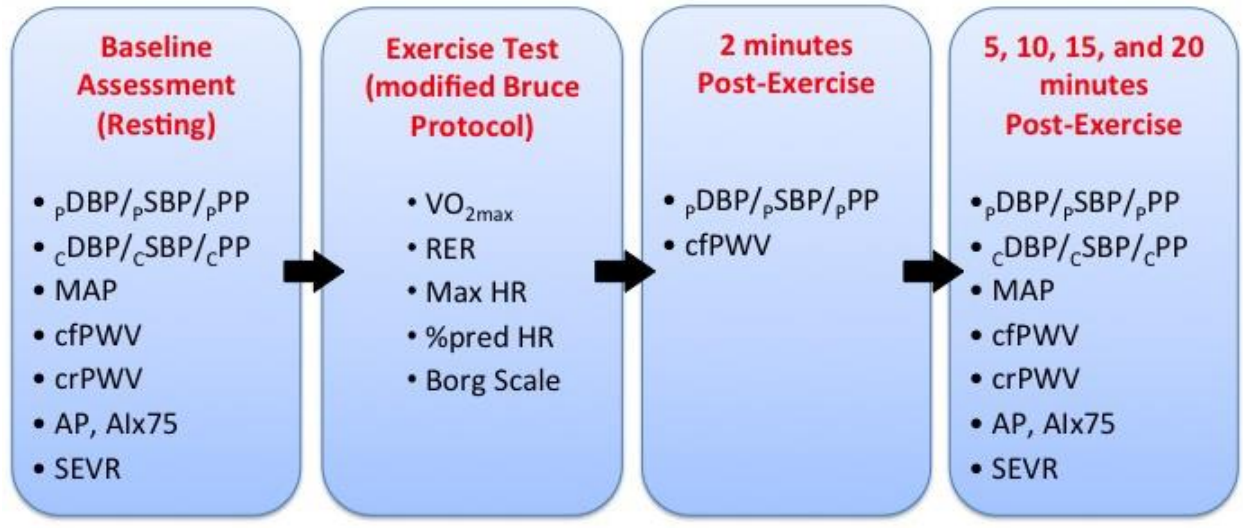
To ensure the accuracy these measures, the gas analyzers and the flow meter of the metabolic cart were calibrated before each test. Before starting the exercise, resting

cardiorespiratory measurements were collected in an upright position over a 1-minute period of normal breathing.

VO_{2max} was identified at the point of leveling off (plateau) in O_2 consumption, despite an increasing work rate of exercise[96]. VO_{2max} was normalized to the subject's weight and analyzed as mL/kg per minute. Peak metabolic equivalents (peakMETs) were then determined by dividing the calculated VO_{2max} by the resting metabolic rate (one MET) of each subject, as calculated by the Harris-Benedict equation using height (cm), weight (kg) and age (years): male=66.4730 + 5.0033(height) + 13.7516(weight) – 6.7550(age); female=655.0955 + 1.8496(height) + 9.5634(weight) – 4.6756(age)[131].

During exercise, HR was continuously monitored via a 3-lead ECG (Medcard, Medisoft, Sorinne, Belgium) connected to the metabolic cart. The max HR upon exercise cessation was recorded (**Figure F**). The percent of the predicted Max HR (%predMaxHR) was also calculated as follows: [Max HR ÷ (220-Age)] × 100.

Figure F – Summary of Time Points for Arterial Stiffness, Hemodynamic and Cardiorespiratory Measurements



4.4.3 Saliva Collection

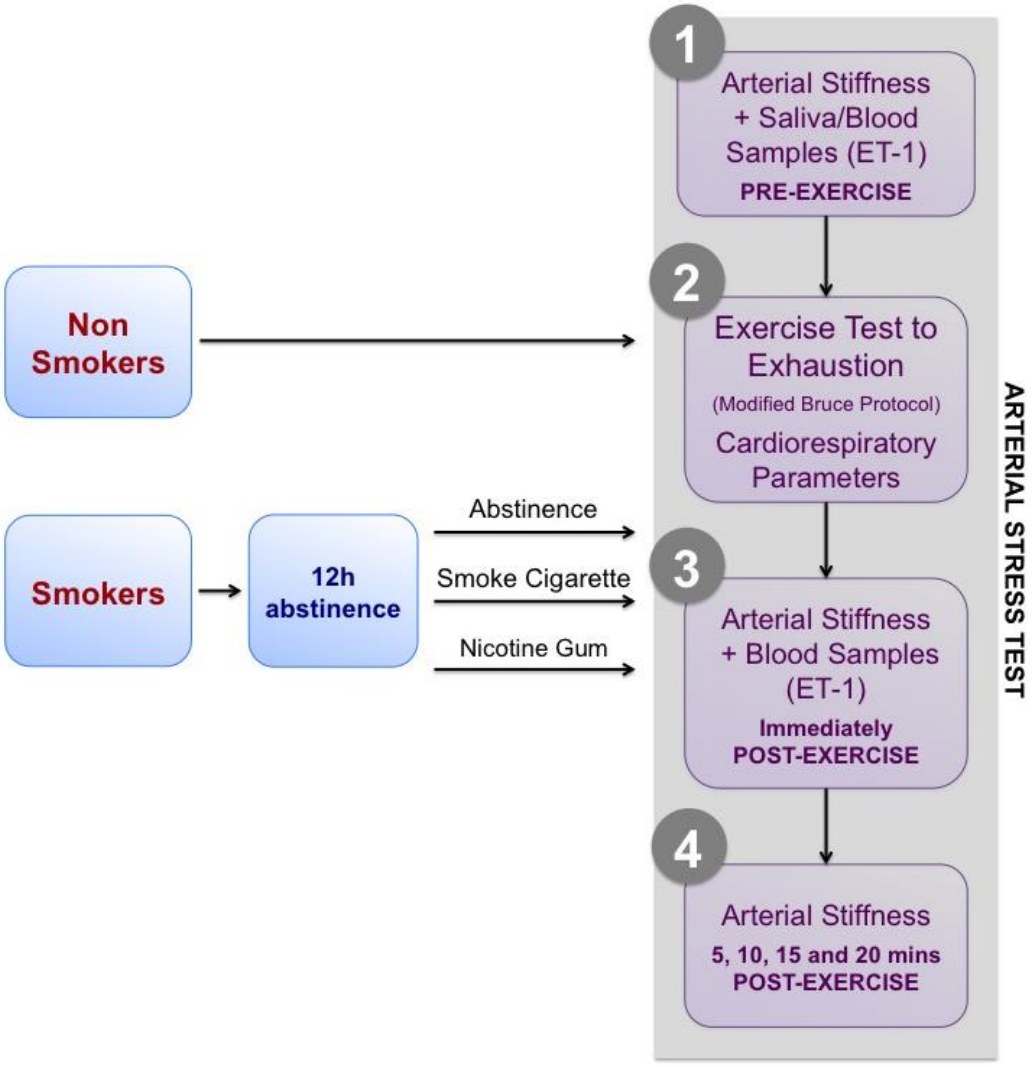
All subjects provided a small sample (5 mL) of saliva in collection vial (Sarstedt, Newton, NC, USA). At the conclusion of the study (December 2015), these samples will be analyzed to measure the levels of cotinine, the principle metabolite of nicotine. Studies have previously demonstrated a strong association between self-reported smoking status and cotinine levels in saliva[132,133]. Therefore, these samples will be used to verify that all smokers have abstained from smoking for at least 12 hours before the assessment. As well, the levels will be tested in non-smokers to ensure they have not been exposed to high levels of second-hand smoke.

4.4.4 Blood Collection

A peripheral venous catheter (Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) was introduced and well secured on the forearm. The subject was required to rest for 30 minutes afterwards to ensure that any acute reaction from the puncture had subsided before blood collection, including acute alterations in ET-1 levels. Blood was then drawn after all measurements of arterial stiffness had been completed before starting exercise (10 mL), and at 3 minutes post-exercise (10 mL). After centrifugation (3000 revolutions per minute x 10 minutes), plasma was processed and stored at -80°C until analysis.

The plasma aliquots were used to quantify plasma ET-1 levels for *Study 2*, and will be available for future analyses when the study is completed. ET-1 levels were quantified in duplicate using a commercially available irisin enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA). The mean minimum detectable dose was 0.087 pg/mL. According to the manufacturer, inter-assay variation was <8%, while intra-assay variation was <4%.

Figure G – Summary of SMOKELESS Study Protocol Components



4.5 Statistical Analyses

Study 1

Descriptive statistics were used to present baseline characteristics. An analysis of normality of the continuous variables was performed using Kolmogorov-Smirnov test. A comparison of categorical variables (sex) between smoker and non-smokers was performed using a chi-squared test.

To estimate differences in the immediate response of vessel hemodynamics to exercise, the relative change between pre- and post-exercise (2 minute or 5 minute) values was calculated as [post-pre/pre-values]. The absolute change [post-pre values] was calculated for parameters with both positive and negative values since the relative difference could not be calculated.

Between group (non-smoker vs. smokers) and within group (smokers on the 3 conditions) comparisons of resting vessel hemodynamic parameters, exercise parameters and the exercise-induced response of vessel hemodynamic parameters (relative/absolute change) were analyzed using PROC GENMOD generalized estimating equations (GEE). GEE was chosen for these analyses for its ability to factor in the dependency of observations by specifying a "working correlation structure". Therefore, GEE is highly capable of modelling correlated data that arises from longitudinal observations, and as is the case in this study, multiple measurements within smokers at 3 different points in time. Furthermore, it allowed for the inclusion of time-dependent covariates. As such, these analyses were performed with and without adjustments for relevant covariates. Resting parameters of vessel hemodynamics were adjusted for age, sex, BMI, as well as MAP and resting HR for certain parameters when indicated (detailed information provided in Study 1 table legends). Exercise parameters were adjusted for age, sex, and BMI. When the model did not converge after adjusting for all variables, the complexity of the model was reduced

by adjusting for selected variables. If the model did not converge with any variables, values were presented as unadjusted.

To estimate the impact of smoking on overall vascular function after physical stress, area under the curve (AUC) values were calculated for vessel hemodynamic parameters measured at baseline, 2, 5, 10, 15, and 20 minutes. The baseline AUC was subtracted from the total AUC for each parameter. The use of AUC simplified the statistical analyses by transforming the multivariate data into a univariate space. By subtracting the baseline portion of the AUC we are able to compare differences in the AUC irrespective of the baseline values, allowing the comparison of the 'vascular reserve'. PROC mixed general linear models was used for the comparison of the AUC values for all parameters between smokers and non-smokers. All AUC analyses were performed with and without adjustments for important covariates such as age, sex, BMI and exercise time, as well as MAP and resting HR for certain parameters when indicated. At this stage, comparisons of AUC parameters between smokers on the 3 conditions were performed without any covariate adjustments.

The level of statistical significance was set at $P<0.05$. All tables and figures indicate relevant adjustments. SAS version 9.3 was used (SAS Institute, Cary, NC, USA).

Study 2

Descriptive statistics were used to present baseline characteristics. Kolmogorov–Smirnov test was used to check normal distribution of all parameters, and non-parametric tests were used as appropriate. A comparison of categorical variables (sex) between smoker and non-smokers was performed using a chi-squared test.

Between group comparisons (smokers and non-smokers) of arterial stiffness, cardiorespiratory, and ET-1 values (pre-exercise, post-exercise, absolute difference [post-pre

levels], relative difference [post-pre/pre levels]) were performed using a Mann-Whitney test. Within group comparisons of arterial stiffness, cardiorespiratory and ET-1 values amongst smokers on the 3 conditions were compared using Friedman's test. We further performed Spearman's rank correlations between relative change in ET-1 levels and the relative change in cardiorespiratory parameters (post-pre values/pre-values).

The level of statistical significance was set at $P<0.05$. IBM SPSS Statistics version 20.0 (Armonk, NY, USA) was used.

5.0 RESULTS

5.1 Study 1

5.1.1 Subject Characteristics

Baseline characteristics are presented in **Table 1.1**. We included 123 young, active, healthy smokers (n=43) and non-smokers (n=80). Mean age was 28.2 ± 7.1 years and BMI was 23.1 ± 3.2 kg/m^2. There were no significant differences in age, BMI, waist:hip ratio or IPAQ score between smokers and non-smokers. However, there was a significant difference in the distribution of men and women between smokers and non-smokers, with a greater number of men than women in the smoker group (P=0.048). Smokers reported a median 4.5 pack-year history, and were all either light (n=39) or moderate (n=4) smokers, where moderate smoking is defined as having a pack-year history between 20 and 39, and light smoking corresponds to 19 or less[134].

Table 1.1 - Subject Baseline Characteristics

	Non-Smokers (n=80)	**Smokers (n=43)**	***P* value**
Age (years)	27.5 ± 7.0	29.5 ± 7.3	0.130
Sex (men/women)	43/37	31/12	**0.048**
BMI (kg/m^2)	22.8 ± 2.9	23.6 ± 3.6	0.143
Waist:hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.411
IPAQ score	2244.0 [1328.3-5001.8]	2202.0 [1448.3-3382.8]	0.609
Pack-years	-	4.5 [3.0-10.4]	-

BMI, body mass index; IPAQ, international physical activity questionnaire
Pack-years = # of packs smoked per day × # of years as a smoker, where 1 pack=20 cigarettes
*Presented as median [interquartile range], all other values are mean ± SD
Bolded values indicate significance (P<0.05)

5.1.2 Resting Vessel Hemodynamics

Resting vessel hemodynamic for non-smokers and smokers are presented in **Table 1.2**. Smokers had significantly elevated **resting HR** after acute smoking and nicotine compared to their chronic condition ($P<0.001$ and $P=0.002$, respectively), as well as compared to non-smokers (both $P<0.001$). The elevated HR observed after acute smoking was also significantly greater than after nicotine exposure ($P<0.001$).

Smokers had significantly higher $_{P}$**SBP** after acute smoking compared to the chronic condition ($P<0.001$) and nicotine condition ($P=0.0017$), as well as non-smokers ($P=0.004$). In regards to central BP, $_{C}$**SBP** was significantly higher in smokers after acute smoking compared to non-smokers ($P=0.007$) and there was a trend for increased $_{C}$SBP after acute smoking compared to the chronic condition ($P=0.062$). Similarly, **MAP** was significantly higher after acute smoking compared to non-smokers ($P=0.037$), and trended towards significance after acute smoking compared to the chronic condition ($P=0.056$). Both $_{P}$**PP** and $_{C}$**PP** were significantly higher after acute smoking compared to non-smokers ($P=0.022$ and $P=0.030$, respectively). **PPA** was significantly lower in chronic smokers, as well as after nicotine exposure, compared to non-smokers ($P<0.001$ and $P=0.012$, respectively). However, PPA was significantly higher after acute smoking compared to the chronic and nicotine conditions ($P<0.001$ and $P=0.012$, respectively) and yielded values that were comparable to non-smokers.

Smokers had significantly higher **AP** under all 3 conditions compared to non-smokers ($P<0.001$, $P=0.027$, and $P<0.001$, respectively). Similarly, **AIx** followed the same trend on the 3 conditions ($P<0.001$, $P=0.007$, and $P<0.001$, respectively). **AIx75** was also significantly higher in smokers on the 3 conditions (all, $P<0.001$) compared to non-smokers. Among smokers AP

and AIx were significantly higher after acute smoking compared to chronic smoking (P=0.032 and P=0.004, respectively), and the values were intermediate after nicotine exposure.

There was no significant difference in **SEVR** between chronic smokers and non-smokers. However, smokers exhibited significantly lower SEVR after acute smoking and nicotine compared to their chronic condition (P<0.001 and P<0.001, respectively), and non-smokers (P<0.001 and P=0.004, respectively).

crPWV was higher in smokers under all 3 conditions compared to non-smokers (P=0.035, P<0.001, and P=0.044, respectively), however significance was lost after adjustment for age, sex, and MAP (the model did not converge with BMI and resting HR). Nevertheless, smokers after acute smoking had significantly higher crPWV than non-smokers, even after adjustment (P=0.012). **cfPWV** was also higher in smokers under all 3 conditions compared to non-smokers (P=0.040, P<0.001, and P=0.006, respectively), however once again significance was lost after adjustment for age, sex, BMI, resting HR and MAP.

Table 1.2 - Resting Vessel Hemodynamic Parameters – Smokers vs. Non-Smokers

	Non-Smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N
HR	59.76 ± 0.88	61.84 ± 0.96	71.31 ± 1.28*[&]	65.72 ± 1.15*	0.109	**<0.001**	**<0.001**
$_{P}$SBP	108.03 ± 0.89	107.75 ± 1.18	112.43 ± 1.20*[&]	109.03 ± 1.25	0.856	**0.004**	0.520
$_{P}$DBP	67.34 ± 0.68	67.51 ± 0.99	67.95 ± 1.02	68.04 ± 0.99	0.894	0.623	0.564
$_{P}$PP	40.72 ± 0.92	40.22 ± 1.16	44.45 ± 1.28	40.96 ± 1.00	0.743	**0.022**	0.861
$_{C}$SBP	93.15 ± 0.76	94.35 ± 1.00	96.31 ± 0.90	95.31 ± 1.09	0.346^	**0.007**	0.105^
$_{C}$DBP	68.23 ± 0.72	67.82 ± 0.92	69.20 ± 1.01	69.37 ± 1.00	0.732	0.436	0.360
$_{C}$PP	24.91 ± 0.57	26.46 ± 0.69	27.09 ± 0.78	26.02 ± 0.63	0.093	**0.030**	0.206
MAP	79.59 ± 0.69	79.96 ± 0.93	82.00 ± 0.94	81.25 ± 0.97	0.753	**0.037**	0.165^
PPA	1.64 ± 0.01	1.53 ± 0.02	1.64 ± 0.02*[&]	1.58 ± 0.02*	**<0.001**	0.931	**0.012**
AP	0.30 ± 0.28	2.43 ± 0.36	1.46 ± 0.40*	2.05 ± 0.35	**<0.001**	**0.027**	**<0.001**
AIx	0.88 ± 0.97	9.26 ± 1.27	5.28 ± 1.24	7.66 ± 1.27	**<0.001**	**0.007**	**<0.001**
AIx75	-5.21 ± 1.00	3.26 ± 1.30	2.69 ± 1.21	3.03 ± 1.31	**<0.001**	**<0.001**	**<0.001**
SEVR	169.99 ± 2.24	166.40 ± 1.98	154.27 ± 2.90*[&]	160.28 ± 2.40	0.239*	**<0.001**	**0.004**
crPWV	7.96 ± 0.10	8.16 ± 0.13	8.34 ± 0.11	8.16 ± 0.18	0.244^	**0.012**	0.331^
cfPWV	6.35 ± 0.11	6.48 ± 0.10	6.63 ± 0.12	6.48 ± 0.10	0.411^	0.106^	0.413

AIx, augmentation index (%); AIx75, augmentation index adjusted to heart rate of 75 beats per minute (%); AP, augmentation pressure (mmHg); $_{C}$SBP, central systolic blood pressure (mmHg); $_{C}$DBP, central diastolic blood pressure (mmHg); $_{C}$PP, central pulse pressure (mmHg); cfPWV, carotid-femoral pulse wave velocity (m/s); crPWV, carotid-radial pulse wave velocity (m/s); HR, heart rate (beats per minute); MAP, mean arterial pressure (mmHg); $_{P}$SBP, peripheral systolic blood pressure (mmHg); $_{P}$DBP, peripheral diastolic blood pressure (mmHg); $_{P}$PP, peripheral pulse pressure (mmHg); PPA, pulse pressure amplification; SEVR, subendocardial viability ratio (%).

Values are adjusted and presented as mean ± standard error (SE)

Bolded values indicate significance ($P<0.05$)

^$P<0.05$ unadjusted

* $P<0.05$ vs. chronic smoking (CS)

[&] $P<0.05$ vs. nicotine (N)

Resting blood pressures adjusted for age, sex, and BMI

Resting AP, AIx, SEVR, and cfPWV adjusted for age, sex, BMI, resting HR and MAP

Resting crPWV adjusted for age, sex, and MAP

Resting AIx75 and HR adjusted for age, sex, BMI, and MAP

5.1.3 Exercise Parameters

Exercise parameters for non-smokers and smokers are presented in **Table 1.3**. Maximum exercise time was significantly lower in smokers on all 3 conditions compared to non-smokers (*P*=0.015, *P*=0.009, and *P*=0.017, respectively). Furthermore, smokers on all 3 conditions were not able to achieve comparable max HR upon exercise termination as non-smokers (*P*<0.001, *P*=0.001, and *P*=0.001, respectively), and similarly did not achieve a comparable percentage of their age-predicted max HR as non-smokers (*P*=0.004, *P*=0.006, and *P*=0.035, respectively). VO_{2max} was lower in smokers on all 3 conditions compared to non-smokers, although non-significantly. However, there was a trend for lower VO_{2max} after the acute smoking condition compared to non-smokers (*P*=0.065). We observed no significant differences in peak METs between groups. Among smokers, there were no significant differences in exercise parameters (exercise time, VO_{2max}, max HR, %predMaxHR, and peak METs), however they were highest on the nicotine day compared to the acute smoking and chronic smoking conditions.

Table 1.3 - Exercise Parameters – Smokers vs. Non-Smokers

	Non-Smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N
Exercise Time	16.2 ± 0.3	15.3 ± 0.3	15.2 ± 0.2	15.3 ± 0.2	**0.015**	**0.009**	**0.017**
Max HR	190.1 ± 1.1	182.1 ± 1.8	182.0 ± 1.9	184.0 ± 1.4	**<0.001**	**0.001**	**0.001**
%predMaxHR	98.7 ± 0.5	95.4 ± 1.0	95.8 ± 1.0	96.6 ± 0.9	**0.004**	**0.006**	**0.035**
VO_{2max}	47.4 ± 1.1	44.1 ± 1.6	45.3 ± 1.7	46.0 ± 1.7	0.065	0.306	0.511
Peak METs	14.7 ± 0.7	12.9 ± 0.8	13.4 ± 0.7	13.6 ± 0.8	0.184	0.296	0.397

Values are adjusted and presented as mean ± SE
Bolded values indicate significance (*P*<0.05)
Exercise time (minutes), adjusted for age, sex and BMI
Max HR, maximal HR (beats/minute), adjusted for age, sex, BMI and resting HR
%predMaxHR, percent of age-predicted maximal heart rate (%), unadjusted
VO_{2max}, maximal oxygen consumption (mL/kg per minute), unadjusted
Peak METs, peak metabolic equivalents, adjusted for age only

5.1.4 Exercise-Induced Changes in Vessel Hemodynamics

HR was significantly increased from baseline values immediately post-exercise (2 minutes) in all groups (P<0.001 for all). The relative increase in HR in response to exercise was significantly lower in smokers under all 3 conditions compared with non-smokers (P<0.001 for all). Among smokers, the increase in HR was significantly lower in smokers after acute smoking compared to their chronic condition (P<0.001). Furthermore, smokers on all 3 conditions exhibited a significantly lower HR AUC compared to non-smokers (**Figure 1.1**).

Exercise led to a significant increase in $_{P}$SBP, $_{P}$PP, $_{C}$SBP, and $_{C}$PP in smokers and non-smokers (all, P<0.001). Smokers after acute smoking demonstrated a significantly lower AUC than non-smokers for $_{P}$SBP, $_{P}$PP, $_{C}$SBP, and $_{C}$PP (P=0.013, P=0.005, P=0.045, and P=0.006 respectively). Among smokers, the relative increase in PPA in response to exercise was significantly lower in smokers after acute smoking compared to their chronic condition (P=0.008), however no differences in the AUC were observed.

AIx75 was significantly increased post-exercise (5 minutes) in all groups (P<0.001 for all), however smokers on all 3 conditions did not achieve a comparable increase as non-smokers (P=0.003, P<0.001, and P=0.001, respectively) (**Figure 1.2**). Furthermore, the AIx75 AUC was significantly lower in smokers on all 3 conditions compared to non-smokers (all, P<0.0001).

SEVR was significantly decreased post-exercise (5 minutes) in all groups (all, P<0.001). Smokers on all 3 conditions did not achieve comparable decrease in SEVR in response to exercise as non-smokers (all, P<0.001). Furthermore, the decrease in SEVR was significantly lower in smokers after acute smoking and nicotine exposure compared to their chronic condition (P<0.001 and P=0.010, respectively). Correspondingly, smokers on all 3 conditions had a lower

SEVR AUC compared to non-smokers (P=0.002, P<0.001, and P=0.002, respectively) (**Figure 1.3**).

cfPWV was significantly elevated at 2 minutes post-exercise in all groups (all, P<0.001). Although there were no significant differences in the response of cfPWV to exercise between chronic smokers and non-smokers, the relative increase in cfPWV after exercise was significantly lower on the acute smoking condition compared to non-smokers (P=0.010) (**Figure 1.4**)

Table 1.4 - Relative and Absolute Change in Vessel Hemodynamics Immediately Post-Exercise

	Non-Smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N
Relative Change (%)							
HR	79.23 ± 2.76	62.83 ± 2.97	50.99 ± 2.71*	58.66 ± 2.83*	**<0.001**	**<0.001**	**<0.001**
$_{P}$SBP	50.84 ± 1.96	50.88 ± 2.97	47.50 ± 2.46	47.88 ± 2.33	0.991	0.299	0.347
$_{P}$DBP	-3.69 ± 1.63	0.81 ± 2.01	-1.10 ± 2.14	0.035 ± 1.83	0.080	0.337	0.126
$_{P}$PP	148.49 ± 6.92	141.58 ± 11.07	128.66 ± 9.88	129.49 ± 6.78	0.615	0.228	0.954
$_{C}$SBP	8.61 ± 1.06	7.24 ± 1.32	7.61 ± 1.47	6.45 ± 1.18	0.421	0.593	0.193
$_{C}$DBP	-5.18 ± 1.10	-3.09 ± 1.58	-1.17 ± 1.41	-4.79 ± 1.33	0.302	0.029	0.827
$_{C}$PP	50.15 ± 3.66	36.89 ± 5.82	34.74 ± 6.80	38.77 ± 5.26	0.064	0.055	0.094
MAP	2.19 ± 0.98	2.64 ± 1.24	3.38 ± 1.19	1.55 ± 0.98	0.777	0.446	0.654
PPA	2.27 ± 1.05	5.02 ± 1.34	0.73 ± 1.19*	4.66 ± 1.33	0.115	0.336	0.163
SEVR	-53.25 ± 1.66	-46.47 ± 1.46	-36.79 ± 1.79*	-43.28 ± 1.30*	**0.003**	**<0.001**	**<0.001**
crPWV	4.01 ± 1.12	3.63 ± 1.70	1.94 ± 2.06	2.57 ± 1.95	0.856	0.390	0.534
cfPWV	49.98 ± 2.46	47.09 ± 3.30	39.11 ± 3.44*	42.92 ± 3.26	0.491	**0.010**	0.087
Absolute Change							
AP	0.08 ± 0.37	-0.03 ± 0.46	0.97 ± 0.38	0.37 ± 0.33	**0.030**	0.417	0.090
AIx	0.16 ± 1.08	-2.40 ± 1.24	2.62 ± 1.06*	-0.77 ± 1.09	0.131^	0.110	0.550
AIx75	17.61 ± 1.17	10.50 ± 1.20	11.62 ± 1.13	11.43 ± 1.06	**<0.001**	**<0.001**	**<0.001**

AIx, augmentation index (%); AIx75, augmentation index adjusted to heart rate of 75 beats per minute (%); AP, augmentation pressure (mmHg); $_{C}$SBP, central systolic blood pressure (mmHg); $_{C}$DBP, central diastolic blood pressure (mmHg); $_{C}$PP, central pulse pressure (mmHg); cfPWV, carotid-femoral pulse wave velocity (m/s); crPWV, carotid-radial pulse wave velocity (m/s); HR, heart rate (beats per minute); MAP, mean arterial pressure (mmHg); $_{P}$SBP, peripheral systolic blood pressure (mmHg); $_{P}$DBP, peripheral diastolic blood pressure (mmHg); $_{P}$PP, peripheral pulse pressure (mmHg); PPA, pulse pressure amplification; SEVR, subendocardial viability ratio (%).
Relative Change (%) calculated as [(post – pre)/pre × 100]
Absolute Change calculated as [post – pre] for parameters where relative change could not be calculated because of negative pre-exercise values
Values are adjusted and presented as mean ± SE
Bolded values indicate significance (P<0.05)
* P<0.05 vs. chronic smoking
^ P<0.05 unadjusted
Blood pressures adjusted for age, sex, and BMI and exercise time
AP, AIx, SEVR, cfPWV, crPWV adjusted for age, sex, BMI, resting HR, MAP and exercise time
AIx75 and HR adjusted for age, sex, BMI, MAP and exercise time

Table 1.5 - Area Under the Curve of Vessel Hemodynamics in Response to Exercise – Smokers vs. Non-Smokers

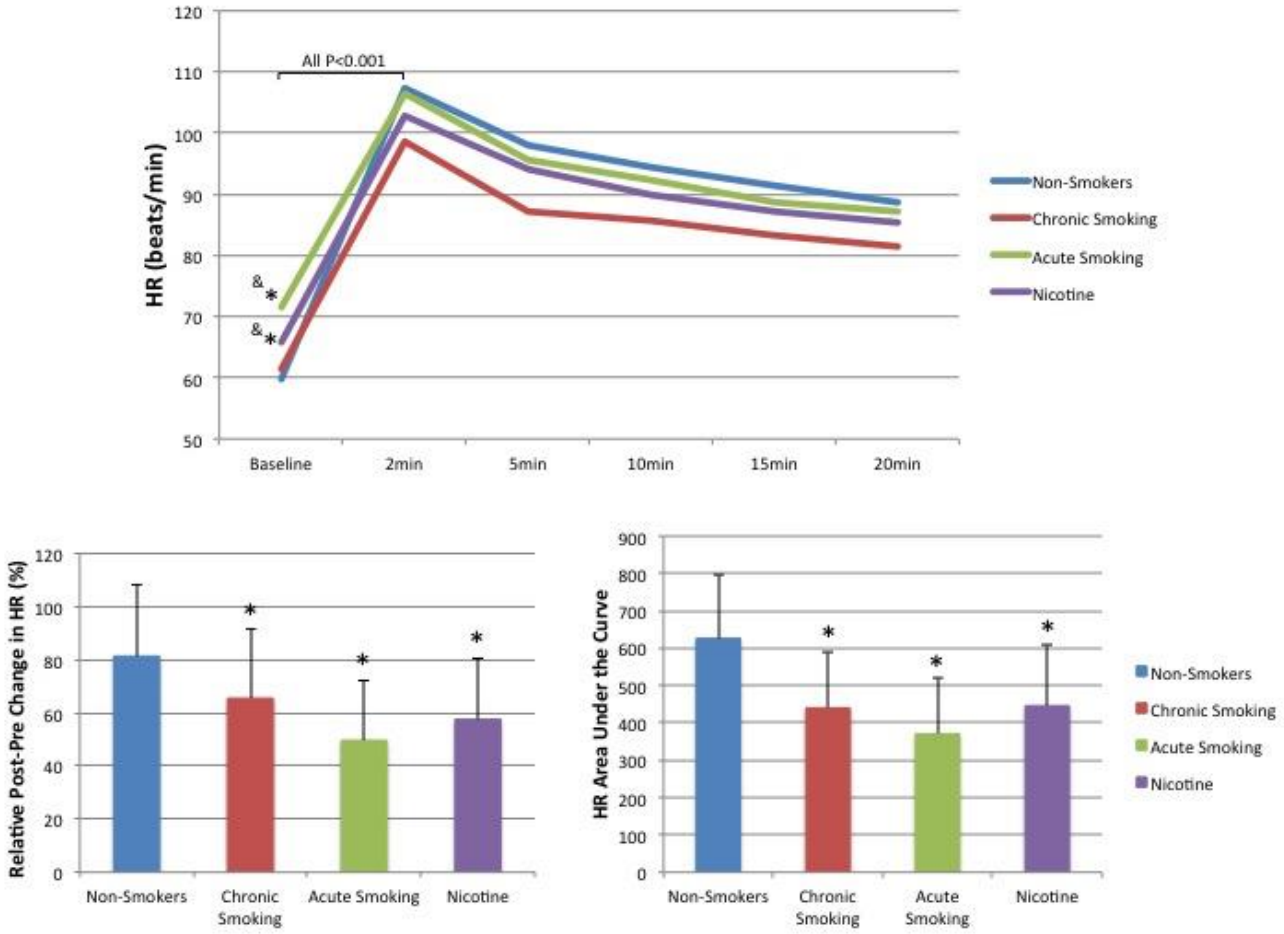
	Non-Smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N	*P* value CS vs. AC. vs. N
HR	619.7 ± 16.8	455.1 ± 22.4	382.3 ± 24.2	452.8 ± 23.8	**<0.001**	**<0.001**	**<0.001**	**0.001**
$_{P}$SBP	173.9 ± 14.7	150.4 ± 19.7	99.7 ± 20.8	149.9 ± 18.0	0.352	**0.013**	0.357	**0.045**
$_{P}$DBP	-40.3 ± 10.8	-13.3 ± 14.7	-15.9 ± 16.4	-30.3 ± 14.4	0.629	0.170	0.621	0.806
$_{P}$PP	214.1 ± 16.8	180.5 ± 22.7	115.6 ± 26.3	180.2 ± 21.6	0.248	**0.005**	0.272	**0.047**
$_{C}$SBP	31.6 ± 9.2	15.8 ± 12.9	-4.2 ± 12.8	9.7 ± 12.3	0.332	**0.045**	0.164	0.216
$_{C}$DBP	-17.3 ± 8.8	-1.7 ± 12.4	-7.6 ± 14.7	-16.7 ± 13.0	0.318	0.527	0.953	0.876
$_{C}$PP	49.6 ± 9.1	19.2 ± 12.7	-2.1 ± 14.2	25.1 ± 12.3	0.060	**0.006**	0.145	0.150
MAP	-1.4 ± 8.0	6.4 ± 11.3	-4.4 ± 11.7	-5.8 ± 11.0	0.583	0.950	0.718	0.553
PPA	1.2 ± 0.2	1.6 ± 0.3	1.1 ± 0.3	1.3 ± 0.3	0.178	0.916	0.700	0.213
AP	-15.3 ± 4.7	-21.6 ± 6.4	-7.1 ± 7.7	-17.6 ± 6.5	0.439	0.353	0.821	0.181
AIx	-56.7 ± 15.4	-84.5 ± 21.1	-37.3 ± 21.5	-72.2 ± 20.8	0.301	0.531	0.602	0.057
AIx75	177.3 ± 15.4	87.9 ± 20.2	78.0 ± 21.0	92.6 ± 19.8	**0.001**	**0.002**	**0.002**	0.222
SEVR	-1339.2 ± 38.0	-1121.1 ± 53.2	-844.1 ± 64.5	-1024.4 ±54.31	**0.002**	**<0.001**	**0.002**	**<0.001**
crPWV	3.5 ± 1.0	0.30 ± 1.4	-1.1 ± 1.9	1.6 ± 1.8	0.076	0.134	0.492	0.421
cfPWV	10.5 ± 1.1	11.4 ± 1.6	9.8 ± 2.3	12.2 ± 1.6	0.657	0.811	0.368	0.106

AIx, augmentation index (%); AIx75, augmentation index adjusted to heart rate of 75 beats per minute (%); AP, augmentation pressure (mmHg); $_{C}$SBP, central systolic blood pressure (mmHg); $_{C}$DBP, central diastolic blood pressure (mmHg); $_{C}$PP, central pulse pressure (mmHg); cfPWV, carotid-femoral pulse wave velocity (m/s); crPWV, carotid-radial pulse wave velocity (m/s); HR, heart rate (beats per minute); MAP, mean arterial pressure (mmHg); $_{P}$SBP, peripheral systolic blood pressure (mmHg); $_{P}$DBP, peripheral diastolic blood pressure (mmHg); $_{P}$PP, peripheral pulse pressure (mmHg); PPA, pulse pressure amplification; SEVR, subendocardial viability ratio (%).

Values are presented mean ± SE
Bolded values indicate significance ($P<0.05$)
Blood pressures adjusted for age, sex, and BMI and exercise time
AP, AIx, SEVR, cfPWV, crPWV adjusted for age, sex, BMI, resting HR, MAP and exercise time
AIx75 and HR adjusted for age, sex, BMI, MAP and exercise time
P values (CS vs. AS vs. N) are unadjusted

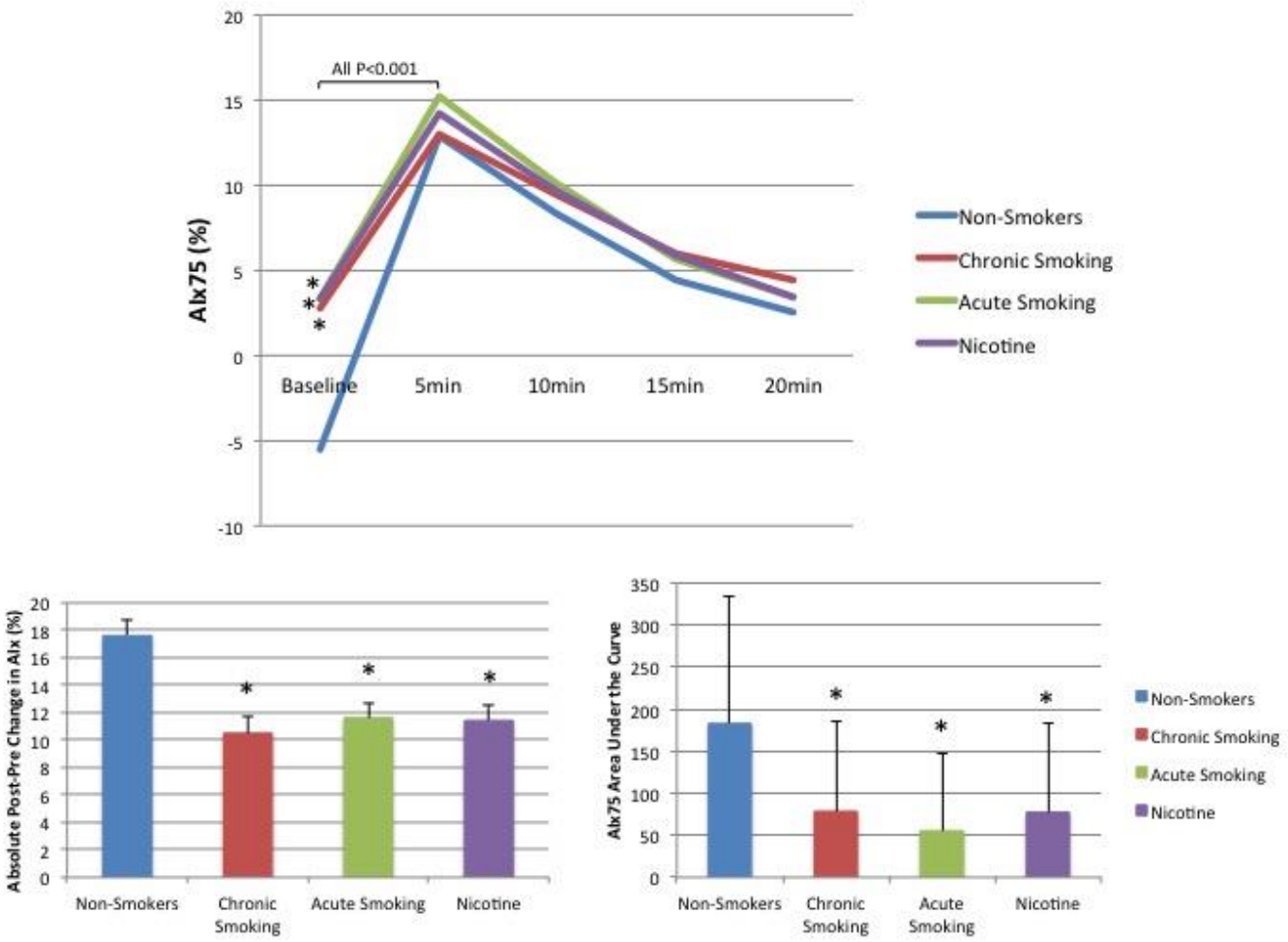
Study 1: Figures

Figure 1.1 - Exercise-Induced Changes in HR in Non-Smokers and Non-Smokers



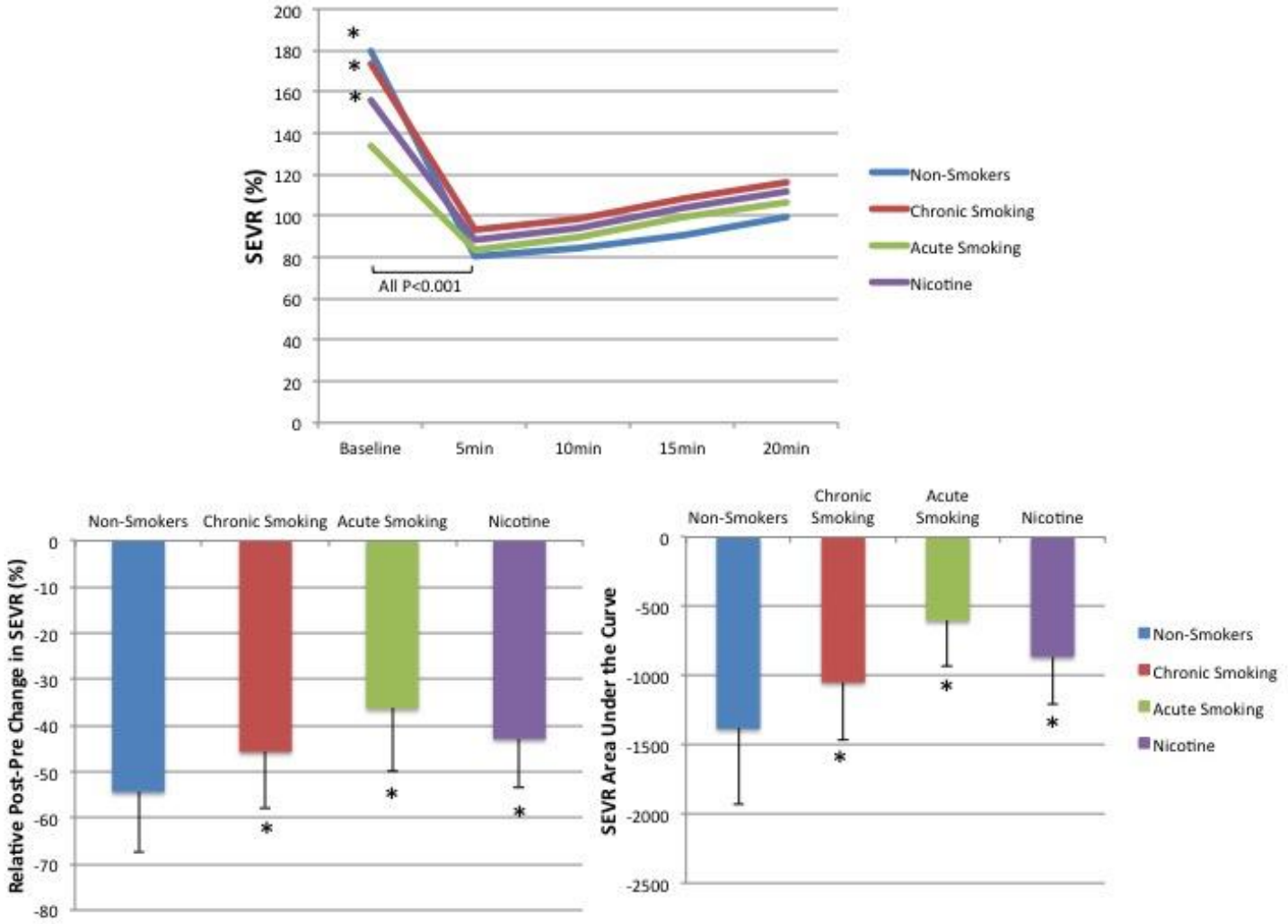
HR, heart rate (beats per minute)
*P<0.05 compared to non-smokers (adjusted)
&P<0.05 compared to chronic smoking (adjusted)

Figure 1.2 - Exercise-Induced Changes in AIx75 in Non-Smokers and Non-Smokers



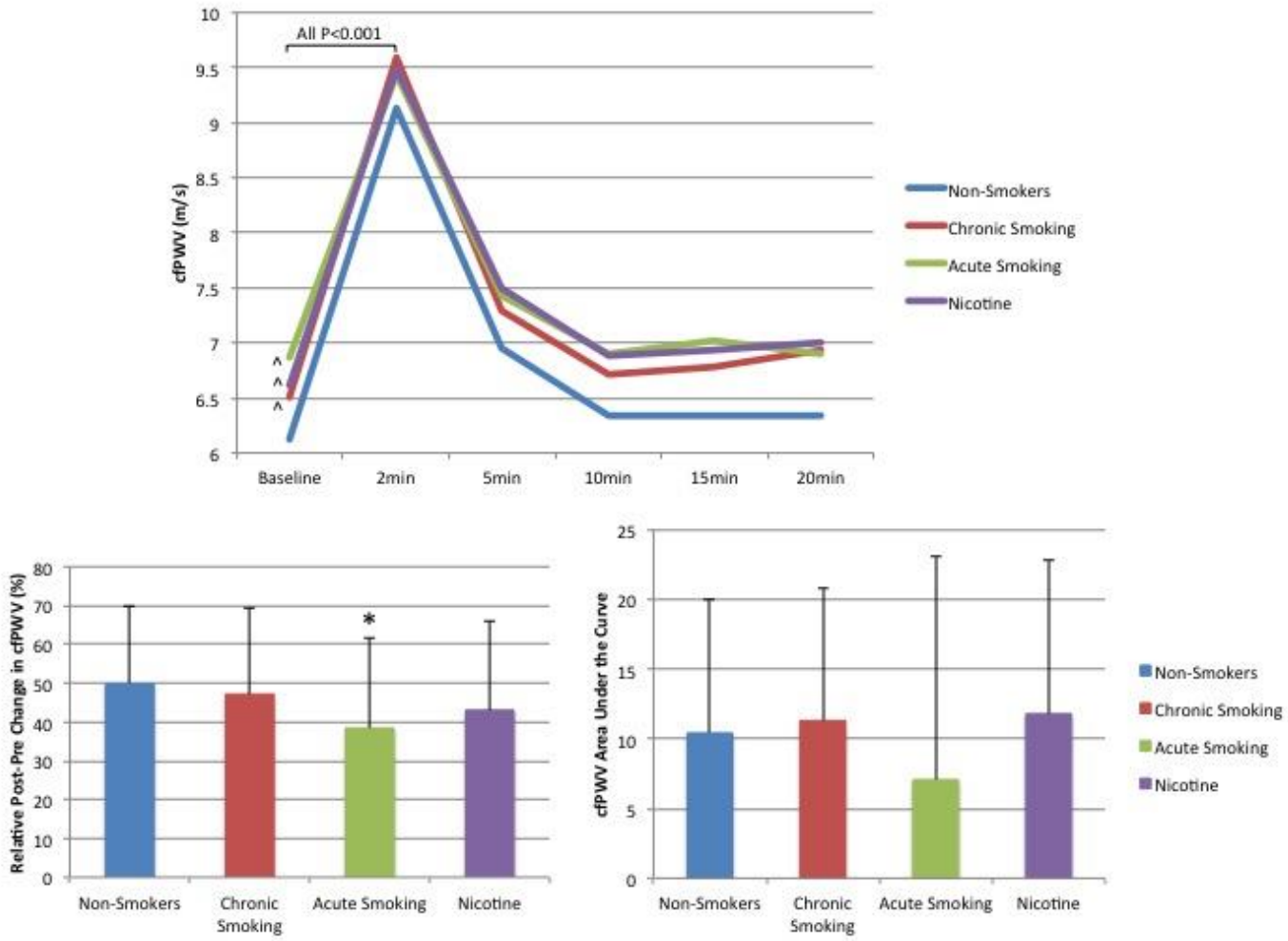
AIx75, augmentation index corrected for a heart rate of 75 beats per minute (%)
*P<0.05 compared to non-smokers (adjusted)

Figure 1.3 - Exercise-Induced Changes in SEVR in Non-Smokers and Non-Smokers



SEVR, subendocardial viability ratio (%)
*P<0.05 compared to non-smokers (adjusted)

Figure 1.4 - Exercise-Induced Changes in cfPWV in Non-Smokers and Non-Smokers



cfPWV, carotid-femoral pulse wave velocity (m/s)
^ *P*<0.05 vs. non-smoker (unadjusted)
**P*<0.05 vs. non-smoker (adjusted)

5.2 Study 2

5.2.1 Subject Characteristics

We selected 35 smokers and 35 non-smokers from the larger population included in Study 1. Subjects were matched for age, sex and BMI. Mean age was 29.1±7.3 years, and BMI was 23.9±3.4 kg/m^2. There were no significant differences between the waist:hip ratio or IPAQ score between smokers and non-smokers.

Table 2.1 - Subject Baseline Characteristics

	Non-Smokers (n=35)	**Smokers (n=35)**	***P* value**
Age (years)	28.6 ± 7.2	29.1 ± 7.4	0.614
Sex (men/women)	28/8	25/10	0.580
BMI (kg/m^2)	23.6 ± 3.2	24.2 ± 3.6	0.438
Waist:hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.884
IPAQ score*	2479.5 [1503.1-6293.3]	2199.0 [1328.3-4972.5]	0.644
Pack-years*	-	5.0 [3.0-11.0]	-

BMI, body mass index; IPAQ, international physical activity questionnaire
Pack-years = # of packs smoked per day × # of years as a smoker, where 1 pack=20 cigarettes
*Presented as median [interquartile range], all other values are mean ± SD

5.2.2 Resting and Exercise Parameters

There were no significant differences in exercise parameters (exercise time, max HR post-exercise, VO_{2max}, and peak METs) between smokers and non-smokers, or between smokers on the 3 conditions. Comparing smokers on the 3 conditions (chronic vs. acute vs. nicotine), smokers after acute smoking had significantly higher resting $_{P}$SBP (P=0.002) and HR (P<0.001).

Table 2.2 - Resting and Exercise Parameters for Non-Smokers and Smokers

	Non-Smokers	Chronic Smoking	Acute Smoking	Nicotine
Resting HR	60.5 ± 7.0	61.6 ± 6.2	71.6 ± 8.9*	65.5 ± 7.7
Resting $_{P}$SBP	108.6 ± 8.4	109.7 ± 8.6	114.5 ± 9.8*	111.0 ± 9.8
Resting $_{P}$DBP	67.9 ± 6.0	68.1 ± 6.2	69.1 ± 8.3	69.5 ± 7.0
Exercise time	15.9 ± 2.7	15.4 ± 2.5	15.3 ± 2.6	15.4 ± 2.8
Max HR	187.1 ± 10.5	182.1 ± 13.9	183.4 ± 14.1	183.8 ± 13.1
VO_{2max}	47.4 ± 11.1	44.9 ± 10.2	44.3 ± 10.4	45.5 ± 10.2
Peak METs	14.3 ± 3.1	13.6 ± 2.7	13.5 ± 2.8	13.8 ± 2.7

HR, heart rate (beats per minute); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg), Exercise time (minute), Max HR, maximal heart rate (beats per minute); VO_{2max}, maximum oxygen consumption (mL/kg per minute); Peak METs, peak metabolic equivalents

All values are presented as mean ± SD
*P<0.05 compared to non-smokers, all other values NS

5.2.3 Endothelin-1 Levels at Rest and in Response to Exercise

Post-exercise ET-1 levels were significantly lower than pre-exercise levels in non-smokers (P<0.001) and smokers under all 3 conditions: chronic smoking, acute smoking and nicotine (P=0.005, P<0.001, P=0.001, respectively) (**Figure 2.1**).

There were no differences in pre-exercise ET-1 levels between non-smokers and smokers after 12-h abstinence. However, there was a trend for increased levels pre-exercise after acute smoking (P=0.064) when compared to chronic smoking. When dividing the smoking population into two groups based on pack-years (light: median [interquartile range], 3.3 [1.9-4.0] vs. heavy smokers: 11.0 [7.8-15.5]) heavy smokers had significantly greater levels of ET-1 pre-exercise than light smokers on the acute smoking day (P=0.046). There were however, no significant differences between post-exercise ET-1 levels between non-smokers and smokers.

Both the absolute (P=0.007) and relative decrease in ET-1 levels (P=0.004) was significantly smaller in smokers on their abstinence day compared with non-smokers. Moreover, after acute smoking, smokers had greater absolute and relative decreases in ET-1 levels post-exercise (P=0.047 and P=0.060, respectively) compared to chronic smoking (**Figure 2.2**).

Table 2.3 - Endothelin-1 Levels for Non-Smokers and Smokers

	Non-Smokers	Chronic Smoking	Acute Smoking	Nicotine	*P* value NS vs. CS
ET-1 pre-exercise	1.5 ± 0.4	1.41 ± 0.40	1.6 ± 0.5^	1.5 ± 0.5	0.488
ET-1 post-exercise	1.1 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.4	0.122
ET-1 absolute difference	-0.4 ± 0.3	-0.2 ± 0.4	-0.4 ± 0.5	-0.3 ± 0.5	**0.007**
ET-1 relative difference (%)	-0.3 ± 0.2	-0.1 ± 0.3	-0.2 ± 0.3	-0.1 ± 0.4	**0.004**

ET-1, endothelin-1
Absolute difference calculated as (post – pre ET-1 levels)
Relative difference calculated as (post – pre ET-1 levels/pre ET-1 levels)
All values are presented as mean± SD, and expressed as pg/mL
Bolded values indicate significance (P<0.05)
^P=0.064 compared to chronic smoking

5.2.4 Cardiorespiratory Parameters

We compared the relative change of all cardiorespiratory parameters in response to maximal exercise between chronic smokers and non-smokers (**Table 2.4**). Interestingly, non-smokers had a greater relative increase in Vt (P=0.021), $FeCO_2$ (P=0.050), VO_2 (P=0.005), VCO_2 (P=0.004), and PEF (P=0.003) compared to chronic smokers. However, comparing smokers on 3 conditions (chronic smoking vs. acute smoking vs. nicotine exposure) we observed no significant differences in the exercise-induced response of cardiorespiratory parameters.

5.2.5 Endothelin-1 Levels in Relation to Cardiorespiratory Parameters

The decrease in ET-1 observed in non-smokers in response to exercise was significantly associated with exercise induced-changes in Ti (r_s=0.444, P=0.008), Ttot (r_s=0.365, P=0.034), RR (r_s=-0.379, P=0.027), VI (r_s=-0.370, P=0.031) and PIF (r_s=-0.352, P=0.041).

Table 2.4 - The Relative Change in Cardiorespiratory Parameters in Response to Exercise for Non-Smokers and Smokers

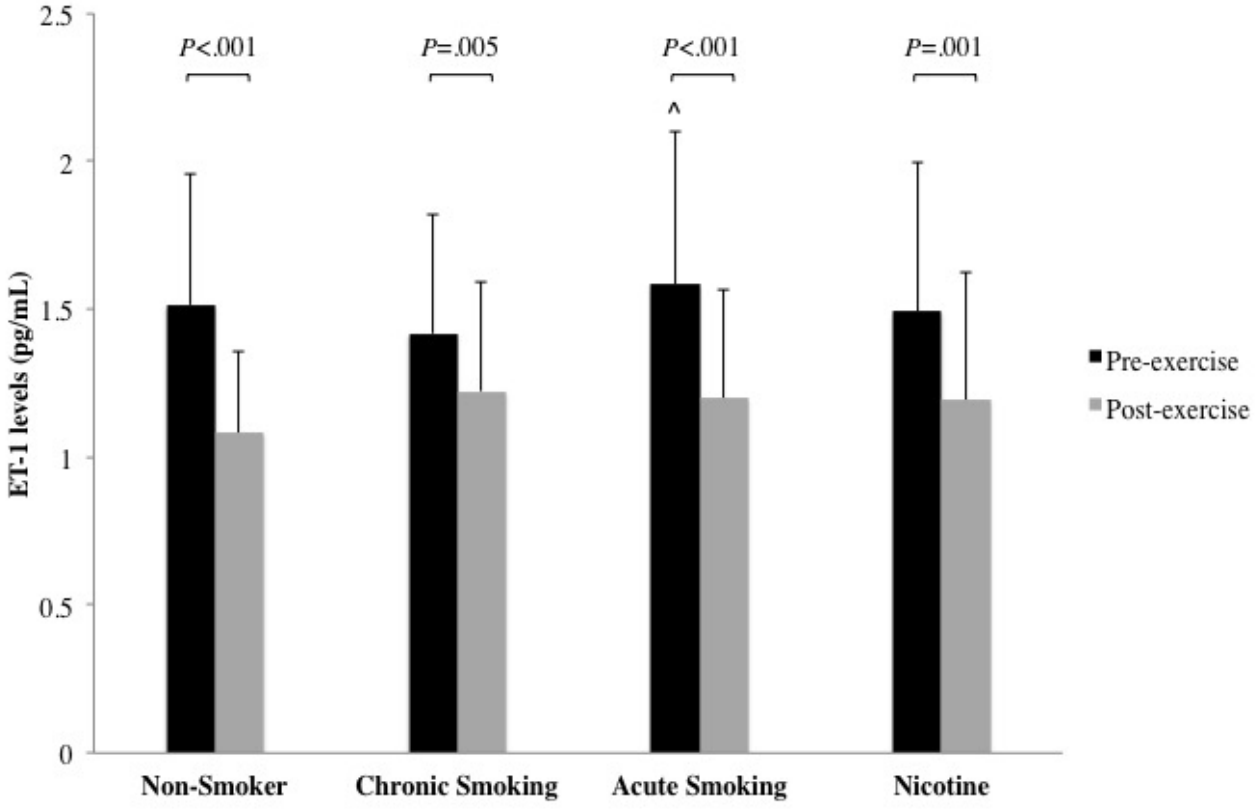
Relative Difference	Non- smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS
HR	1.23 ± 0.30	1.24 ± 0.34	1.11 ± 0.37	1.17 ± 0.27	0.723
Ti	-0.58 ± 0.17	-0.64 ± 0.12	-0.63 ± 0.11	-0.61 ± 0.11	0.202
Ttot	-0.67 ± 0.11	-0.70 ± 0.10	-0.69 ± 0.10	-0.67 ± 0.09	0.291
RR	2.23 ± 1.01	2.46 ± 1.06	2.20 ± 0.96	2.09 ± 0.91	0.525
VE	9.20 ± 2.53	8.37 ± 2.84	8.45 ± 3.17	9.13 ± 3.49	0.074
Vt	2.28 ± 0.72	1.87 ± 0.99	2.10 ± 1.09	2.43 ± 1.17	**0.021**
VI	9.08 ± 2.58	8.42 ± 2.84	8.46 ± 3.21	8.72 ± 3.12	0.134
FeO_2	-0.03 ± 0.04	-0.01 ± 0.03	-0.01 ± 0.04	-0.01 ± 0.03	0.160
$FeCO_2$	0.44 ± 0.22	0.33 ± 0.18	0.37 ± 0.20	0.40 ± 0.20	**0.050**
VO_2	9.10 ± 2.02	7.67 ± 2.36	7.53 ± 2.30	8.30 ± 3.17	**0.005**
VCO_2	13.33 ± 3.23	11.21 ± 3.69	11.63 ± 4.25	12.90 ± 5.22	**0.004**
RER	0.42 ± 0.19	0.41 ± 0.16	0.46 ± 0.18	0.49 ± 0.18	0.701
$PETO_2$	0.03 ± 0.05	0.05 ± 0.06	0.05 ± 0.07	0.05 ± 0.05	0.455
$PETCO_2$	0.17 ± 0.15	0.13 ± 0.12	0.13 ± 0.12	0.14 ± 0.12	0.457
PIF	6.66 ± 1.93	6.33 ± 2.33	6.06 ± 2.26	6.50 ± 2.06	0.381
PEF	8.75 ± 2.40	7.20 ± 2.52	7.16 ± 3.00	7.80 ± 3.22	**0.003**
FiO_2	-0.01 ± 0.00	-0.01 ± 0.00	-0.01 ± 0.00	-0.01 ± 0.00	0.995
$FiCO_2$	0.86 ± 0.89	0.87 ± 0.60	0.90 ± 0.59	0.88 ± 0.59	0.325
VDA	0.82 ± 0.41	0.73 ± 0.42	0.75 ± 0.38	0.93 ± 0.40	0.195

$FeCO_2$, fraction of expired CO_2 (%); FeO_2, fraction of expired O_2 (%); $FiCO_2$, fraction of inspired CO_2 (%); FiO_2, fraction of inspired O_2 (%); HR, heart rate (beats per minute); PEF, peak expiratory flow (L/s); $PETCO_2$, partial pressure of end-tidal CO_2 (mmHg); $PETO_2$, partial pressure of end-tidal O_2 (mmHg); PIF, peak inspiratory flow (L/s); RR, respiratory rate (breaths/minute); Ti, inspiratory time (seconds); Ttot, total time for a tidal volume cycle (seconds); VCO_2, carbon dioxide production (L/minute); VDA, anatomical dead space (mL); VE, exhaled minute ventilation (L/minute); VI, inspired minute ventilation (L/minute); VO_2, oxygen consumption (L/minute); Vt, tidal volume (L).

All values are mean ± SD
Bolded values represent significant differences between non-smokers and chronic smokers

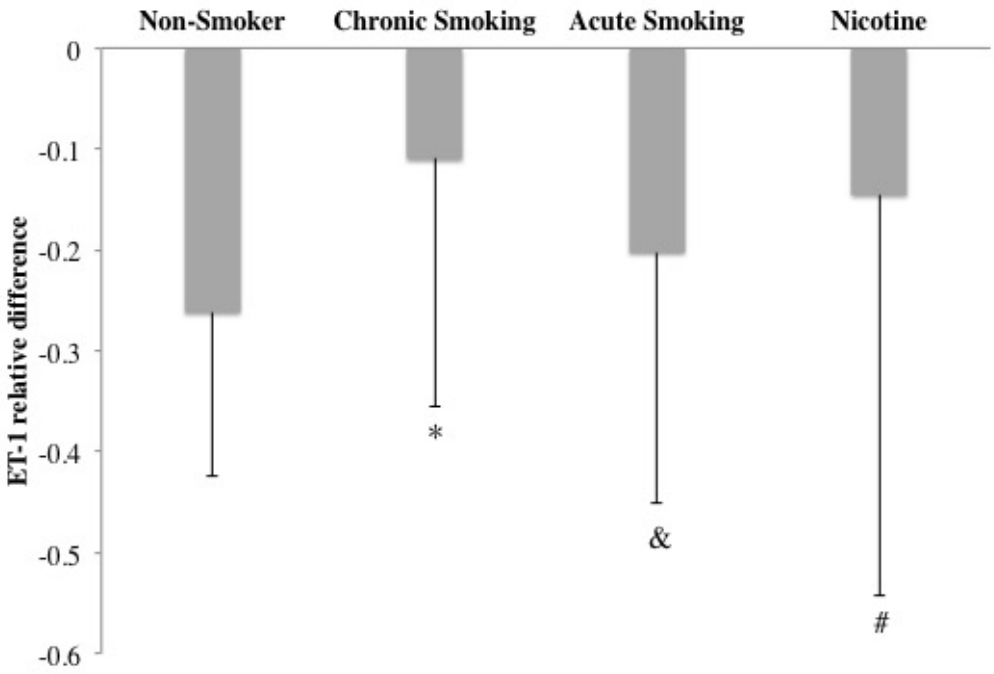
Study 2 : Figures

Figure 2.1 - Endothelin-1 Levels Pre- and Post-Exercise for Non-Smokers and Smokers



ET-1, endothelin-1
^*P*=0.064 compared to chronic smoking pre-exercise ET-1 levels

Figure 2.2 - The Response of Endothelin-1 to Maximal Exercise



ET-1, endothelin-1
Relative difference calculated as (post – pre levels/pre levels)

Relative decrease in ET-1 (above graph): **P*=0.004 vs. Non-Smoker, $^{\&}$*P*=0.060 vs. Chronic Smoking, $^{\#}$*P*=0.053 vs. Chronic Smoking

Absolute decrease in ET-1: **P*=007 vs. Non-Smoker, $^{\&}$*P*=0.047 vs. Chronic Smoking, $^{\#}$*P*=0.074 vs. Chronic Smoking

6.0 DISCUSSION

6.1 Study 1

We have demonstrated that both acute and chronic smoking have detrimental effects on resting vessel hemodynamics in young healthy smokers compared to non-smokers. Importantly, by examining the response of the arteries to acute physical stress, we have been able to identify further differences between smokers and non-smokers that were not present at rest. The *arterial stress test* has revealed that smokers have an impaired ability of the arteries to adapt to the increased demands during acute physical stress. This study has also been the first to examine the chronic and acute effects of smoking, as well as nicotine exposure on vessel hemodynamics in the same group of smokers. This has allowed us to uncover a significantly impaired response of vessel hemodynamics to acute smoking, as well as establish a role for nicotine in this altered response.

Despite similar levels of baseline physical activity, smokers on all 3 conditions failed to achieve comparable **exercise time**, **max HR**, and **VO_{2max}** as non-smokers. These findings are in agreement with several other studies that have observed smoking-related impairments in exercise tolerance, and aerobic capacity, as well as an altered HR response[95,115-117,121]. A large body of evidence has demonstrated that decreased exercise time and VO_{2max} in smokers is largely a function of reduced O_2 carrying capacity[135-138]. It is well understood that smokers chronically have higher levels of HbCO in their blood; non-smokers typically have less than 2% HbCO, however chronic smokers can have HbCO levels between 5 and 10%[93,136]. Ekblom and Huot observed that HbCO levels of 7% led to a 9% reduction in VO_{2max} of young healthy non-smoking subjects after CO inhalation[135]. Interestingly, Horvath et al. further demonstrated that the critical level at which HbCO influenced VO_{2max} in young healthy subjects after CO inhalation

was as low as 4.3%[136]. Such levels of HbCO also significantly reduced exercise time in these individuals. More recently, Kobayashi et al. demonstrated similar findings in a population of young healthy smokers, whereby HbCO levels of 5.5% in smokers led to a significantly lower VO_{2max} and exercise time[138]. It has also been suggested that increased HbCO significantly reduces O_2 transport to the mitochondria via myoglobin due to the far greater affinity of myoglobin for CO than O_2[137]. While we have not measured HbCO levels in our study population we have observed lower exercise time as well as a trend for lower VO_{2max} in smokers that may be attributed to increased HbCO levels and a decreased O_2 carrying capacity of the blood. In contrast to these studies, others have reported no significant differences in physical fitness of VO_{2max} between smokers and non-smokers in populations of young highly active individuals such as elite sportsmen and military conscripts[125,126], suggesting that physically active individuals are less susceptible to the destructive effects of smoking on exercise capacity. However, we have demonstrated smoking-related impairments in exercise capacity despite including an overall active population of smokers and non-smokers in our study (as determined by high IPAQ scores) in both groups.

Smoking is also known to affect the ability to increase one's HR in response to exercise[124]. In our study, smokers on all 3 conditions had a significantly lower **max HR** compared to non-smokers. Although this attenuated response does not meet the requirements of chronotropic incompetence (defined as the inability to reach 85% of the maximal age-predicted HR) we have still seen that smokers on all 3 conditions failed to achieve a comparable **%predMaxHR** as their non-smoking counterparts. These findings are in line with other studies in young smoking individuals[74,76,95,121]. It has been proposed that this impaired exercise-induced HR response in smokers could be a function of chronic increases in sympathetic tone, and a corresponding

down-regulation of β-adrenergic receptors[124,139].

Among smokers **VO_{2max}**, **exercise time**, **max HR** and **%predMaxHR** were highest after nicotine exposure. Although this did not reach significance, the higher values that we observed are in line with findings from other studies demonstrating that nicotine administration during exercise delays fatigue and significantly improves exercise time during stress testing[140]. Nicotine exerts these effects through the activation of the sympathetic nervous system (through β-adrenergic receptors) leading to increased catecholamine release, and subsequent increases in HR, BP, stroke volume, cardiac output, coronary blood flow, as well as skeletal muscle vasodilation[141]. Despite nicotine's ability to improve performance in chronic smokers, it still exerts harmful cardiovascular effects, and could be responsible for the diminished performance upon abstinence from nicotine[142].

Through similar mechanisms, nicotine is also primarily responsible for the acute increase in **resting HR** and **$_{P}$SBP** observed in smokers on the acute smoking condition. Studies have confirmed elevated plasma nicotine levels immediately after smoking or chewing nicotine gum[37,143], and have also demonstrated a dose-related effect of nicotine on increases in HR and BP[144]. Our findings are also in line with other studies that observed acute increases in resting HR and $_{P}$SBP in young smokers[74,76,95,121].

We observed higher resting values of **PWV** in chronic smokers in both the central elastic arteries (**cfPWV**), as well as the peripheral muscular arteries (**crPWV**), however, significance was lost after adjustment for covariates such as age, sex, and MAP, as well as BMI and resting HR in the case of cfPWV. Yet, this may be a result of over adjustment since there were no significant differences in age or BMI between smokers and non-smokers in our study population. While smoking has been associated with increased PWV[81,82,86,145] the majority of studies

examining the effects of smoking on various measures of PWV in younger healthy individuals have not found significant differences between chronic smokers and non-smokers[83-85]. On the other hand, acute smoking increased both cfPWV and crPWV, however significance only remained for crPWV after appropriate adjustments for MAP. Therefore, we can infer that the increased arterial stiffness in the muscular arteries in response to smoking occurs independently of acute smoking-induced increases in MAP. Lemogoum et al. have reported similar findings of increased cfPWV and crPWV in response to acute smoking in a young population of habitual smokers[75]. Interestingly, the increase in PWV in their study was directly related to plasma cotinine levels, the principle metabolite of nicotine. In response to acute exercise, we observed a significant increase in cfPWV in all groups. On the other hand, crPWV increased to a smaller extent, and did not yield significance. Both cfPWV and crPWV subsequently decreased towards baseline levels by 10-15 minutes. These findings are in agreement with other studies investigating the effect of acute bouts of exercise on central stiffness[106], as well as previous studies at the Vascular Health Unit[104]. Comparing the relative increase in cfPWV at 2 minutes revealed that smokers had a significantly diminished increase after acute smoking in comparison to their chronic condition. The relative increase in response to nicotine was intermediate between acute smoking and chronic smoking. Therefore, this suggests that acute exposure to cigarette smoke, perhaps mediated in part by nicotine, leads to a blunted ability of the arteries to accommodate the increased demands of exercise.

We have also demonstrated increased systemic arterial stiffness in smokers, as evidenced by significantly higher resting values of **AIx75** in smokers on all 3 conditions compared to non-smokers. Non-smokers exhibited 'healthy' negative values of AIx75, indicating no augmentation of central systolic pressure in the aorta upon the return of the reflected wave. However, smokers

exhibited significantly more positive values of AIx75 revealing increased wave reflection in the aorta, and subsequently increased **$_{C}$SBP**. Our results showed higher unadjusted $_{C}$SBP in smokers under all 3 conditions compared to non-smokers. While significance was lost after adjustment for age, sex, and BMI on the chronic and nicotine condition, we still observed significantly greater $_{C}$SBP after acute smoking. In response to acute exercise, we have observed a significant increase in AIx75 in smokers and non-smokers, which is in line with other studies[146,147]. We further observed marked differences in the exercise-induced increase in AIx75, whereby non-smokers were able to increase from very negative values to similar peak values as chronic smokers. As a result, non-smokers exhibited a significantly greater AUC in response to exercise. The increase in the speed and magnitude of wave reflections as indicated by higher values of AIx could be the result of increased ET-1 mediated vasoconstriction of the arteries to renal and splanchnic circulations[100]. Therefore, a greater exercise-induced increase in AIx in non-smokers compared to chronic smokers could reflect a superior redistribution of blood flow through the combined actions of NO and ET-1[100].

Our results also contribute to the current evidence that chronic smoking is associated with lower resting values of **PPA**[72,76]. This is considered to be the result of increased wave reflection, which disproportionately increases the $_{C}$PP over peripheral $_{P}$PP. On the other hand, acute smoking led to a significant increase in PPA reaching similar levels to non-smokers. However, this acute increase in PPA is likely HR dependent, since a rise in HR directly influences the timing of wave reflection. Wilkinson et al. have previously demonstrated through atrial pacing that increasing HR leads to a significant and linear rise in PPA as a consequence of reduced wave reflection, and a diminished augmentation of $_{C}$SBP[58].

At rest, there were no significant differences in **SEVR** between chronic smokers and non-

smokers. However, SEVR was significantly decreased after acute smoking compared to the chronic smoking condition, as well as non-smokers. Once again, this may be partly attributed to the elevated HR in response to acute smoking. Increased HR not only increases myocardial demand for O_2, but it has also been shown to reduce the time for diastolic filling and myocardial perfusion[148,149]. Moreover, acute smoking can further impact the supply of O_2 through CO and ROS[5]. Therefore, lower SEVR in smokers after acute smoking is indicative of a diminished ability to achieve an optimal ratio of myocardial O_2 supply vs. demand. Immediately after acute exercise, there was a significant reduction in SEVR among all groups, with the greatest decrease observed in non-smokers. While such a decrease in SEVR would be considered detrimental at rest, non-smokers may be able to stress themselves more and better maximize the ability of their cardiovascular system to meet increasing O_2 demands during exercise. This is evidenced by the fact that the SEVR in non-smokers decreased by more than half to levels of approximately 80% (**Figure 1.3**). Therefore, non-smokers may be able to get closer to the point of ischemia without actually becoming ischemic (SEVR<50%). Furthermore, despite adjustments for exercise time, the AUC was significantly greater (more negative) in non-smokers compared to chronic smokers, as well as smokers after acute smoking and nicotine exposure. Among smokers, acute smoking further inhibited the favourable exercise-induced decrease in SEVR leading to a significantly lower AUC.

Overall, the effect of nicotine on various indices of vessel hemodynamics has yielded an effect that is intermediate between the chronic and acute conditions. While nicotine (via catecholamine release and increased HR) may contribute to many of the smoking-associated detrimental effects on many of these parameters, other components of the cigarette smoke have evidently also contributed to the smoking-related alterations in vessel hemodynamics. As

discussed, CO leads to significant impairments in the O_2 carrying capacity of the blood, and reduces the delivery to the working tissues, including the heart. Furthermore, free radicals contained within the cigarette smoke can acutely reduce NO bioavailability, leading to an impaired vasodilatory response and increased arterial stiffening. Smoking can also acutely induce oxidative stress, leading to endothelial dysfunction, and increased arterial stiffness[75].

Important strengths of this study include the implementation of strict inclusion/exclusion criteria. The inclusion of young healthy individuals in our study has allowed us to minimize important confounding effects such as age, BMI, medications, hypertension and other cardiovascular or inflammatory diseases and isolate the effect of smoking and nicotine alone on vessel hemodynamics and exercise capacity. As a result, we observed no significant differences in age or BMI in our study population. However, we have taken a conservative approach by still including them as covariates in our model. Moreover, our inclusion of relatively light smokers (median 4.5 packs/year) in our study has allowed us to evaluate a less-likely scenario to elicit vascular alterations than if we had included heavy smokers with more long-term vascular damage. Furthermore, no previous studies have assessed the acute and chronic effect smoking, as well as the effects of nicotine exposure alone in the same group of smokers. This has allowed us to examine the direct effects of nicotine on arterial stiffness, separately from the other harmful compounds contained within cigarette smoke. While it would have been preferable to also assess the acute effect of nicotine on arterial stiffness in non-smokers, the MUHC Research Ethics Board deemed this unethical. Furthermore, the use of AUC in our study has not only allowed us to quantify the 'vascular reserve' (including the recovery phase), but it has also strengthened our statistical approach in handling this data. Calculation of the AUC has allowed us to create a single variable that summarizes multiple measurements over time, and importantly has increased

the power of our analyses by not sacrificing the individual contributions of each measurement time point[150].

This study also has certain limitations. We were not able to include equal numbers of smokers and non-smokers in our study. However, including a larger number of non-smokers (n=80) allowed us to increase our sample size substantially allowing us to attain a high-degree of statistical power despite a 2:1 ratio[151]. Furthermore, since we enrolled consecutive eligible subjects we were not able to match for age, sex, or BMI. While there were no significant differences in age, or BMI, we did observe significant differences in the sex distribution between smokers and non-smokers. Therefore, we adjusted for sex in all of our analyses and further adjusted for age and BMI since our sample size allowed us to. Due to the technical limitations of applanation tonometry under circumstances of vigorous exercise, we were not able to measure arterial stiffness during exercise, only immediately post-exercise. However, the purpose of the study was to examine the maximal response of the arteries to acute physical stress. Due to time constraints we were only able to measure select indices of arterial stiffness at the 2-minute time point; however we chose to measure cfPWV since it is the most reliable measure of central stiffness. For the analyses presented herein we have only ensured by self-reporting that smokers abstained from smoking for 12-hours before each of their assessments. However, we will validate these responses by measuring the levels of cotinine, the principle metabolite of nicotine, in collected saliva samples upon termination of the SMOKELESS study.

Since our study included women, we initially ensured that all women were examined during the early follicular phase of the menstrual cycle to minimize the effect of hormonal changes on arterial stiffness, as the evidence was controversial at the time[152,153]. However, during this time, a parallel study at our Vascular Health Unit demonstrated that the natural menstrual cycle (early

follicular, late follicular and luteal phases) had no effect on arterial stiffness in young healthy women (n=36)[154]. These findings were also in line with an earlier smaller study by Robb et al. (n=10), which also showed no effect of the natural menstrual cycle on arterial stiffness and hemodynamics[155]. Therefore, from this point onwards, women in the SMOKELESS study (n=16 out of 33) were assessed during any phase of the menstrual cycle. Furthermore, taking into consideration existing evidence regarding the adverse cardiovascular effects of oral contraceptive pills (OCPs), women taking OCPs were initially excluded from the study. Subsequently, the same parallel study at our Vascular Health Unit compared arterial stiffness and hemodynamics in OCP users and OCP nonusers and demonstrated significantly higher $_{C}$SBP and $_{P}$SBP in the late follicular and luteal phases of the menstrual cycle, however observed no effect of OCPs on parameters of arterial stiffness[154]. As a result, women taking OCPs were included in the SMOKELESS study (n=4 out of 33).

In summary, smokers at rest had significantly elevated AP, AIx, AIx75, and decreased PPA compared to non-smokers. Smoking a single cigarette significantly increased HR, SBP, and PPA, while significantly lowered SEVR. In response to acute maximal exercise, smokers failed to achieve comparable exercise time, max HR, and VO_{2max} as non-smokers. Furthermore, the *arterial stress test* revealed that smoking appears to affect the ability of the vasculature to adapt to increased demands as chronic smokers did not achieve comparable exercise-induced changes in AIx and SEVR as non-smokers. Furthermore, acute smoking significantly affected smokers' ability to increase cfPWV in response to exercise. Therefore, the increase in central artery stiffness (cfPWV and AIx), and the decrease in SEVR, while detrimental at rest, may represent a beneficial physiological response to physical stress that is negatively affected by smoking.

6.2 Study 2

This study provides clear evidence for an acute decrease of circulating ET-1 in response to acute maximal exercise in young, active, healthy individuals. Furthermore, we have demonstrated for the first time a differential response of ET-1 to acute maximal exercise between non-smokers and smokers examined under 3 different conditions; chronic smoking, acute smoking, and nicotine.

The pre-exercise ET-1 levels were not significantly different between non-smokers and chronic smokers, however there was a trend for increased ET-1 levels after acute smoking when compared to chronic smoking. Although non-significant, the pre-exercise levels of ET-1 on the nicotine day were intermediate between the acute response to cigarette smoking and the chronic smoking condition. Previous studies have reported elevated plasma levels of ET-1 within 10 minutes of cigarette smoking or nicotine exposure alone[30,32,33]. Haak et al. studied 10 healthy male smokers, and found that smoking a high-tar cigarette led to a significant increase in venous ET-1 levels within 10 minutes, as well as increased HR and SBP[32]. Goerre et al. also demonstrated an acute increase (within 10 minutes) of venous ET-1 levels in young smokers (n=12) after cigarette smoking[30]. Subjects continued to smoke for the next 8-h (on average 15.6 cigarettes), but they found no significant increase in ET-1 levels at the 4-h or 8-h time-point[30]. They concluded that response of ET-1 levels to smoking is only transitory, occurring within the first 10 minutes as a response to hypoxia, or the direct effects of smoke components, such as CO, or tar[30]. However, they found no significant changes in ET-1 levels in non-smokers (n=11) receiving nicotine transcutaneously[30]. In contrast, a study by Letizia et al. in healthy, young non-smokers (n=10) did observe a significant increase in ET-1 levels within 15 minutes of chewing nicotine gum (2 mg)[33]. We found only a trend for increased ET-1 levels after acute smoking.

However, in our study, as per protocol blood was only drawn ≈ 25 minutes after cigarette smoking or nicotine gum, which may have resulted in a lower acute increase of ET-1 in response to both smoking and nicotine.

Exercise is known to cause a significant redistribution of blood flow, with increased blood flow (vasodilatation) to working muscles, and decreased blood flow (vasoconstriction) to the splanchnic circulation[103]. ET-1 has been shown to play an important physiological role in the redistribution of blood during exercise to ensure adequate perfusion of the exercising muscle [100]. Maeda et al. first demonstrated this effect in exercising rats by showing that the natural decrease in blood flow to internal organs, and corresponding increase to exercising muscles was abolished by the administration of an ET_A-receptor antagonist[100]. We found that ET-1 levels were significantly lower after an acute bout of exercise in non-smokers and smokers under all 3 conditions. While ET-1 may be acting locally on ET_A receptors to increase vasoconstriction of the arteries to internal organs, circulating ET-1 could be acting on ET_B receptors on the endothelium in the working limbs to enhance dilatation through the release of NO and PGI_2. When ET-1 acts on the ET_B receptor it is very rapidly internalized with the receptor and removed from the circulation[156]. The increased levels of NO in the circulation in response to exercise also have a direct inhibitory action on circulating ET-1, and could therefore explain the rapid decrease in ET-1 levels observed in our study[157,158]. Additionally, enhanced pulmonary blood flow during maximal exercise may be responsible for the rapid clearance of ET-1 from the circulation by the ET_B receptors in the lungs, therefore contributing to a decrease in circulating ET-1[159,160]. Since blood was taken within 3 minutes of acute exercise, we are likely capturing the initial vasodilator response of ET-1, which may then be followed by an increase in ET-1 levels after exercise, as reported by other studies[102,161]. For instance, a study by Maeda et al. measured

plasma ET-1 before and 30 minutes after low and high intensity cycle exercise (90% and 130% ventilatory threshold, respectively)[161]. Under both conditions they found a significant increase in ET-1 levels 30 minutes after exercise, with a greater increase observed after high intensity exercise. A study by Richter et al. in healthy young men found that exhaustive cycling led to a significant decrease in ET-1 within the first 30 minutes of exercise, which was followed by a gradual increase to resting levels by the termination of exercise[162]. These findings lend evidence to the biphasic response of ET-1 to exhaustive exercise[162].

In response to exercise, non-smokers had a significantly greater decrease in ET-1 levels than chronic smokers. The diminished decrease we observed in smokers could perhaps be explained by a shortage of bioavailable NO, either due to reduced NO synthesis and/or enhanced NO breakdown, two known consequences of chronic smoking[31]. As a result, smokers have been shown to have reduced basal NO-mediated vasodilation even at a young age before clinical signs of disease are present[36]. Therefore, the diminished decrease in ET-1 levels in smokers compared to non-smokers could be attributed to smokers' stunted ability to naturally increase NO levels during exercise. Furthermore, the greater decrease of ET-1 observed in non-smokers may be explained by more efficient pulmonary elimination of ET-1. For example, in our study, non-smokers demonstrated a greater exercise-induced increase in $FeCO_2$, Vt, VO_2, VCO_2, and PIF, compared to chronic smokers. This is similar to another study by Kobayashi et al., which also found significant differences in the cardiorespiratory response to exercise between young smokers and non-smokers, reporting higher VE, VE/CO_2, RR, Vt, and a faster HR recovery post-exercise in non-smokers[138]. These findings provide evidence that non-smokers may have superior gas exchange capacity compared to smokers, allowing for greater clearance of ET-1 by the lungs in response to exercise. Furthermore, in our study, we observed a relationship between

the decrease in ET-1 and exercise induced-changes in Ti, Ttot, RR, VI and PIF in non-smokers, indicating there may indeed be a link between respiratory capacity and ET-1 clearance in the lungs.

Among smokers on the 3 conditions, acute smoking led to the greatest decrease in ET-1, compared to chronic smoking, while the decrease was intermediate after nicotine exposure. Through action on the acetylcholine nicotinic receptors, cigarette smoking and nicotine exposure is known to acutely increase endogenous NO levels[163,164]. Therefore, the greater ET-1 decrease after acute smoking and nicotine vs. the chronic condition may be a function of a decreased ET-1 release into the circulation in response to increased inhibitory action of NO. Additionally, smoking one cigarette has been shown to cause a transient increase in airway blood flow, while nicotine, administered as both a nasal spray and oral inhaler, did not have these effects[165]. Therefore, it is possible that this increased blood flow after acute smoking facilitated a greater elimination of ET-1 in the pulmonary circulation, leading to an overall decrease in ET-1 that was greater than the chronic and nicotine conditions.

Limitations of this study include a relatively small sample size (n=70), however, previous studies on ET-1 had sample sizes closer to n=20[30,32,33,102,112,161,166-168]. Furthermore, the several measurement time-points/conditions greatly increased the power of the current study. We also ensured smokers and non-smokers were matched for sex, age, and BMI, and implemented strict exclusion criteria. Our protocol was designed to specifically study the response of ET-1 immediately after maximal exercise. In future studies we will measure ET-1 at several additional time points during the recovery period following exercise. Due to the study design and logistics we were not able to measure ET-1 earlier than 25 minutes (to avoid capturing the effect of the venopuncture on ET-1 level) and we may possibly have missed the acute response to smoking

and nicotine. However, due to hospital restrictions, the subjects could not have smoked nearer to the blood draw, and a resting period was necessary before obtaining the blood sample. Future studies could also focus on obtaining blood at several time points after cigarette smoking to determine how soon the "single cigarette effect" occurs. This could provide insight whether the cerebral effect of nicotine[169] could be influencing the subsequent systemic response within the vessels and ET-1 release. Furthermore, we have only speculated a role for nicotine in altering the response of ET-1 to exercise in smokers, however we could not test this response in the non-smoker group, since it was deemed unethical by the MUHC Research Ethics Board. Our findings would also be strengthened with simultaneous measurements of NO end-products, nitrite and nitrate, which would help to explain whether the rapid decrease in ET-1 that we have observed is a function of increased NO levels, or whether it simply reflects increased ET-1 uptake and/or cleavage. Also, plasma ET-1 levels do not adequately reflect local ET-1 levels. Since ET-1 binds tightly with its receptors with a low dissociation rate, only 20% of ET-1 accumulates in the blood, and therefore we are not capturing the complete response of ET-1 to exercise, smoking and nicotine[170]. Unfortunately, this is an inherent limitation of studying ET-1 levels in human subjects. Lastly, by only measuring ET-1 in our study we are not directly assessing endothelial dysfunction. Incorporating FMD in future studies could be used to more directly assess endothelial dysfunction in these young smokers. Interestingly, local isometric tests, such as a handgrip test, could be used to increase shear stress within the vessel for FMD assessment. Similar to our current design of using maximal exercise to stress the vascular system, handgrip exercise in conjunction with FMD testing has been used to identify underlying endothelial dysfunction in young healthy smokers[171].

In summary, we have clearly demonstrated that acute exercise to exhaustion leads to a significant decrease in ET-1 levels in young, active healthy individuals. However, we have also shown that smoking status may affect the response of ET-1 to exercise. Despite being young, active and otherwise healthy, chronic smokers were not able to achieve a comparable decrease in ET-1 as non-smokers. We propose this may be in part due to an underlying shortage of bioavailable NO and/or impaired respiratory clearance. However, future studies will be needed to confirm this, as well as to address the underlying mechanisms of this differential response. In line with previous research, we observed a trend for increase ET-1 in smokers after acute smoking, demonstrating that even one cigarette can trigger acute alterations in the vasculature and alter the response of ET-1 to exercise. By incorporating exhaustive acute exercise into our study design as a vascular stressor, we have taken a novel approach to provide evidence of an altered ET-1 response in smokers, which otherwise would not have been detected at rest. Despite our conservative approach of selecting young, active and relatively light smokers, we were still able to demonstrate an impaired cardiorespiratory response in smokers. Therefore, we have shown that our study design may serve as a useful model to identify early sub-clinical signs of endothelial dysfunction in young, otherwise healthy smokers.

7.0 FUTURE DIRECTIONS

An important future direction of this study includes the completion of a longitudinal follow up of both groups of smokers and non-smokers at 6-month intervals for 2 years. At each visit, all subjects undergo the same *arterial stress test* to access changes in resting vessel hemodynamics and the 'vascular reserve' over time. Smokers are assessed after 12-hours abstinence to evaluate the continuing chronic damage associated with smoking. This component of the study is still ongoing, but will allow for an evaluation of the progression of smoking-related vascular damage over time, and will help establish whether changes in smoking habits (increasing or decreasing pack-years) have a dose-related effect on the extent of vascular changes over time. Furthermore, since smoking cessation has been associated with significant reductions in cardiovascular risk relatively soon after cessation, another important aim of the longitudinal component of the study is to assess whether smoking cessation is associated with improvements in vascular health over the 2-year study period. As such, former smokers who had recently quit smoking (1-3 months) have been recruited and are currently being followed every 6 months for 2 years.

Future work will also include a complete analysis of cardiorespiratory parameters in the full study (Study 1). Since differences in exercise time, HR response, and VO_{2max} (trend) were noted in Study 2, a complete analysis of all cardiorespiratory parameters could further inform our findings on the negative implications of smoking on the cardiorespiratory response to acute physical stress in young healthy individuals. Additional blood has also been collected for analyses of other vascular markers including NO end-products as well as specific endothelium-derived circulating microRNAs that have been associated with vascular damage and are dysregulated in response to smoking. These blood markers have not yet been examined in young smokers at rest or after physical stress. In this context, they have the potential to improve our

understanding of the mechanisms through which smoking affects arterial stiffness and endothelial dysfunction in young healthy individuals.

Beyond the scope of this study, there is still a critical need to better understand the role for nicotine, and the many other toxic components of cigarette smoke on the development and progression of endothelial dysfunction and arterial stiffness. Recently there has been growing evidence demonstrating that endothelial dysfunction and arterial stiffness are to some extent reversible, especially in young individuals[172]. Therefore future research should also focus on the development of therapeutic strategies that might reverse these effects and improve endothelial function in these individuals.

An important future clinical direction would be to examine the predictive value of the *arterial stress test* in identifying younger individuals who are at a greater risk of poor cardiovascular outcomes. Currently, CVD risk scores only include smoking as a binary risk factor (presence or absence of smoking) and therefore can often underestimate a person's risk. As such, the quantification of the 'vascular reserve' through the *arterial stress test* provides the opportunity to greatly improve risk assessment in younger, clinically asymptomatic individuals. Therefore, future studies should aim to test the applicability of this model, as well as define appropriate reference values.

8.0 CONCLUSIONS

In conclusion, the findings from these studies demonstrate that acute and chronic cigarette smoking leads to an altered endothelial and vessel hemodynamic response even in young healthy relatively light smokers. On a chronic basis, smokers have increased central arterial stiffness and impaired exercise capacity. Acute smoking, mediated primarily through nicotine, further aggravates vessel hemodynamics by significantly increasing HR, BP, and PPA while significantly lowering SEVR. Taken together, these results substantiate existing evidence that smoking through its acute alterations in vessel hemodynamics triggers an overall deterioration of vascular health, even in young smokers. By incorporating exercise as a vascular stressor in our study (*arterial stress test*), we have taken a novel approach to provide further evidence of dysfunction in smokers, including an altered ET-1 and cardiorespiratory response, as well as a blunted ability of the arteries to respond to the increased physical demands.

Altogether, we have also shown that the *arterial stress test* may serve as a useful tool to identify vascular impairment in young healthy smokers at an early stage before clinical signs of dysfunction become apparent. Our ability to quantify these changes through the 'vascular reserve' may allow us to better monitor clinical risk, and could provide an opportunity for more targeted and individualized interventions at the pre-clinical level. However, most importantly, our findings stress the need to encourage smoking cessation in these young individuals imminently in order to prevent further vascular impairment, and adverse outcomes later on in life.

APPENDICES

Appendix A – SMOKELESS Study Advertisements

Centre universitaire de santé McGill
McGill University Health Centre

CIHR IRSC

"SMOKELESS" STUDY
Looking for MEN and WOMEN

- Study conducted at the Vascular Health Unit of the Montreal General Hospital by Dr. Stella Daskalopoulou
- Looking for healthy **SMOKERS**, **NON-SMOKERS**, and **RECENT FORMER SMOKERS (have recently quit) aged 20-45 years**
- Smokers come in for 3 separate assessments and non smokers only once (each assessment = 1.5hrs)
- Involves a questionnaire, safe and painless arterial pulse measurements on the skin, exercise on a treadmill and small samples of blood and saliva
- **Compensation** will be provided

For additional information please contact Alexandra Cooke, study coordinator at: cigarette.smoking.study@gmail.com or 514-934-1934 ext. 42478.

Centre universitaire de santé McGill
McGill University Health Centre

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ÉTUDE "SMOKELESS"
RECHERCHONS HOMMES et FEMMES

- Étude menée à l'Hôpital général de Montréal par Dr. Stella Daskalopoulou
- Recherchons **FUMEURS**, **NON-FUMEURS** et récents **EX-FUMEURS âgé de 20-45** en bonne santé
- Comprenant **1 ou 3** évaluations d'environ 1.5 heures
- Remplir un questionnaire, une évaluation non-invasive de votre rigidité artérielle, de l'exercise sur tapis roulant et petits échantillons de sang et salive
- **Compensation** offerte

Pour plus d'information, veuillez contacter Alexandra Cooke, coordonnatrice de l'étude à cigarette.smoking.study@gmail.com ou 514-934-1934 ext. 42478

Appendix B – Eligibility Questionnaire

The Cigarette Smoking Study – Participant Information and Questionnaire

Name:	
Phone Number:	
E-mail:	

Instructions
This questionnaire will help the study researchers determine if you are eligible to participate in the study. Please fill out this questionnaire completely and e-mail it to cigarette.smoking.study@gmail.com.

Please note that all of your answers will only be read by the study researchers and are completely confidential.

Introduction
This research study will be investigating the function of the arteries in smokers and non-smokers before and after exercise. The principal investigator of the study is Dr. Stella Daskalopoulou from the Department of Medicine at the Montreal General Hospital.

Study Procedures
Below you will find the study protocol depending on if you are a **Smoker, Non-Smoker, or Former Smoker**.

Non-Smokers
Non-smokers initially need only to come in for **1 assessment on one day**. Non-Smokers are also expected to be available for a follow-up assessment every 6 months for a period of 2 years. The study protocol is as follows:

1. Fill out a questionnaire and consent form (approximately 20 minutes).
2. Arterial stiffness measurements (approximately 20 minutes)
    a. In this part of the tests you will lie down on a bed and we will take your blood pressure.
    b. Next we will measure your arterial stiffness non-invasively by placing a pen-shaped device on your skin above three arteries: radial artery (on your wrist), carotid artery (on your neck), and femoral artery (on your inner thigh). Because the femoral artery is in an awkward spot, we usually ask that you wear loose shorts so we can access it easily.
    c. During these procedures we will also have you hooked up to an electrocardiogram (ECG). We will place 4 electrodes on the chest and abdomen**.**
3. A small sample sample of blood will be drawn by a Research Nurse.
4. Exercise test (approximately 10-20 minutes).
    a. In this part of the test you will run on a treadmill and we will increase the speed and the incline of the treadmill every 3 minutes until you are exhausted. During this portion of the assessment we use a Metabolic Cart to record properties of your exhaled air.
    b. Your exhaustion point is based on when you think you cannot run anymore.
5. Blood Pressure and arterial stiffness measurements are repeated (aproximately 20 minutes) after exercise.
6. A small sample of blood will be drawn by a Research Nurse.

Recent Former Smokers
The study protocol is exactly the same as that for Non-Smokers (shown above). Recent Former Smokers are expected to be available for a follow-up assessment every 6 months for a period of 2 years.

Smokers
Smokers must initially come in for **3 assessments on three seperate days**. The study protocol for smokers is the same as that for Non-Smokers except that before exercise you will either smoke a cigarette, not smoke a cigarette, or chew nicotine gum. You will do one of these on each test day and will only need to fill out a questionnaire on the first day. Smokers are expected to be available for a follow-up assessment every 6 months for a period of 2 years.

The compensation for this study is $30/assessment.

If participating in this study interests you please fill out the questionnaire below.

1. How did you learn about our study?
   - ☐ McGill Ads
   - ☐ Kijiji
   - ☐ Craigslist
   - ☐ Posters
   - ☐ Heard from others
   - ☐ Other (please specify)

2. Are you presently a cigarette smoker?
   - ☐ Yes
   - ☐ No

   If **YES**, how many cigarettes do you smoke per day? How many years have you smoked for?

3. Have you ever stopped smoking or tried to quit smoking?
   - ☐ Yes
   - ☐ No

   If **YES**, when and for how long (provide dates if you can)?

4. If you are a non-smoker have you ever smoked **Cigarette or Sheehsa** in your life ?
   - ☐ Yes
   - ☐ No

   If **YES**, please indicate, what and when did you smoke, for how long, and how many cigarettes/sheesha did you smoke (provide dates if you can)?

5. Part of the assessment restrictions is that you cannot smoke for 12 h before the tests. Can you abstain from smoking for 12 h before the tests (keep in mind that we can schedule you early in the morning if you prefer)?
   - ☐ Yes
   - ☐ No

6. Where are you from/born?

7. What is the ethnic background of your biological mother?

8. What is the ethnic background of your biological father?

9. What is your birth date (DD/MM/YYYY)? _____/_____/_____

10. What is your height and weight? Height: Weight:

11. Do you have any kind of a restrictive diet (i.e.: vegetarian, vegan, any kind of food restrictions, any kind of food allergies)

☐ Yes

☐ No

If yes, please explain:

12. Do you play sports/exercise regularly?

☐ Yes

☐ No

If yes, please ellaborate and tell us what types of exercise you do and how many times per week you do it:

13. Are you a student?

☐ Yes

☐ No

If yes, what year are you in?

14. How many years are you planning on being in Montreal for? years

15. Are you in Montreal for the summers?

☐ Yes

☐ No

16. When are your availabilities/the best times for you to come in? (check all that apply)

Monday	☐AM	☐PM
Tuesday	☐AM	☐PM
Wednesday	☐AM	☐PM
Thursday	☐AM	☐PM
Friday	☐AM	☐PM

17. Please indicate how often you use the following illicit drugs:

Type	Frequency of Consumption				N/A Not Applicable
	Daily	Weekly	Monthly	Other (Please Specifiy)	
Marijuana	☐	☐	☐		☐
Cocaine	☐	☐	☐		☐
MDMA	☐	☐	☐		☐
Heroin	☐	☐	☐		☐
Other Please specify:	☐	☐	☐		☐

18. Are you a coffee drinker?

☐ Yes

☐ No

19. One of the assessment restrictions is that you cannot consume caffeine for 12 h before the tests. Can you abstain from coffee for 12 h before the tests?

☐ Yes

☐ No

20. How would you rate your health:

☐ Excellent

☐ Very good

☐ Good

☐ Poor

☐ Very poor

21. What medications do you take? Please list all of them including over the counter medications (i.e. gravol, tylenol, aspirin), oral contraceptives (birth control), antibiotics, or <u>any</u> other type of medications.

22. Do you take any kind of nutritional supplements? Please list them.

23. Has a doctor ever told you that you have high blood pressure?

☐ Yes

☐ No

If yes, when?

24. Has a doctor ever told you that you have high cholesterol?

☐ Yes

☐ No

If yes, when?

25. Has a doctor ever told you that you have diabetes?

☐ Yes

☐ No

If yes, when?

26. Have you ever had any of the medical procedures done or do you have any of these conditions? Please note, that most of these you will probably never have heard of unless you or a family member has had them.

MEDICAL CONDITIONS	YES	NO
· Neurological disease (muscle or nerves)	☐	☐
· Lung disease (emphysema, bronchitis or asthma)	☐	☐
· Thyroid disease	☐	☐
· Migraine headache(s)	☐	☐
· Peptic ulcer/stomach problems	☐	☐
· Bowel disease (colitis, diverticulitis or irritable colon)	☐	☐
· Kidney disease	☐	☐
· Arthritis (joint pain) / rheumatoid artritis / lupus or other autoimmune disease	☐	☐
· Cancer, (specify type):	☐	☐
· Osteoporosis (fragile bones)	☐	☐
· Digestive system (cirrhosis, hepatitis, pancreatitis, gallbladder)	☐	☐
· Depression, anxiety or other emotional problems	☐	☐
· Blood disorders/ anemia, thrombocytosis?	☐	☐
· Alcoholism	☐	☐
· Heart attack or myocardial infarction	☐	☐
· Irregular heart rhythm	☐	☐
· Angina or chest pain from heart disease	☐	☐
· Heart defects from childhood	☐	☐
· Congestive heart failure	☐	☐
· Blocked arteries / neck or brain	☐	☐
· Heart valve problems (which valve)	☐	☐
· Blocked arteries in legs	☐	☐
· Rheumatic fever	☐	☐

· Thrombophlebitis / clot in leg veins	☐	☐
· Stroke / mini stroke	☐	☐
· Aortic aneurysm	☐	☐
· Clot in lungs	☐	☐
· Coronary angioplasty or stent placement	☐	☐
· Coronary artery bypass surgery	☐	☐
· Valve surgery	☐	☐
· Artificial pacemaker or defibrillator	☐	☐
· Surgery or angioplasty for carotid or peripheral disease	☐	☐

24. If you are **female**:
    a. Do you have regular periods? ☐ Yes ☐ No
    b. When is the start of your next period (please provide a date)?
    c. How long is your cycle (i.e. how many days between the start of your periods)?
    d. How many days do your periods usually last

Thank you very much for your time. Please e-mail this questionnaire back to cigarette.smoking.study@gmail.com. We will look over your answers and determine if you are eligible for the study. If you are eligible we will contact you and set up an appointment.

Appendix C – Consent Form

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INFORMED CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

MP-CUSM-08-020 GEN - Acute and Chronic Effect of Smoking on Vessel Hemodynamics and Metabolic Parameters at Rest and Exercise

RESEARCHERS

Main Investigator: Stella S. Daskalopoulou, MD, MSc, PhD, Division of General Internal Medicine
Co-Investigator: Francesco Carli, MD, MPhil, FRCPC, Department of Anesthesia

SPONSOR

The Research Institute of McGill University Health Centre (RI MUHC) will sponsor this study. The research will be conducted at the Montreal General Hospital (MGH).

INTRODUCTION

You are being asked as a smoker, a non-smoker (healthy volunteer) or a former smoker to participate in a research study designed to assess the acute and chronic effect of smoking on the function of the body at rest and exercise.

Before you decide to participate, it is important to carefully read through and understand the content of this consent form. Make sure all your questions are answered and take your time making a decision. If you decide to participate in this study, you will be asked to sign this consent form.

PURPOSE OF THE STUDY

Smoking causes hardening of the arteries and compromises the ability to exercise. The purpose of this study is to assess the effect of smoking, both acute and chronic, on the arteries of young healthy people without any known health problems, at rest and after exercise.

STUDY PROCEDURES

If you agree to take part in this study, you will be asked to undergo the following procedures, which are only for the purpose of this study.

For all participants:

At your initial clinical visit and again at 6 month invervals for 2 years (6, 12, 18 and 24 months):

- Fill out a questionnaire regarding your past medical history, medication, health status, family history, lifestyle habits, as well as reproductive history (for women). The questionnaire will take approximately 15 minutes to complete;
- Your blood pressure, heart rate, weight, height, waist circumference and hip circumference will be measured;
- Your arterial function before and after exercise will be measured. Exercise refers to an incremental exercise test on a treadmill to volitional exhaustion;
- 6ml (1 teaspoon) of blood will be drawn from you before and after exercise;
- A small sample of saliva will be taken;
- The functional parameters of your heart and lungs during exercise will be measured;

Additional visits if you are a current smoker:

Your initial clinical visit will consist of **three** appointments on **three** different days:

- For all three appointments, you will be asked to abstain from smoking for at least 12 hours prior to your appointment.

- At each of the three appointments you will also be asked to do one of the following, in random order: **a)** only abstain from smoking; **b)** abstain from smoking and smoke a cigarette on site; **c)** abstain from smoking and chew nicotine gum on site.
- At each appointment your arterial function before and after exercise will be measured again as will the functional parameters of your heart and lungs during exercise.

You will be asked to come in for one follow-up visit at 6, 12, 18 and 24 months at which time you will also abstain from smoking for at least 12 hours.

The duration of these procedures is approximately 90 minutes (1 and 1/2 hour) per session. All tests are non-invasive.

In addition:

Some of you will be asked to come in for an additional session(s) to perform a less intense exercise program on a treadmill and/or bicycle. The same rest/exercise procedures will be repeated as described above.

Your participation in the research study will be arranged at your convenience.

The procedures explained above are only for research purposes.

POTENTIAL RISKS

The only known risks associated with this study are with the drawing of blood. The taking of blood samples may cause some discomfort, fainting, formation of a small blood clot or swelling of the vein on surrounding tissue, bleeding from the puncture site, and /or rarely an infection. There is a possibility that you may faint, however, precautions will be taken to ensure your safety should this occur.

Foreseeable harm is minimal but possible with exercise. Some physical injury may occur while exercising on the treadmill. To minimize the risk of injury a trained person will familiarize you with the equipment prior to your exercise session.

POTENTIAL BENEFITS

You should not expect any direct benefits from participating in this study. However, the information collected during this research study may benefit future subjects.

INDEMNIFICATION

The MUHC, the RI MUHC, and the investigators would not be able to offer compensation in the unlike event of any injury resulting from your participation in this research study. However, you are not giving up any of your legal rights by signing this consent form and agreeing to participate in this study.

COST

There will be no cost to you for participating in this study.

COMPENSATION

Compensation of $30 (per visit) for expenses (e.g., transportation costs, snacks) will be provided.

CONFIDENTIALITY AND ACCESS TO MEDICAL RECORDS

The team of researchers of the MUHC will consult your medical file to take note of the relevant data to this research project.

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COST

There will be no cost to you for participating in this study.

COMPENSATION

Compensation of $30 (per visit) for expenses (e.g., transportation costs, snacks) will be provided.

CONFIDENTIALITY AND ACCESS TO MEDICAL RECORDS

The team of researchers of the MUHC will consult your medical file to take note of the relevant data to this research project.

All information obtained during this study will be kept strictly confidential. Your name will be coded and the code list will be locked in a filing cabinet in the investigator's office with limited access. The results from this study may be published, and other physicians participating in this research study may have access to your records related to this research study; however, your identity will not be revealed in the combined results. In order to verify the research study data, monitors from the United States Food and Drug Administration (FDA), the Canadian Therapeutic Products Directorate (TPD), or the Quality Assurance Officer at the MUHC-Research Ethics Boards may review these records.

By signing this consent form, you give us permission to release information regarding your participation in this study to these entities. Your confidentiality will be protected to the extent permitted by applicable laws and regulations.

VOLUNTARY PARTICIPATION AND/OR WITHDRAWAL

Your participation in this research project is voluntary. Your decision not to participate in the study or to withdraw from it will not have any impact on the quality of care and services to which you are entitled or your relationship with the researcher in charge of the project and the other caregivers. You can also withdraw from the project at any moment, without giving any reason, by informing the researcher in charge of the project or one of the members of the research team. If you withdraw or are withdrawn from the project, the information that was already collected in the course of the project will be stored as long as necessary, to ensure your safety as well as the safety of the other research subjects and to meet the regulatory requirements.

CONTROL OF THE ETHICAL ASPECTS OF THE RESEARCH PROJECT

The Research Ethics Board of the MUHC approved this research project and guarantees the follow-up. In addition, it will first approve any review and amendment made to the information/consent form and to the study protocol.

STUDY RECORDS RETENTION POLICY

For security purposes, especially to be able to communicate with you rapidly, your family name, first name, coordinates and the start and end date of participation in the project would be stored for one year after the completion of the project in a separate registry maintained by the researcher in charge of the project or by the institution.

You have the right to consult your study file in order to verify the information gathered and to rectify it if necessary, as long as the project researcher or the institution holds this information. However, in order to protect the scientific integrity of the research project, you would have access to certain information only once this project has come to an end.

QUALITY ASSURANCE PROGRAM

The MUHC implemented a Quality Assurance Program that includes active continuing review of projects (on site visits) conducted within our establishment. Therefore, it must be noted that all human subject research conducted at the MUHC or elsewhere by its staff, is subject to MUHC Routine and Directed Quality Improvement Visits.

CONTACT INFORMATION AND/OR QUESTIONS

If you have any questions regarding the study, you should contact the investigator:

Dr Stella S. Daskalopoulou at 514-934-1934, ext 42295.

If you have any questions regarding your rights as a research subject in the study, enquires are appropriately directed to the Ombudsman for the MUHC This person is Lynne Casgrain at 514-934-1934, ext. 48306.

DECLARATION OF CONSENT

I have read the content of this consent form, and I agree to participate in this research study. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I will be given a copy of this signed consent form. By signing the consent form, I have not given up any of my legal rights.

Participant's Signature _____ Date _____

Printed Name _____

I have explained the research to the participant and, to the best of my knowledge, the participant has understood the proposed research and freely consented to research participation.

Investigator's Signature _____ Date _____

Printed Name _____

Appendix D – SMOKELESS Datasheet

Non-Smoking Protocol

Subject ID:	
Birthdate (DD/MM/YYYY):	
Height (cm):	
Weight (kg):	
Waist Circ. (cm):	
Hip Circ. (cm)	

Date:	
Time:	
Room Temp (˚C):	
Room Humidity :	

☐ **Saliva Sample**

☐ **Blood Sample (PRE)**

☐ **Trials picked**

Time since Caffeine:_____

Alcohol:_____

Smoking:_____

Physcial activity:_____

Phase 1: Resting Hemodynamic Measurements

Reading	1 (Discard)	2	3
Brachial BP			
Average	**XXXX**		

Central BP SBP/DBP(MAP)PP		
AP		
AIx		
AIx (HR Corr.) HR		
crPWV HR		
cfPWV HR		
SEVR		

Anthropometric Measurements

Carotid dist. (cm)	
Radial dist. (cm)	
Femoral dist. (cm)	

Phase 2: Exercise Metabolic Measurements

Time (min)	Heart Rate	Speed (mph)	Incline (%)	Borg Rating Pulmonary	Borg Rating Legs
0		3.0	0		
1					
2					
3		1.7	10		
4					
5					
6		2.5	12		
7					
8					
9		3.4	14		
10					
11					
12		4.2	16		
13					
14					
15		5.0	18		
16					
17					

Time to Exhaustion	
Max HR	
Max RER	
VO_{2max}	

☐ Blood Sample (POST)

☐ Borg Scale (POST)

Phase 3: Post-Exercise Hemodynamic Measurements

Measurement	2 min.	5 min.	10 min.	15 min.	20 min.
cfPWV HR					
BP					
Central BP (MAP) PP	XXX				
AP	XXX				
AIx	XXX				
AIx (HR corr.) HR	XXX				
crPWV HR	XXX				
SEVR	XXX				

Appendix E – SMOKELESS Questionnaire

Acute and Chronic Effects of Smoking on Vessel Hemodynamics and Metabolic Parameters at Rest and Exercise ❒

The Effect of Ovarian Hormones and the Menstrual Cycle on Vessel Hemodynamics (CYCLIC) ❒

Date: __/__/__ (Day/Month/Year) Patient code # ⌊⌊⌊

Ethnic Background

1. Were you born in Canada?
   ❒ Yes ❒ No, specify country of birth:
2. When did you move to Canada? Year: _____ ❒ N/A
3. People living in Canada come from many different cultural and geoethnic backgrounds. Are you?
   - ❒ South Asian (e.g. East Indian, Pakistani, Sri Lankan, etc.)
   - ❒ Southeast Asian (e.g. Cambodian, Indonesian, Laotian, Vietnamese, etc.)
   - ❒ European (Anglosaxon, French, Italian, Greek, Spanish, German, Slavic, Scandinavian, etc)
   - ❒ Chinese
   - ❒ Filipino
   - ❒ Latin/Hispanic American
   - ❒ African (African /African American)
   - ❒ Arab
   - ❒ Japanese
   - ❒ Korean
   - ❒ Indigenous / Aboriginal / Native American
   - ❒ Middle Eastern / North African (Afghan, Algerian, Moroccan, Egyptian, Iranian, Iraqi, Israeli, Palestinian, Syrian, Tunisian, Turkish etc.)
   - ❒ Caucasian

   Other (specify): _____
4. What is the cultural and racial background of your mother? _____
5. What is the cultural and racial background of your father? _____
6. What is your mother tongue? _____

Health in General

7. How would you rate your health in general, compared to people your age?

❒ Excellent ❒ Very Good ❒ Good ❒ Poor ❒ Very Poor

Medication Use

8. Do you take aspirin? ❒ Yes ❒ No
   If yes, when did you start? _____/____
   Month/Year
9. Check the aspirin that you are taking:
   ❒ Adult (325 mg)
   ❒ Baby (80 mg)
   ❒ Other (specify):_____
10. How often do you take the aspirin?
   ❒ Once per day
   ❒ Number of times per week: _____
   ❒ Other (specify): _____

11. List all **prescribed** medications that you are currently taking (including aspirin, contraceptives and hormonal replacement therapy)

❒ I am not taking any prescribed medications

Name of medication	Dose	Route*	Number of times per day	Start Date (month/year)

*by mouth, by injection, patch, syrup, pills, suppository, etc

12. What other medications are you currently taking? (Check all that apply)

❒ I am not taking any non prescribed medications including nutritional supplements, vitamins or herbal remedies

❒ Tylenol	❒ Vitamin A	❒ Folic acid	❒ Garlic
❒ Allergy medication	❒ Vitamin B	❒ Calcium	❒ Selenium
❒ Cough syrup	❒ Vitamin C	❒ Omega-3	❒ Ginseng
❒ Multiple vitamins	❒ Vitamin E	❒ Ginkgo biloba	❒ Chamomile

❒ Other(s) (specify): _____

Family Medical History

13. Are your parents alive?

Mother ❒ Yes Age: _____ ❒ No Age at time of death: _____

Father ❒ Yes Age: _____ ❒ No Age at time of death: _____

14. If they have passed away, indicate the cause of death:

Mother: _____

Father: _____

15. How many brothers and/or sisters do you have that are alive?

Brother 1	Age: _____	Sister 1	Age: _____
Brother 2	Age: _____	Sister 2	Age: _____
Brother 3	Age: _____	Sister 3	Age: _____

16. If you a have sibling (s) who have passed away, indicate:

Cause of death of brother(s)	Age at time of death
1.	
2.	
3.	
Cause of death of sister(s)	**Age at time of death**
1.	
2.	
3.	

17. For each of your natural parent(s) and sibling(s), check the appropriate box with respect to each of the following disorders that they have or have had in the past:

	Brothers		Sisters		Mother	Father
	Yes	Number of brothers	Yes	Number of sisters	Yes	Yes
Heart attack/Angina						
Heart attack/Angina ***before*** 55 years old for women or 45 for men						
High blood pressure						
Stroke						
High cholesterol						
Diabetes						
Circulation problems in legs						

YOUR PAST Medical History

18. In the past, have you had any of these **diseases or procedures**? (Check all that apply)

	Yes	No	Year		Yes	No	Year
Heart attack or myocardial infarction				Irregular heart rhythm			
Angina or chest pain from heart disease				Heart defects from childhood			
Congestive heart failure				Blocked arteries / neck or brain			
Heart valve problems				Blocked arteries in legs			
Rheumatic fever				Thrombophlebitis / clot in leg veins			

	Yes	No	Year		Yes	No	Year
Stroke				Aortic aneurysm			
Clot in lungs							
Coronary angioplasty or stent placement				Coronary artery bypass surgery			
Valve surgery (which valve)?				Artificial pacemaker or defibrillator			
Surgery or angioplasty for carotid or peripheral disease							

19. In the past, have you had any of the following **medical conditions**? (Check all that apply)

	Yes	No	Year		Yes	No	Year
Neurological disease (muscle or nerves)				Lung disease (emphysema, bronchitis or asthma)			
Thyroid disease				Migraine headache(s)			
Peptic ulcer/stomach problems				Bowel disease (colitis, diverticulitis or irritable colon)			
Kidney disease				Arthritis (joint pain)			
Cancer, (specify type):				Osteoporosis (fragile bones)			
Digestive system (cirrhosis, hepatitis, pancreatitis Gallbladder)				Depression, anxiety or other emotional problems			
Blood disorders/ anemia				Alcoholism			

Others (describe): _____

_____

Hypertension

20. Has a health professional ever told you that you have had high blood pressure or hypertension? ❒ Yes ❒ No, **if no, go to question # 25**
21. When did he/she first tell you? _____/_____
    Month / Year
22. Have you ever been treated for high blood pressure with medication?
    ❒ Yes ❒ No, **if no go to question # 25**
23. When did you start taking medication for high blood pressure? _____/_____
    Month / Year
24. Are you still taking medication for high blood pressure?
    ❒ Yes ❒ No, when did you stop? _____/_____
    Month / Year

High Cholesterol

25. Has a health professional ever told you that you have had high cholesterol?
    ❒ Yes ❒ No, **if no go to question # 30**
26. When did he/she first tell you? _____/_____
    Month / Year
27. Have you ever been treated for high cholesterol with medication?
    ❒ Yes ❒ No**, if no go to question # 30**
28. When did you start taking medication for high cholesterol? _____/_____
    Month / Year
29. Are you still taking medication for high cholesterol?
    ❒ Yes ❒ No, when did you stop? _____/_____
    Month / Year

Diabetes

30. Has a health professional ever told you that you have diabetes?
    ❒ Yes ❒ No, **if no go to question # 36**
31. When did he/she first tell you? _____/_____
    Month / Year
32. Have you ever been treated for diabetes with medication?
    ❒ Yes ❒ No, **if no go to question # 36**
33. What kind of medication did you take to treat diabetes?
    ❒ Pills ❒ Insulin injections ❒ Both
34. When did you start taking this medication for diabetes?
    ❒ Pills _____/_____ ❒ Insulin injections _____/_____
    Month / Year Month / Year
35. Are you still taking medication for diabetes?
    ❒ Yes, what kind: ❒ Pills ❒ Insulin injections ❒ Both
    ❒ No, when did you stop?
    ❒ Pills _____/_____ ❒ Insulin injections _____/_____
    Month / Year Month / Year

Tobacco consumption

36. Have you ever smoked?
    - ❒ No**, if no go to question # 46**
    - ❒ Yes, when did you start? _____/_____ *or* at what age did you start? _____ years
      Month / Year
37. Do you smoke now?
    - ❒ Yes ❒ No, when did you stop? _____/_____
      Month / Year
38. Do you smoke marijuana?
    - ❒ No ❒ Yes If YES, do you add tobacco to your marijuana ❒ No ❒ Yes
39. What types of tobacco do you, or have you, use (d) the most?
    - ❒ Cigarettes ❒ Pipe ❒ Cigars ❒ Other (specify): _____

40. If you smoke now, even if you have stopped smoking at several occasions in your life, a) how many years did you smoke all together? ___ year(s)
    b) on average, how many cigarettes do you smoke per day? _____
41. If you are currently smoking, where do you smoke the most?
    - ❒ Home ❒ Social outing ❒ Work ❒ Other (specify): _____
42. How long after you wake up in the morning do you smoke your first cigarette?_____
43. If you are currently smoking, have you ever tried to quit smoking? ❒ Yes ❒ No
    If yes, a) how many times have tried to quit smoking? _____
    b) how are you trying to quit?
    - ❒ Cessation counseling clinic
    - ❒ By yourself, no external help
    - ❒ Patches
    - ❒ Gum
    - ❒ Other (describe): _____

44. If you have stopped smoking throughout your life,
    a) how many years did you smoke all together? ___ year(s)
    b) how many cigarettes have you smoked, on average, per day? _____
45. If you have stopped smoking, how did you manage to quit?
    - ❒ Cessation counseling clinic
    - ❒ By yourself, no external help
    - ❒ Patches
    - ❒ Gum
    - ❒ Other (describe): _____

46. Are you exposed to second-hand smoke (to be in the presence of a smoker on a regular basis)? ❒ Yes ❒ No
    If yes, where are you exposed to second-hand smoke and how often? (Check all that apply)
    - ❒ Home ____ hours per day
    - ❒ Work ____ hours per day
    - ❒ Social ____ hours per week

Recreational drug consumption

47. Have you ever taken illicit drugs? ❒ Yes ❒ No, **if no go to question # 51**
If yes, what type (s) of drugs? Describe_____

48. Do you take these illicit drugs now? ❒ Yes ❒ No
If yes, what type (s) of drugs? Describe _____

49. Have you ever taken cocaine? ❒ Yes ❒ No
If yes, when was the first time? _____/_____
Month / Year
when was the last time? _____/_____
Month / Year

50. During this period, how frequently did you use it?
❒ Daily ❒ Weekly ❒ Monthly ❒ Less than monthly

51. Have you ever taken Viagra? ❒ Yes ❒ No
If yes, when was the first time? _____/_____ when was the last time? ____/_____
Month / Year Month / Year

52. During this period, how frequently did you use it?
❒ Daily ❒ Weekly ❒ Monthly ❒ Less than monthly

Food consumption/dietary habits

53. Have you ever been on a special diet? ❒ Yes ❒ No

54. Are you following any special diet now? ❒ Yes ❒ No
If yes, which of the following: (Check all that apply)

Low fat diet	❒	Low salt diet	❒	Low cholesterol diet	❒
Weight reducing diet	❒	Vegetarian diet	❒	Diabetic diet	❒
"Atkins Diet"	❒	"Slim Fast"	❒	"The Zone"	❒
"Weight Watchers"	❒	"Dean Ornish Diet"	❒	"Montignac"	❒

Other: _____ Do not know name (describe):_____

55. Did a doctor recommend that you change your diet? ❒ Yes ❒ No

56. Do you drink coffee regularly?
❒ Yes, how many cups per day? _____ cups / day ❒ No

57. Check usual coffee type:
❒ Caffeinated ❒ Decaffeinated ❒ Both

58. Do you drink tea regularly?
❒ Yes, how many cups per day? _____ cups / day ❒ No

59. Not counting juice, how often do you eat fruit?
Per day: _____ Per week: _____
Per month: _____ Per year: _____ Never ❒

60. How often do you eat green salad?
Per day: _____ Per week: _____
Per month: _____ Per year: _____ Never ❒

61. Not counting potato chips, how often do you eat potatoes?
Per day: _____ Per week: _____
Per month: _____ Per year: _____ Never ❒

62. How often do you usually eat carrots?
Per day: _____ Per week: _____
Per month: _____ Per year: _____ Never ❒

63. Not counting green salad, carrots or potatoes, how often do you usually eat other vegetables?
Per day: _____ Per week: _____
Per month: _____ Per year: _____ Never ❒

64. How many times a week do you eat a "home cooked meal" at home or somewhere else (not including restaurants or frozen dinners)?
Lunch: ____ times at home or somewhere else /week
Dinner: ____ times at home or somewhere else /week

65. How many times a week do you eat meals at a restaurant?
Lunch: ____ times at a restaurant /week
Dinner: ____ times at a restaurant /week

66. Do you drink alcoholic beverages? ❒ Yes ❒ No

67. If yes, do you drink: (Check all that apply)
❒ Regularly (every day)
❒ Socially (on certain occasions)
❒ Heavily (more than usual) from time to time

68. If yes, how many glasses of each per week:
❒ Wine, sherry, port (1 glass = 4 oz.) _____ Glasses/week
❒ Beer, ale, etc. (1 bottle = 12 oz.) _____ Bottles/week
❒ Spirits or hard liquor (1 drink = 1½ oz.) _____ Drinks/week

69. How many years have you been drinking these amounts?
_____ years ❒ less than 1 year

70. Have you ever been a heavy drinker in the past?
❒ Yes, for how many years? _____ ❒ No

Physical activity habits

71. How physically demanding is your daily job (including caregivers)?
❒ Not at all ❒ Mild ❒ Moderately ❒ Very (get out of breath)

72. How physically demanding are your usual daily activities (e.g. house work, getting to work)?
❒ Not at all ❒ Mild ❒ Moderately ❒ Very (get out of breath)

73. On average, how many floors do you climb up per day? (One floor equals ten steps)
_____ number of floors/day

74. On average, how many city blocks _____ or _____km (s) do you walk per day?

75. Do you engage in any regular exercise (e.g. brisk walking, jogging, bicycling, work out at the gym) long enough to work up a sweat?
❒ Yes ❒ No

If yes, how many times per week? _____ for how many minutes? _____

76. How long have you been doing this exercise? _____ months _____ years

77. In the past, have you been doing regular exercise? (e.g. brisk walking, jogging, bicycling, work out at the gym) (Check all that apply)
- ❒ Never
- ❒ On an off for _____ years
- ❒ Continually for _____ years

78. During which season(s) do you exercise? (Check all that apply)
❒ Winter ❒ Spring ❒ Summer ❒ Fall

79. How many times do you do any of the following physical activities?
❒ I do not do any physical activities

Exercise/Sport	**Times per Week**	**Month**	**Year**	**Season***
Gardening or yard work				
Walking for exercise				
Jogging or running				
Home exercises				
Exercise class or aerobics				
Other exercise at the gym (e.g. weights, treadmill)				
Bicycling				
Swimming				
In-line skating or rollerblading				
Golfing				
Fishing				
Baseball or softball				
Soccer				
Tennis				
Volleyball				
Basketball				
Bowling				
Popular or social dance				
Downhill skiing or snowboarding				
Snowshoeing				
Ice skating				
Ice hockey				
Other				

*Add all the seasons that apply W=winter, Sg=spring, Sr =summer, F= fall

Personal Assessment

80. In general, how do you feel about yourself?

I feel:	Strongly agree	Agree	Neither agree nor disagree	Disagree
That I have a number of good qualities				
That I am a person of worth, at least equal to others				
That I am able to do things as well as other people				
That I have a positive attitude toward myself				
All in all, I am satisfied with myself				
All in all, I am inclined to think of myself as a failure				

81. How often , during the past week, did you:	Never	Once in a While	Fairly Often	Very Often
Feel hopeless about the future?				
Feel lonely?				
Have your mind go blank?				
Feel discouraged or "down"?				
Feel tense or under pressure?				
Lose your temper?				
Feel bored or have little interest in things?				
Feel fearful or afraid?				
Have trouble remembering things?				
Cry easily or feel like crying?				
Feel nervous or shaky inside?				
Feel critical of others?				
Feel easily annoyed or irritated?				
Get angry over things that are not too important?				

82. On a scale of 1-10 with 10 being severe stress, how do you rate your level of stress?

	Does not apply	No Stress **1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	Severe Stress **10**
At work	❒										
At home											
Overall											

83. How confident do you feel in managing your stress?

❒ Not confident ❒ A little confident

❒ Moderately confident ❒ Very confident

Education

84. What is your level of education? (Check all that apply)

No School	❒						
Elementary School (Grade)	1	2	3	4	5	6	7
High School (Years)	1	2	3	4	5	Equivalence Certificate	
College (CEGEP/Technical)	❒ Pre-University program ❒ Technology/Professional program						
University (Professional)	❒Bachelor's	❒Masters	❒Doctorate	❒PhD	❒Post-Doc		

Occupation

85. What is your current occupation?_____

86. What is your current occupational status?

- ❒ Full time worker
- ❒ Part time worker
- ❒ Occasional worker
- ❒ Work from home
- ❒ Student
- ❒ Out of work ____ years ____ months
- ❒ Homemaker/caregiver
- ❒ Disabled due to medical condition
- ❒ Retired
- Other _____

87. How many hours do you work outside the house? _____hours per week

Marital Status

88. What is your marital status?

- ❒ Married/common law
- ❒ Never married
- ❒ Separated
- ❒ Divorced
- ❒ Living with someone
- ❒ Widowed
- ❒ Living with a roommate
- ❒ Living alone
- ❒ Other (specify):

89. How many natural children do you have? Boys _____ Girls

90. How many dependents?

- ❒ Spouse
- ❒ Children (number):_____
- ❒ Parents (number):_____
- ❒ Other (specify): _____

Reproductive History and Menopause
For women only
For men, the questionnaire is complete

91. At what age did you begin having your period? Age: _____

92. What was the first date of your last menstrual period?____/_____/____
Day/Month/Year

93. Do you have regular periods? _____ ❒ Yes ❒ No ❒ Not always

94. If you have regular periods, how long is your cycle? Number of days

95. If you do not have regular periods, what is the minimum and maximum number of days of your periods in the past year? Minimum number of days
Maximum number of days _____

96. Are you pregnant right now? _____ ❒ Yes ❒ No ❒ Do not know

97. How many times have you been pregnant (including abortions and miscarriages)? __

98. How many deliveries have you had in total? ___

a) Of your total deliveries, how many still births have you had? ___
b) Of your total deliveries, how many premature babies have you had? ___

During one or more of your pregnancies	Yes	No	Unsure
99. Did you have high blood pressure?			
100. Did you have pre-eclampsia/eclampsia?			
If yes, a) at how many weeks of your pregnancy? _____			
b) were there proteins in your urine?			
101. Did you develop diabetes or pre-diabetes during your pregnancies?			
102. Did you have thrombosis (clots) during or after your pregnancies (including abortions or miscarriages)?			

Birth Control Use

	Yes	No	Unsure
103. Have you ever taken birth control pills?			
104. Are you currently using birth control pills?			

105. If you are using another form of birth control other than the pill, (describe):_____

Menopause

106. Are you menopausal?

❒ Yes, when was your last period? _____/____
Month/Year

❒ No **(the questionnaire is complete)**

❒ Unsure

107. If you are menopausal, what kind of menopause?

❒ Natural menopause

❒ Hysterectomy:

❒ Uterus only ❒ Uterus, both ovaries

❒ Ovaries only ❒ Unsure

❒ Uterus, one ovary

Hormone Replacement Therapy

108. Are you currently taking hormone replacement therapy?

❒ Yes If yes, a) what kind? ❒ Estrogen ❒ Progesterone
❒ Combination ❒ Unsure

b) what form? ❒ Orally_____ ❒ Vaginal cream
❒ Patch ❒ Other (specify): _____

c) when did you start? _____/____ (month/year)

❒ No If no, have you ever taken hormone replacement therapy?

❒ No **(if no, the questionnaire is complete)**

❒ Yes, if yes, when did you start:_____/____

when did you stop:_____/____
Month/Year

109. What is the longest length of time you have used hormone replacement therapy?

___ years ❒ less than 1 year

THANK YOU!

Appendix F – International Physical Activity Questionnaire (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

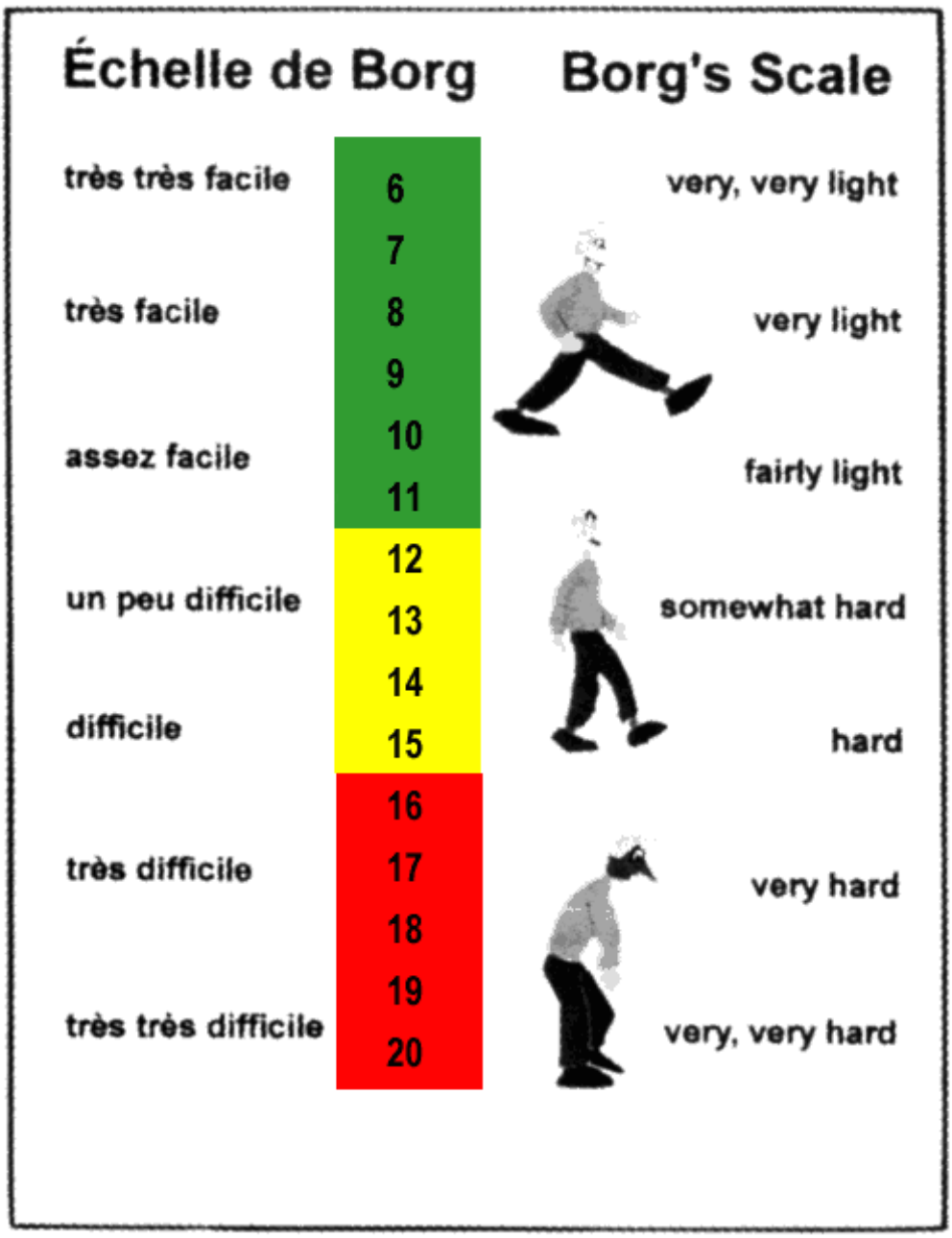
This is the end of the questionnaire, thank you for participating.

Appendix G – Modified Bruce Protocol

Stage	Speed (mph)	Incline (%)	Duration (minutes)
1	3.0	0	3
2	1.7	10	3
3	2.5	12	3
4	3.4	14	3
5	4.2	16	3
6	5.0	18	3
7	5.5	20	3
8	6.0	22	3

Mph, miles per hour

Appendix H – Borg RPE Scale



Appendix I – Study 1 Raw Data

Table 1.2 Raw Data - Exercise Parameters – Smokers vs. Non-Smokers

	Non- smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N
Exercise Time	16.05 ± 2.73	15.33 ± 2.36	15.29 ± 2.51	15.39 ± 2.41	0.121	0.117	0.161
Max HR	189.95 ± 10.63	181.70 ±1 4.17	182.61 ± 14.48	184.09 ± 13.57	**0.007**	**0.003**	**0.013**
VO_{2max}	47.36 ± 10.20	44.08 ± 10.12	45.26 ± 11.31	46.00 ± 11.43	0.065	0.306	0.511
Peak METs	14.21 ± 2.75	13.20 ± 2.55	13.72 ± 3.71	13.93 ± 3.67	**0.032**	0.446	0.660

Exercise time (minutes); Max HR, maximal HR (beats/minute); Peak METs, peak metabolic equivalents; VO_{2max}, maximal oxygen consumption (mL/kg per minute); %predMaxHR, percent of age-predicted maximal heart rate (%)

Values are unadjusted and presented as mean ± SD
Bolded values indicate significance ($P<0.05$)

Table 1.3 Raw Data - Resting Vessel Hemodynamic Parameters – Smokers vs. Non-Smokers

	Non- smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N
HR	59.72 ± 8.20	61.44 ± 6.66	71.55 ± 8.74*	65.77 ± 7.83*	0.204	**<0.001**	**<0.001**
$_{P}$SBP	106.88 ± 8.57	108.47 ± 9.51	113.14 ± 9.94*	109.74 ± 9.87	0.355	**0.001**	0.104
$_{P}$DBP	66.55 ± 6.66	68.00 ± 7.22	68.44 ± 7.73	68.54 ± 7.51	0.270	0.170	0.142
$_{P}$PP	40.33 ± 8.28	40.47 ± 8.18	44.70 ± 9.52	41.21 ± 7.37	0.927	**0.010**	0.540
$_{C}$SBP	92.06 ± 7.39	94.98 ± 7.85	96.99 ± 7.47	95.99 ± 8.49	**0.037**	**<0.001**	**0.010**
$_{C}$DBP	67.41 ± 6.94	68.33 ± 6.87	69.70 ± 7.66	69.86 ± 7.48	0.471	0.100	0.073
$_{C}$PP	24.61 ± 5.02	26.63 ± 4.64	27.29 ± 5.34	26.22 ± 4.35	**0.023**	**0.006**	0.061
MAP	78.69 ± 6.76	80.49 ± 7.16	82.55 ± 7.38	81.80 ± 7.53	0.157	**0.004**	**0.022**
PPA	1.64 ± 0.12	1.53 ± 0.13	1.64 ± 0.15*	1.58 ± 0.16*	**<0.001**	0.917	**0.019**
AP	0.47 ± 3.03	2.51 ± 2.72	1.00 ± 2.94*	1.98 ± 3.22	**<0.001**	0.340	**0.011**
AIx	1.75 ± 11.20	9.43 ± 9.91	3.39 ± 10.33*	7.27 ± 11.25	**<0.001**	0.411	**0.009**
AIx75	-5.52 ± 11.68	2.77 ± 10.95	3.35 ± 10.70	3.29 ± 11.62	**<0.001**	**<0.001**	**<0.001**
SEVR	179.91 ± 36.08	173.25 ± 23.84	133.48 ± 24.45*	155.73 ± 23.22*	0.230	**<0.001**	**<0.001**
crPWV	7.83 ± 0.86	8.22 ± 1.06	8.46 ± 0.81*	8.25 ± 1.24	**0.005**	**<0.001**	**0.006**
cfPWV	6.12 ± 0.98	6.50 ± 0.98	6.50 ± 0.98	6.86 ± 0.10	**0.035**	0.285	**0.043**

AIx, augmentation index (%); AIx75, augmentation index adjusted to heart rate of 75 beats per minute (%); AP, augmentation pressure (mmHg); $_{C}$SBP, central systolic blood pressure (mmHg); $_{C}$DBP, central diastolic blood pressure (mmHg); $_{C}$PP, central pulse pressure (mmHg); cfPWV, carotid-femoral pulse wave velocity (m/s); crPWV, carotid-radial pulse wave velocity (m/s); HR, heart rate (beats per minute); MAP, mean arterial pressure (mmHg); $_{P}$SBP, peripheral systolic blood pressure (mmHg); $_{P}$DBP, peripheral diastolic blood pressure (mmHg); $_{P}$PP, peripheral pulse pressure (mmHg); PPA, pulse pressure amplification; SEVR, subendocardial viability ratio (%).

Values are unadjusted and presented as mean ± SD
Bolded values indicate significance ($P<0.05$)
* $P<0.05$ vs. chronic smoking

Table 1.4 Raw Data - Relative and Absolute Change in Vessel Hemodynamics Immediately Post-Exercise

	Non-Smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N
Relative Change (%)							
HR	81.31 ± 26.90	61.50 ± 25.91	49.65 ± 22.82*	57.56 ± 22.73	**<0.001**	**<0.001**	**<0.001**
$_{P}$SBP	51.40 ± 1	50.47 ± 21.12	46.88 ± 17.31	47.70 ± 16.62	0.803	0.194	0.239
$_{P}$DBP	-2.20 ± 16.15	-0.04 ± 14.33	-1.99 ± 14.21	-0.92 ± 12.54	0.486	0.960	0.609
$_{P}$PP	145.77 ± 57.78	142.98 ± 70.24	130.00 ± 64.06	131.45 ± 45.25	0.838	0.187	0.126
$_{C}$SBP	9.16 ± 8.91	6.94 ± 8.86	7.26 ± 9.64	6.11 ± 7.91	0.192	0.287	0.051
$_{C}$DBP	-4.30 ± 10.13	-3.61 ± 10.21	-1.80 ± 8.73	-5.34 ± 10.12	0.707	0.147	0.586
$_{C}$PP	49.38 ± 31.35	37.21 ± 36.39	35.41 ± 42.08	39.24 ± 34.74	0.066	0.056	0.109
MAP	2.89 ± 8.70	2.23 ± 8.27	2.93 ± 7.96	1.11 ± 7.40	0.690	0.975	0.231
PPA	1.54 ± 8.96	5.41 ± 8.71	1.25 ± 8.47*	5.09 ± 9.23	**0.019**	0.818	0.039
SEVR	-54.27 ± 13.27	-45.61 ± 12.34	-36.21 ± 13.69*	-42.80 ± 10.64*	**<0.001**	**<0.001**	**<0.001**
crPWV	4.32 ± 9.93	3.47 ± 10.78	1.72 ± 13.33	2.36 ± 12.69	0.658	0.264	0.376
cfPWV	49.82 ± 20.20	47.16 ± 22.23	38.46 ± 23.12*	43.05 ± 22.82	0.513	**0.013**	0.102
Absolute Change							
AP	0.31 ± 3.17	-0.13 ± 2.90	0.81 ± 2.45*	-0.07 ± 2.12	**0.032**	0.406	0.094
AIx	0.94 ± 9.82	-2.77 ± 7.78	2.06 ± 7.44	-1.02 ± 8.59	**0.020**	0.440	0.178
AIx75	18.69 ± 10.69	9.94 ± 8.13	10.80 ± 8.35	3.45 ± 8.18	**<0.001**	**<0.001**	**<0.001**

AIx, augmentation index (%); AIx75, augmentation index adjusted to heart rate of 75 beats per minute (%); AP, augmentation pressure (mmHg); $_{C}$SBP, central systolic blood pressure (mmHg); $_{C}$DBP, central diastolic blood pressure (mmHg); $_{C}$PP, central pulse pressure (mmHg); cfPWV, carotid-femoral pulse wave velocity (m/s); crPWV, carotid-radial pulse wave velocity (m/s); HR, heart rate (beats per minute); MAP, mean arterial pressure (mmHg); $_{P}$SBP, peripheral systolic blood pressure (mmHg); $_{P}$DBP, peripheral diastolic blood pressure (mmHg); $_{P}$PP, peripheral pulse pressure (mmHg); PPA, pulse pressure amplification; SEVR, subendocardial viability ratio (%).

Relative Change (%) calculated as [(post – pre)/pre × 100]
Absolute Change calculated as [post – pre] for parameters where relative change could not be calculated because of negative pre-exercise values
Values are unadjusted and presented as mean ± SD
Bolded values indicate significance ($P<0.05$)
* P<0.05 vs. chronic smoking

Table 1.5 Raw Data - Area Under the Curve of Vessel Hemodynamics in Response to Exercise – Smokers vs. Non-Smokers

	Non-Smokers (NS)	Chronic Smoking (CS)	Acute smoking (AC)	Nicotine (N)	*P* value NS vs. CS	*P* value NS vs. AS	*P* value NS vs. N	*P* value CS vs. AC. vs. N
HR	626.1 ± 168.2	439.9 ± 148.6	370.3 ± 147.8	445.8 ± 163.6	**<0.001**	**<0.001**	**<0.001**	**0.001**
$_{P}$SBP	172.3 ± 117.9	148.7 ±140.2	91.1 ±115.7	143.9 ± 102.2	0.329	**0.001**	0.191	**0.045**
$_{P}$DBP	-34.3 ± 93.4	-38.3 ± 101.9	-33.4 ± 99.4	-35.8 ± 100.1	0.828	0.960	0.933	0.806
$_{P}$PP	206.6 ± 145.5	185.5 ± 161.5	124.4 ± 157.6	179.7 ± 136.6	0.470	**0.005**	**0.328**	**0.047**
$_{C}$SBP	34.7 ± 73.6	9.9 ± 88.1	-13.0 ± 58.6	4.1 ± 69.4	0.111	**0.001**	**0.032**	0.216
$_{C}$DBP	-14.3 ± 77.5	-7.2 ± 75.1	-17.9 ± 89.4	-19.3 ± 88.0	0.634	**0.022**	0.755	0.876
$_{C}$PP	49.8 ± 77.2	18.8 ± 82.1	-0.2 ± 76.4	22.1 ± 75.4	**0.047**	**0.001**	0.067	0.150
MAP	1.7 ± 66.9	0.4 ± 76.7	-18.0 ± 62.5	-10.3 ± 69.0	0.925	0.125	0.363	0.553
PPA	1.1 ± 1.8	1.8 ± 1.6	0.1 ± 1.9	1.4 ± 2.3	0.052	0.733	0.340	0.213
AP	-14.8 ± 43.6	-22.6 ± 32.2	-8.8 ± 38.5	-19.3 ± 31.7	0.325	0.470	0.571	0.181
AIx	-54.9 ± 141.3	-87.9 ± 113.7	-38.1 ± 92.3	-78.2 ± 107.3	0.208	0.501	0.366	0.057
AIx75	182.8 ± 150.4	78.6 ± 107.2	55.5 ± 91.1	77.4 ± 104.9	**<0.001**	**<0.001**	**<0.001**	0.222
SEVR	-1378.9 ± 549.7	-1045.7 ± 419.2	-576.2 ± 340.0	-859.2 ± 346.8	**0.001**	**<0.001**	**<0.001**	**<0.001**
crPWV	3.1 ± 9.8	1.0 ± 7.9	-1.8 ± 12.6	1.7 ± 13.0	0.634	0.168	0.483	0.421
cfPWV	10.4 ± 9.6	11.3 ± 9.6	7.1 ± 16.0	11.8 ± 11.0	0.243	**0.023**	0.527	0.106

HR, heart rate (beats per minute), $_{P}$SBP, peripheral systolic blood pressure; $_{P}$DBP, peripheral diastolic blood pressure; $_{P}$PP, peripheral pulse pressure, $_{C}$SBP, central systolic blood pressure; $_{C}$DBP, central diastolic blood pressure; $_{C}$PP, central pulse pressure, MAP, mean arterial pressure (mmHg); PPA, pulse pressure amplification; AP, augmentation pressure (mmHg); AIx, augmentation index (%), AIx75, augmentation index adjusted to heart rate of 75 beats per minute (%);SEVR, subendocardial viability ratio (%); crPWV, carotid-radial pulse wave velocity (m/s); cfPWV, carotid-femoral pulse wave velocity (m/s).

Values are unadjusted and presented as mean ± SD

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