# From Rest to Stressed: An Altered Vascular Hemodynamic Profile in Cigarette Smokers

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**Abstract (English)**

**Background:** Cigarette smokers have increased arterial stiffness at rest, which is exacerbated by acute smoking. The impact of smoking on the ability of the vascular system to respond to increased demands has not, however, yet been investigated. This study aims to estimate the impact of acute and chronic smoking on arterial stiffness, and specifically carotid-femoral pulse wave velocity (cfPWV) and pulse wave analysis (PWA), including subendocardial viability ratio (SEVR), augmentation index (AIx75), pulse pressure amplification (PPA), and cardiorespiratory fitness in response to acute physical stress in young, healthy, light-moderate smokers.

**Methods:** Healthy light-moderate smokers (n=28) and non-smokers (n=34) underwent cfPWV and PWA measurements at rest, and 2, 5, 10, 15, and 20 minutes following a maximal exercise test to exhaustion. Smokers were tested under two conditions, a) after 12 hours abstinence from smoking (to estimate impact of chronic smoking), and b) immediately after smoking one standardized cigarette following 12 hours abstinence from smoking (to estimate impact of acute smoking).

**Results:** Chronic smoking led to increases in cfPWV and AIx75, and a decrease in PPA at rest when compared to non-smokers. Acute smoking led to significantly decreased SEVR, increased PPA and an additional increase in cfPWV when compared to chronic smoking at rest. There were no differences noted between smokers and non-smoker in maximal exercise time, but smokers demonstrated significantly decreased $VO_2$peak, maximal heart rate (HRmax), and increased peak respiratory exchange ratio (peakRER) under both conditions. There were no differences in cardiorespiratory variables between smokers' chronic condition and acute condition. In all groups, exercise caused acute increases in cfPWV, AIx75, PPA. The cfPWV was significantly elevated in acute smoking condition compared to non-smokers at various time points post-exercise. Following exercise there were significant decreases in the SEVR compared to resting values in all groups. The largest decrease in SEVR was noted in non-smokers, and this remained significantly lower than smokers under both conditions at all post-exercise time

points before adjustment, and at 15 and 20 minutes post-exercise after adjustment. The SEVR remained below baseline resting values for the entire recovery period in both smokers and non-smokers alike. Smokers under both conditions also recovered towards resting values of AIx75 faster than non-smokers, while cfPWV also recovered faster in smoker acute condition compared to non-smokers, owing to detrimentally higher resting values of arterial stiffness observed among smokers.

**Conclusion:** Chronic smoking was shown to have detrimental effects on the vascular and cardiorespiratory systems' ability to respond to increased demands in young, healthy men. Acute smoking further exaggerated the altered vascular hemodynamic profile in response to stress among smokers, demonstrating cigarettes' negative implications on the ability of the vascular system to respond to increased demands. Vascular characterization may allow future clinicians to monitor progression of disease, enhance risk profiling, and direct treatment in patient populations at increased risk for developing cardiovascular complications and disease, as with cigarette smokers.

**Abstract (French)**

**Introduction:**Les utilisateurs de tabac ont une rigidité artérielle accrue au repos, ce qui est exacerbée par l'action aigüe du tabagisme. L'impact de la cigarette sur la capacité du système vasculaire à répondre à des demandes physiologiques accrues n'a cependant pas été étudié jusqu'à maintenant. Cette étude s'intéresse à l'impact du tabagisme aigu et chronique sur la rigidité artérielle, et spécifiquement la vitesse de propagation de l'onde de pouls carotido-fémorale (VPOPcf) et les indices de l'analyse de l'onde de pouls (ou *pulse wave analysis*, PWA), tels que le rapport subendocardial de viabilité (ou *subendocardial viability ratio*, SEVR), l'index d'augmentation (ou *augmentation index*, AIx75), l'amplification de la pression pulsée (ou *pulse pressure amplification*, PPA), et la condition cardiorespiratoire en réponse à un stress physique aigu chez des jeunes fumeurs (légers à modérés) en santé.

**Méthodologie :** La rigidité artérielle de fumeurs légers à modérés en santé (n=28) et de non-fumeurs (n=34) a été mesurée au repos, ainsi qu'à 2, 5, 10, 15, et 20 minutes après une épreuve d'effort maximal (jusqu'à épuisement). Les fumeurs étaient évalués sous deux conditions : a) après 12 heures d'abstinence tabagique (afin d'estimer l'impact chronique de la cigarette), et b) immédiatement après avoir fumé une cigarette standardisée suivant une période d'abstinence tabagique de 12 heures (afin d'évaluer l'impact aigu de la cigarette).

**Résultats :** L'effet chronique de la cigarette se traduisit par une augmentation du la VPOPcf et du AIx75, ainsi qu'une diminution de la PPA chez les fumeurs au repos lorsque comparés aux non-fumeurs. L'effet aigu de la cigarette se traduisit par une diminution significative du SEVR, une augmentation de la PPA, et une augmentation additionnelle de la VPOPcf chez les fumeurs lorsque comparés aux non-fumeurs au repos. Aucunes différences furent trouvées entre les fumeurs et les non-fumeurs pour ce qui est de la durée de l'exercice maximal, mais les fumeurs avaient un pic de consommation maximale d'oxygène ($VO_{2peak}$), une fréquence cardiaque maximale (ou *maximal heart rate*, HRmax), ainsi qu'un pic de la mesure du ratio d'échanges gazeux (ou *peak respiratory echange ratio*, peakRER) diminués dans les deux conditions

(tabagisme aigu et chronique). Il n'y avait pas non plus de différences entre les variables cardiorespiratoires chez les fumeurs dans les conditions chroniques et aigües. Chez tous les groupes, l'exercice donna lieu à des augmentations de la VPOPcf, du AIx75, et de la PPA. La VPOPcf était élevée dans les conditions de tabagisme aigu à différentes intervalles après l'exercice. Après l'exercice, tous les groupes démontrèrent une diminution significative du SERVR comparativement avec les valeurs au repos. La plus grande diminution du SEVR fut observée chez les non-fumeurs, ce qui demeura significativement diminué comparativement aux fumeurs en conditions aigües et chroniques à toutes les intervalles de temps avant ajustements, et à 15 et 20 minutes après l'exercice après ajustements. De plus, le SEVR demeura en dessous des valeurs de repos pour la période entière de récupération autant chez les fumeurs que les non-fumeurs. Les valeurs d'AIx75 des fumeurs sous les deux conditions (aigüe et chronique) retournèrent également plus rapidement aux valeurs de repos que celles des non-fumeurs, tandis que seule la VPOPcf des fumeurs en condition de tabagisme aigu retourna aux valeurs de repos comparativement aux non-fumeurs, probablement dû à l'effet nuisible des valeurs accrues de rigidité artérielle au repos des fumeurs.

**Conclusion :** Le tabagisme a démontré avoir un effet chronique nuisible sur la capacité des systèmes vasculaire et cardiopulmonaires à répondre à des demandes physiologiques accrues chez des jeunes hommes en santé. Le tabagisme aigu exacerba également le profile altéré de l'hémodynamie vasculaire en réponse au stress physique chez les fumeurs, démontrant l'implication négative de la cigarette dans la capacité du système vasculaire à répondre à des demandes accrues. La caractérisation vasculaire pourrait aider les futurs cliniciens dans la surveillance de la progression des maladies, la détermination des profils de risque, et la direction des traitements dans les populations à risque de développer des complications et des maladies cardiovasculaires, tels que les fumeurs de tabac.

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## 1.0 Overview

With nearly 25% of the Canadian population considered current smokers, the health burden that results as a direct consequence of cigarettes is staggering [1]. Smoking not only impacts the individual, but society at large. Cigarettes leave in their stead physical afflictions, environmental contaminants, and heavy financial loads, without providing a positive return to human physical health. Chronic cigarette smoking has shown to drastically increase the risk for cardiovascular disease and is the leading preventable cause of death and disability worldwide. [2]

To date, the chronic negative impact of cigarette smoking on the various organ systems, most notably the vascular system, has been well established, as have the acute and chronic benefits of physical activity. However, the impact of acute aerobic stress on the ability of the vascular system to respond to increased demands, in young healthy smokers, has yet to be established.

With recent advances in technology we may now begin to qualify and quantify the impact that multiple stressors place on the vascular system. By measuring such factors as arterial stiffness we may be able to evaluate the detrimental effects of smoking and the effect of acute exercise as a stressor to the vascular system, while also assessing for the severity and progression of cardiovascular complications and disease. Although continually underutilized in clinical practice, measures of arterial stiffness provide insight into systemic vascular health. This vascular characterization becomes even more important within groups at increased risk for cardiovascular disease, as with current smokers.

This thesis aims to shed additional light on this multifaceted interaction by quantifying parameters of arterial stiffness in young, healthy chronic smokers under several conditions before and after acute physical stress as compared to young, healthy non-smokers. The project herein is a stride towards uncovering the relationship between cigarette smoking, acute physical stress, and arterial stiffness such that we, as a society, may be educated to begin to modify our behaviour and our health for the better.

## 2.0 Background

### 2.1 Cigarette Smoking:

*2.1.1 Smoking Statistics in Canada*

Cigarette smoking is one of the most well established cardiovascular risk factors and is the leading preventable cause of death and disability worldwide [1, 2]. In general, smoking rates have been on the decline in recent years, but they still remain quite high, particularly in younger age groups. The Canadian Tobacco Use Monitoring Survey (CTUMS) 2012 recently disclosed that 12% of youth aged 15-19 years are current smokers, increasing to 22% in young adults age 20-24, and decreasing back to 17% in adults above age 25 [3]. Smoking rates are even higher in the United States, where the prevalence of smoking is 25% in adults and adolescents above age 15. These already staggering statistics are further exacerbated in various countries around the world, as exemplified by Southern Europe where multiple countries additionally demonstrate adult and adolescent smoking prevalence well above 30% (Austria, Greece, Hungary, and Czech Republic) [4]. Efforts to stem the detrimental impacts of cigarette smoking are moving in the right direction, as smoking rates in young adults in Canada have decreased from 33% in 2008, to 26% in 2009, to 24% in 2010, and now resting at 22% in 2012 [1, 3]. Although this is a positive trend, young adults smokers still average 12.7 cigarettes each day, corresponding to just over half of a pack. The statistics for young smokers in the province of Quebec are even more staggering than the rest of Canada, with 24% of adults age 20-24 considered regular smokers in the year 2012 [1]. This is particularly unsettling as the global INTERHEART study established that smoking a mere five cigarettes per day significantly increases the risk for acute myocardial infarction in both younger and older populations [5].

*2.1.2 Smoking and Cardiovascular Damage*

It has been extensively demonstrated that cigarette smoking can cause severe cardiovascular damage, endothelial dysfunction, and arterial stiffening [6-8]. Through a variety of pathophysiological mechanisms, cigarette smoke impacts normal blood flow through the arterial system, causing altered vascular hemodynamics at rest and potentially

abnormal response to acute stress [6, 9-12].While the chronic effects of cigarette smoking on the vascular system can be quite substantial, acute tobacco smoking in itself has been shown to similarly transiently impact vascular hemodynamics for upwards of 2 hours [11]. Cigarette smoking, both acute and chronic, has the potential to impact arterial stiffening in a variety of ways, which are elaborated on with detail in Section 5.0 Discussion, including increased oxidative stress; altered lipid metabolism; pro-inflammatory state; insulin resistance; impaired kidney function; and high blood pressure.

### *2.1.3 Review of the Literature*

At rest, the vascular hemodynamic impact of altered physiological states in smokers has been well documented. *Acute smoking* has been implicated in increased arterial stiffness as measured by carotid-femoral pulse wave velocity (cfPWV), AIx, brachial-radial pulse wave velocity (brPWV), and carotid-radial pulse wave velocity (crPWV) [11, 13-15]. Lemogoum et al. demonstrated a significantly increased cfPWV 10 minutes after smoking one cigarette in healthy current smokers, while Vlachopoulos et al. and Mahmud et al. confirmed these findings extended to later time points following acute smoking. Rhee et al. showed that the heart-femoral pulse wave velocity was significantly increased by 7% five minutes after smoking, which returned to baseline values by 15 minutes [16]. Acute smoking has also been shown to increase the β-stiffness index in healthy current smokers [17]. Supplementing these findings, all studies to date investigating arterial distensibility and compliance (using echotracking, Doppler, or ultrasound) demonstrated significantly decreased compliance in all arterial beds under study following acute smoking [18-23]. Thus, acute smoking has a detrimental impact on resting arterial stiffness, even in otherwise healthy individuals.

The literature demonstrates that results of *chronic smoking* on arterial health are similarly grim. Chronic smoking has been directly associated with increased resting brPWV [24], aortic-femoral PWV [25] and cfPWV [26] in young healthy populations. Binder et al. found radial artery stiffness to be significantly higher in young, healthy chronic smokers compared to non-smokers [27], while Yufu et al. [28] also demonstrated significantly increased baPWV among chronic smokers. A history of smoking has also been associated with increased resting AIx across various populations and age groups [29-32]. Previous

studies have also found AIx to be independently correlated with smoking status in healthy smokers [30-32]. These results demonstrate that chronic smoking may negatively impact vessel hemodynamics at rest, even in younger populations. Similar to results seen with acute smoking, measures of arterial distensibility and compliance using ultrasonography are significantly decreased with chronic smoking. Two studies reported decreased distensibility [33] or compliance [34] in smokers compared to non-smokers. The β-stiffness index, another measure of arterial distensibility, has also shown to be significantly increased in chronic smokers [35, 36].

There is sparse, although significant, evidence relating the impact of acute and chronic smoking on the subendocardial viability ratio (SEVR). As will be further discussed, the SEVR reflects the oxygen supply:demand ratio of the myocardium. Our research team published a paper titled 'Altered Arterial Stiffness and Subendocardial Viability Ratio in Young Healthy Light Smokers after Acute Exercise' [37], wherein we investigated both resting, and post-exercise differences in the SEVR between current smokers and non-smokers. The results indicated that at rest, there were non-significant differences in the SEVR between smokers after 12 hours of smoking abstinence and non-smokers (163.7 ± 26.8% vs. 173.4 ± 44.8, respectively). Acute smoking caused a significant decrease in the SEVR (163.7 ± 26.8% to 130.2 ± 20.0%, P=0.03) among smokers. These results confirm previous work by Fennessy et al. [38], and Frimodt-Moller et al. [39], that have demonstrated an association between cigarette smoking and significantly diminished SEVR.

In contrast to the aforementioned, other studies found no resting differences in various parameters of arterial stiffness with chronic smoking [20, 28, 29, 33, 38, 40, 41]. This controversy in evidence provides the impetus to better clarify the relationship between chronic smoking and altered arterial stiffness. There is a particularly substantial lack of evidence in regards to the *chronic* impact of smoking on the 'gold-standard' measure of cfPWV in younger populations. Furthermore, all studies to date investigated the impact of smoking on arterial hemodynamics at rest. Previously, no study had yet investigated the impact of smoking on changes in arterial stiffness *following acute physical stress* in young, healthy individuals.

## 2.2 Arterial Stiffness and Hemodynamic Analysis

### *2.2.0 Overview*

Arterial stiffness has long been used as a marker to assess the severity and progression of cardiovascular disease [42-45], and has strong predictive value (cfPWV, Aix, and PPA) for cardiovascular events [46-53]. Its use is recommended by the Task Force for the Management of arterial hypertension of the European Society of Hypertension and European Society of Cardiology [54] in the assessment of arterial function, yet is underutilized in clinical practice and research settings. This is particularly important, as the assessment of arterial stiffness is a composite indicator of arterial health and can help direct clinical approach to treatment [52, 55]. In cases of disease, or within groups at higher risk for CVD, as with current smokers, this vascular characterization becomes even more important.

Arterial stiffness can be acutely and chronically impacted by alterations in vessel wall properties. An increased ratio of collagen to elastin, vascular calcification, and smooth muscle cell necrosis may each contribute to alter arterial functional characteristics. These changes primarily occur over a period of time, are accelerated in various pathological states (such as hypertension and atherosclerosis) [55], and may even compound through time. Thus, any lifestyle factor associated with the development of hypertension and atherosclerosis, like cigarette smoking, would further contribute to vascular abnormalities and arterial stiffening [15]. Arterial stiffness has also been shown to be acutely impacted by a variety of other factors, including but not limited to; caffeine [11], alcohol [56], mental stress [12], and acute aerobic exercise [37, 57-79], the latter of which contributes positively to arterial stiffening.

There have been a variety of methods proposed to assess changes in hemodynamic parameters at rest and under conditions of acute and chronic stress (Doppler, ultrasound, etc.). The method used for the purpose of this thesis incorporated the technique of applanation tonometry with the SphygmoCor software system. Applanation tonometry allows for the quantification of specific vascular hemodynamic variables, including cfPWV, SEVR, augmentation index corrected to a heart rate of 75 beats per minute

(AIx75), and PPA, both at rest and in response to acute stress. The SphygmoCor system techniques used to record measures of arterial stiffness are further elaborated in section 4.0 Methods.

### *2.2.1 Carotid-Femoral Pulse Wave Velocity*

The cfPWV is widely considered the 'gold-standard' in the measurement of arterial stiffness and reflects the viscoelastic properties of the aorta and large central arteries [42, 49]. The cfPWV is a particularly appealing variable owing to the following; its ease in measurement, its high reliability and reproducibility, and the vast amount of supporting evidence demonstrating associations with cardiovascular disease risk, independent of traditional risk factors [42, 46, 49, 52, 53].

The SphygmoCor system used in this study has been well validated and has shown to capture highly reproducible measures in a variety of populations, both diseased and healthy [74, 80-82]. Although a single operator conducted all measurements for this particular study (myself), the SphygmoCor system has shown high levels of both inter- and intra-operator reproducibility in assessment of PWV and PWA measures. "Intra-class correlation coefficients ranged from 0.92 to 0.98" [83]. Similarly, the SphygmoCor system has multiple levels of quality control, making the interpretation of a 'good' vs. 'bad' measurement quite objective. Included in the software's quality control are analyses of:

a) The quality of radial waveform pulse overlay in the calculation of PWA measures (specifically, analysis of pulse height, shape, and diastolic variation)

b) The 'Operator Index,' which provides a score out of 100 based on the quality of waveform capture, and

c) The standard deviations associated with PWV measurements.

Measurements that did not reach quality control standards implemented at the initiation of the study were repeated. Any one of the following was cause for repetition of a measurement; Operator Index $\underline{\leq}90$; standard deviations $\geq$10% for cfPWV value; PWA deviations >5% in pulse height, shape, or diastolic variation.

The various software available to compute PWV use differing algorithms to determine what is deemed the 'foot', or beginning, of the pulse wave, and PWV measurements are highly dependent on which algorithm is used. PWV values can vary by as much as 5-15% depending on which algorithm is applied to the data [84]. The SphygmoCor system uses what is termed the 'intersecting tangent algorithm' to determine the foot of the pulse wave, which is said to "[use] gating to the R-wave of the electrocardiogram for the 2 sites" [85], and other algorithm specifics are otherwise poorly elaborated in the literature and by the manufacturer for commercial reasons.

CfPWV measurements are also substantially impacted by the technique used to determine arterial length. The two most common methods of determining path length in measuring cfPWV are the 'direct' and 'subtraction' methods. The direct method involves simply measuring the straight-line distance between the carotid and femoral arterial sites, but this tends to underestimate the total arterial length travelled by the pulse wave. The second method, used herein, takes into account the extra distance covered by the pulse wave through the ascending aorta. This method requires one to calculate the distance by subtracting the sternal notch-to-carotid arterial site distance from the sternal notch-to-femoral arterial site distance. This method is not the 'gold-standard' magnetic resonance imaging method of determining artery length [86], but it has previously shown to provide the best approximation to arterial lengths determined invasively [86, 87]. The method chosen to determine path length is important, as differences in path length can result in changes in PWV of up to 30% [88, 89]. Ideally, in the future, measurements of cfPWV can be standardized through the use of common algorithms and path length measurements.

There are important transient and chronic physiological features that may also impact measures of cfPWV. These include structural alterations of the arterial wall and numerous 'functional factors'. Briefly, with further elaboration in section 5.0 Discussion, as we age, and also accelerated by chronic cigarette smoking, elastin fibers in the arterial wall begin to degenerate [90, 91]. This results from a combination of reduced elastin synthesis and an increase in elastase activity, leading to degeneration of elastic components in the vessel walls and reducing pulse buffer capacity. Zieman et al. recently

described how arterial stiffening was associated with pathologically determined collagen, and broken-down elastin contents in vessel walls [91]. From the ages of 45-54 cfPWV increases by as much as 0.07m/s per year, rising to 0.2m/s per year above age 55 [92]. This is particularly important considering that a 1m/s increase in cfPWV is associated with a 14% increase in total CV events [53].

Multiple physiological stressors may also acutely and chronically alter the impact that elastin and collagen fibers have on PWV in various populations. In cases of chronically increased blood pressure, as in hypertension, there is increased collagen biosynthesis to counteract chronically increased vascular pressures, leading to increased cfPWV [93]. You et al. recently demonstrated a significant positive correlation between cfPWV and collagen content of the ascending aorta, and an inverse correlation between cfPWV and elastin content in individuals with coronary artery disease [94]. Even when blood pressure is controlled in hypertension, structural alterations remain and continue to impact arterial hemodynamics.

As there are both acute and chronic factors that significantly impact vascular hemodynamics, it is an extremely useful tool in the assessment of patient populations and for use in clinical research settings. This is particularly true for the cfPWV, since it has been shown to have true value in the prognosis of cardiovascular disease, while PWV measures taken between other arterial sites (such as the brachial-ankle PWV) have little comparable evidence [95]. Recently, the Framingham Heart Study demonstrated that a 1 standard deviation increase in cfPWV was associated with a 48% increase in arterial disease risk, independent from individual vascular risk factors [52]. A meta-analysis also found that a 1m/s increase in cfPWV was associated with an age-, sex-, and risk factor-adjusted risk increase of 14%, 15%, and 15% in total CV events, CV mortality, and all-cause mortality, respectively [53]. In this meta-analysis a 1 standard deviation increase in cfPWV was similarly associated with respective increases of 47%, 47%, and 42% [53].

These findings are particularly significant, as smoking has been shown to acutely and chronically increase cfPWV. Lemogoum et al. [14] demonstrated increased cfPWV in chronic smokers 10 minutes following a cigarette smoking (5.9 ± 1.7 m/s to 7.0 ± 1.5 m/s, P=0.02). After adjustment for MAP, Vlachopoulos et al. [11] noted a 0.8m/s

increase in cfPWV 1 hour after cigar smoking and the effect lasted for 2 hours ($P<0.0001$). Sham smoking yielding no significant change. Mahmud and Feely similarly noted cfPWV to be significantly increased for 15 minutes after acute smoking in both current and non-smokers ($P<0.05$), with a greater increase occurring in the current smokers [15]. The evidence pertaining to chronic effects of smoking on cfPWV are more scarce, yet increased aortic-femoral arterial stiffness (a surrogate measure of cfPWV) was found to be elevated in smokers compared to non-smokers in various studies [25, 26]. Cigarette smoking was also found to be a significant predictor of increased aortic PWV in healthy adults [25]. Three studies, however, found no correlation between smoking status and cfPWV [29, 30, 41], thus the chronic effects of smoking on arterial hemodynamics need to further be confirmed.

Although differing in potential mechanisms of action, acute physical stress, such as treadmill or bicycle exercise, has been shown to cause immediate changes in PWV. The degree of this change is related to the time post-exercise at which the measurement is taken, and the arterial segment being analyzed. This concept is further explored and discussed in Section 2.3.

### *2.2.2 Subendocardial Viability Ratio*

The subendocardial viability ratio, also termed the Buckberg Index, was initially derived in the 1970s via invasive hemodynamic measures in large animals [96]. It has since continued to gain prominence as a sensitive measure of myocardial perfusion in humans and its use extends to both clinical practice and research endeavours.

The SEVR is considered a 'pressure-time integral' derived from central pressures measured in the aorta and the left ventricle [96]. The systolic portion of the aortic pressure wave reflects the pressure that the myocardium must work against to produce sufficient cardiac output, and is the product of time spent in systole *and the afterload of the heart*. This portion of the central pressure wave is often termed the systolic pressure-time index, or SPTI, and is also known as the Sarnoff tension-time index, or TTI. Subsequently, the diastolic portion of the aortic pressure wave reflects the coronary driving pressure and the time spent in the diastolic phase of the cardiac cycle, and is

responsible for the perfusion of the subendocardium with each heartbeat. This phase of the central pressure wave is termed the diastolic pressure-time index, or DPTI. The SEVR is calculated as the ratio between oxygen supply and demand (SEVR = DPTI/SPTI).

Multiple studies have confirmed that the SPTI is directly correlated to the *oxygen consumption* of the heart [97, 98]. New software algorithms have been developed that bypass such prior necessities proposed initially by Sarnoff, as directly measuring vessel wall tensions. As such, current literature will preferentially refer to this portion of the pressure curve as the systolic *pressure*-time index (rather than the *tension*-time index) [99].

Early experiments revealed that comparison of the area under the curve of the systolic and diastolic portions of the central aortic pulse wave have strong predictive value in determining myocardial oxygen supply and demand [96, 99]. This ratio of supply:demand, often expressed as a percentage, is founded on well-known physiologic principles and allows clinicians to determine if the myocardium is being sufficiently perfused, or if there is clinically relevant myocardial ischemia. This is critical in the evaluation of coronary blood flow and myocardial function. It has been suggested that the critical SEVR ratio, below which there is a clear indication for reduced coronary blood flow, is approximately 0.4-0.5 (or 40-50%) [100]. Below a ratio of 0.5, there is a clear discrepancy between subendocardial and subepicardial flow, resulting in insufficient subendocardial perfusion [99, 100]. Above critical levels, the degree of subendocardial perfusion is fairly constant, and thus there is no clear direct relationship between increased SEVR and increased coronary blood flow. However, clinically, *low* resting SEVR has been associated with coronary artery disease, severity of type-1 and type-2 diabetes, impaired kidney function, a decrease in coronary flow reserve, and perhaps most importantly, a history of smoking [38, 39, 101-104].

Cigarette smoking causes increased resting arterial stiffness, which has been associated with significantly increased SPTI and decreased SEVR [105]. The aortic pulse buffering capacity is reduced in cases of increased central arterial stiffness, as the large elastic arteries begin to rely more on collagen fibers (in deference to elastin) in sustaining

pressure loads through the arterial tree. The speed of pulse transit from reflection sites is thus increased, causing much of the pressure-time integral to be 'left-shifted' from the diastolic to the systolic phase of the cardiac cycle. This represents increased myocardial oxygen consumption resulting from increases in myocardial afterload. Thus, a history of smoking may result in decreased SEVR at rest or abnormal SEVR response to stress. With exercise there are increased demands placed on the heart, acutely impacting the SPTI and SEVR. Referring to the modified Laplace formula, which states that wall stress = (radius*pressure)/(2*wall thickness), one can see that stress is directly related to ventricular radius and pressure. As physical workloads increase, as in acute exercise, there are increases in central pressures driving blood flow to muscles in the periphery in combination with increases in venous return. The expected myocardial oxygen consumption (related to wall stress) will thus increase according to the Laplace formula, decreasing SEVR to below resting levels as the SPTI rises.

When the heart contracts in systole, coronary arteries are compressed into the ventricular wall. There is highly reduced blood flow to the subendocardial layers, and thus a lack of coronary perfusion [100]. During diastole, however, the coronary vessels are allowed to expand and provide the subendocardial layers with required blood flow and associated oxygen delivery. Coronary perfusion is therefore considered to occur entirely in diastole [100], and is thus directly related to the oxygen supply of the heart.

Blood flow in the subendocardium during diastole is dependent on several factors, including; coronary artery diastolic pressure [106]; the driving pressure between coronary arteries and small coronary vessels [107]; time in diastole [108]; and blood viscosity [107], all of which have been shown to be acutely altered with smoking, and under conditions of acute stress.

It has been shown that in cases of acute and chronically increased arterial stiffness, as with smoking, the DPTI of the aortic pressure curve is significantly decreased [105]. This occurs concomitant to increases in the SPTI, resulting primarily from a reduced ability of the aorta to buffer outgoing systolic pulse waves, and a more rapid return of reflected pressure waves from the periphery. The Rotterdam study also demonstrated that increased PWV was associated with such decreases in DPTI and increases in SPTI [105].

In our laboratory, the SEVR parameter is captured using the technique of applanation tonometry as outlined in section 4.3.3. The high inter- and intra-operator reproducibility of SEVR measures, through the use of applanation tonometry and PWA, has been previously verified in a variety of clinical settings [80, 109-111]. Therefore, the measurement of SEVR through applanation tonometry should be considered "a sensitive, reproducible and validated measure of the balance between the supply and demand for myocardial oxygen and of the adequacy of subendocardial perfusion" [82].

### *2.2.3 Augmentation Index*

The augmentation index is widely considered a composite measure of arterial stiffness, primarily representing systemic pulse wave reflections at the aorta. With each contraction of the left ventricle in systole, a pulse wave is produced that travels away from heart towards peripheral vascular sites. At these peripheral sites (be it arterial bifurcations, terminal arterioles, atherosclerotic plaques etc.), the successive pulse waves reflect and return back towards the heart, but with a reduced pressure (similar to a tennis ball being thrown against the wall, returning in the opposing direction and with less force). When the *reflected* waves return to the aorta their pressures superimpose on top of the *outgoing* central aortic systolic pressure wave, causing *pressure augmentation*. This augmentation, or overlap of pressure, begins at the end of systolic phase, and remains for the whole diastolic portion of the cardiac cycle. The moment in time both pulses meet at the aorta is termed the 'inflection point,' and the amount of pulse overlap (in mmHg) is termed the augmentation pressure (AP). The augmentation index is simply the AP expressed as a percentage of the entire aortic pulse pressure (aortic PP = central systolic - diastolic pressure), and essentially represents the incidence of wave reflection on the total pulse pressure [112].

The augmentation index is thus calculated as follows: AIx(%)=(AP/aorPP)*100

Salvi demonstrates how we may clinically get both positive and negative values for AIx based on the location of the inflection point. If the inflection point occurs *after* the peak systolic pressure, then the AIx will be negative because the reflection does not augment the peak pressure, and rather returns during the diastolic portion of myocardial

contraction to enhance myocardial perfusion. In the case where the reflection occurs before peak systolic pressure then we will have a positive AIx, and this is related to an increased myocardial afterload, and work for the heart [100]. In this way AIx is a continuous variable that can be either positive or negative, with more negative values indicating reduced incidence of wave reflection at the aorta and reduced myocardial workloads. The AIx has also shown to be impacted significantly by individual's heart rate at time of measurement. As the heart rate increases, as with aerobic exercise or acute smoking, the percentage of time spent in systole decreases relative to the entire cardiac cycle [113]. Since systole is shortened, the reflected waves begin to return later into diastole, resulting in less central pressure augmentation and lower values of AIx. Thus, AIx and heart rate are inversely related, demonstrated by a 3.9% decrease in AIx for every 10 beat per minute increase in heart rate [21, 114]. It has been previously demonstrated that chronic smoking leads to increased AIx in both younger and older populations [15, 29, 32, 38], while acute smoking acts to immediately decrease the AIx, primarily mediated through an increase in the heart rate [11, 14, 15].

The degree of overlap at the aorta (and thus AIx) is closely related to both the timing of the reflections' return (ie. at what point in time of the cardiac cycle), and the size and distribution of the reflected waves in the periphery [100]. These factors are additionally impacted by a variety of physiological factors that should be considered in the interpretation of AIx, including: sex, as women have shown to have significantly higher AIx at rest [60]; height, which influences the path length and distance to reflection sites [115]; arterial stiffness, which alters the kinetics of the reflected waves [116]; size and variability in reflected waves, discussed below; heart rate, often inversely associated with the AIx, also discussed below [100]. Smoking and acute aerobic exercise are both associated with increases in central arterial stiffness and heart rate, contributing to altered AIx profiles seen in various groups of chronic smokers [15, 29, 32, 38].

Each individual has unique vascular anatomical features that impact the size and variability of reflections in the periphery. As such, outgoing pulses may reflect off of a variety of sites, including:

a) Arterial bifurcations (which cause one incoming wave to result in two outgoing waves, and one reflected wave),

b) Terminal arterioles, such as those found in the tips of the fingers (which cause reflected waves only). They define systemic vascular resistance and are likely the largest contributor to reflections,

c) Atherosclerotic plaques, such as those found in the aorta or carotid or femoral arteries (which cause one incoming wave to split into one outgoing and one reflected). Plaques cause vessel narrowing and act as an obstacle to laminar flow.

In addition to specific sites of reflection, AIx is also directly, and importantly, impacted by the heart rate of the individual as previously described. Since heart rates at rest, and in response to stress, differ between individuals, the AIx is often 'normalized' to a heart rate of 75 beats per minute (AIx75). With this normalized variable inter- and intra-individual comparisons of AIx may be made independent from the strong influence of heart rate. In this context, the AIx75 may be used as a more revealing and useful measure in clinical research settings. The SphygmoCor system software calculates this adjusted variable from the derived aortic waveform, and is based on the following equation formulated by the company: AIx75 = AIx - 0.39*(75-HR).

With such a range of variables impacting AIx, this measure should not be used on its own to evaluate arterial stiffness, but rather should be used in conjunction with other more sensitive measures, such as the cfPWV. It is important to note that although related, discrepancies between cfPWV and AIx have been previously noted [117].

Thus, under normal physiological conditions, in young, healthy individuals, the reflected waves contributing to AIx play a positive role on the cardiovascular system. The more negative the AIx, the more beneficial to the individual, as central systolic pressures are not augmented. Later return of the reflected waves will help to maintain high diastolic blood pressures and adequate coronary blood flow, without increasing the cardiac afterload [100]. In contrast, with higher values of AIx, myocardial work increases as there is a larger arterial pressure to overcome in systole, while coronary flow and perfusion during diastole is decreased.

Various studies have previously detailed the association between acute smoking and AIx [11, 14, 15, 29, 32, 38]. Lemogoum et al. demonstrated AIx to be increased as early as 10 minutes following cigarette smoking in healthy current smokers (7.7± 9.0% to 14.8 ± 18.0%, P=0.01) [14]. Vlachopoulous et al. demonstrated more lasting effects, with AIx elevated by 6.1% one hour following cigar smoking, remaining significantly elevated for another hour [11]. Mahmud and Feely also similarly noted that AIx was increased for 15 minutes following acute smoking in both current smokers and non-smokers alike [15]. Chronic smoking has been associated with increased AIx in a multitude of studies [11, 15, 29, 32, 38], and has been independently correlated with smoking status in both men and women [31]. Rehill et al noted a significantly higher AIx in chronic smokers compared to non-smokers (17.25% vs. 11.75%, respectively, P=0.004) [30], confirmed by the results of Mahmud and Feely, who studied AIx in *younger populations* of current and non-smokers [15]. Since the impact of chronic smoking is more apparent with a greater history of smoking, the majority of studies have investigated arterial hemodynamics in older adult smokers. However, this is cause for concern as the number of factors impacting vessel hemodynamics increases as we age. Thus, the effects of chronic smoking on the vascular system are yet to be fully established in young, healthy individuals, without concurrent CVD risk factors (apart from current smoking). Similarly, no other study has to date investigated the impact of acute aerobic exercise on the AIx in smokers, which could provide valuable insight into potentially altered hemodynamic changes with stress in smokers.

It has previously been demonstrated that AIx75 is acutely elevated in the first 5 minutes following aerobic exercise under multiple conditions [60], while diastolic AIx (which is negatively correlated to AIx) was decreased at multiple sites following cycling and running exercises [67]. Similarly, another study demonstrated an AIx75 that was significantly elevated for 15 minutes post-exercise [37]. At 20 and 30 minutes after cycling exercise, AIx was shown to be significantly reduced compared to resting levels in resistance trained men [62], while Munir et al. demonstrated significant decreased central AIx 15-60 minutes post-exercise [67].

### *2.2.4 Pulse Pressure Amplification*

Largely resulting from differences in the timing of peripheral pulse reflections, the pulse pressure amplification (PPA) is defined as the increase in blood pressure observed from the ascending aorta out towards peripheral vascular sites in the arterial tree [118]. It is well document that there exists variation in blood pressure across the arterial system; systolic blood pressure progressively increases from the aorta out to peripheral sites, while diastolic and mean arterial pressure remain fairly constant [119]. The result, in young, healthy individuals, is a pulse pressure (PP) that is wider in peripheral sites than that observed at the aorta. This widening of PP in young, healthy individuals is beneficial because it allows for a decrease in myocardial work during systole, while also allowing for increased cardiac perfusion in diastole. 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, exemplifying the aforementioned [120].

> In other words, (peripheral SBP -peripheral DBP) > (aortic SBP - aortic DBP), or peripheral PP > aortic PP.

Thus, the pulse pressure is *amplified*. In laboratory settings this parameter is calculated from brachial blood pressures taken via sphygmomanometry, and the derived central aortic pressures obtained through applanation tonometry. Reference values for clinical brachial PP have been elucidated. Asmar et al. found the $50^{th}$ percentile for PP in men and women aged 21-55 to be 50mmHg [121]. It has been shown that PP greater than 60mmHg is associated with increased risk for cardiovascular disease [122], while a brachial PP increase of 10mmHg is associated with a 14% increase in risk for heart failure, 12% increase in coronary artery disease risk, and 6% increased risk for all-cause mortality in individuals over 65 years old [123].

As the vessels in our vascular system narrow from the aorta to the periphery, resistance increases inversely to the fourth power of vessel radius decrease, according to Poiseuille's Law. With such increasing resistances, the cardiovascular system must provide greater pressures to achieve adequate perfusion to the essential organs and working muscles. These required driving pressures, and thus the PPA, can be further

*increased* by a variety of factors, such as an increase in heart rate [124] and changes in posture (impacting the level of the heart with respect to the brachial bifurcation where peripheral BP is measured) [125]. PPA is also shown to *decrease* with age [126]. As we get older, central pressures increase disproportionately to those taken peripherally and thus the difference between aortic and peripheral PP's narrow, decreasing PPA. It is thus very important that not only brachial pressures but also pressures occurring at the aorta be used in the interpretation of vascular hemodynamic abnormalities, as central aortic pressures have proven to be a better predictor than peripheral pressures in the evaluation of cardiovascular disease outcomes. These two sites of arterial pressure are related, but have clinically important differences. It has been shown that despite demonstrating similar reductions in peripheral BP, antihypertensive medications may have altogether differing effects on reductions of aortic pressure depending on the medications prescribed [127].

Since blood flow through the heart, coronary, and carotid arteries is closely related to central pressures, alterations to these variables with stress are of the utmost importance. Through analysis of the PPA, one can verify if the individual under study generates normal levels of aortic pressure with each cardiac cycle, or if increased aortic pressures are causing the required work of the myocardium to increase. Central blood pressures have previously been correlated with intima-media thickness [128] and left-ventricular workload [129], both of which are independent predictors of mortality, and thus implicate abnormally low PPA in death and disease. Smoking has been strongly associated with increased intima-media thickness and central aortic pressure, and may partially explain reduced PPA at rest, previously found among chronic smokers [130].

A variety of additional factors may impact the increase in BP from the aorta towards the periphery. As transit time for reflected pulses decrease inversely with increasing arterial stiffness, the outgoing and reflected pulse waves meet earlier and earlier in the cardiac cycle at the aorta, augmenting *central systolic BP*. Similarly, the closer the site of measurement is to areas of reflection, the more the reflected wave will superimpose over the outgoing wave in the systolic phase and increase site SBP. In healthy populations with low arterial stiffness and slower pulse transit time the reflected component will have

a greater peripheral contribution to systolic pressure (in the radial artery, for example) than aortic pressure. Reflections occur nearer the site of measurement and therefore reflections contribute more to the systolic component of the pulse wave. As we age, or with smoking, there is also an increase in the ratio of collagen to elastin fibers in the vessel walls. This causes stiffening of the arteries (increased cfPWV), and an altered return of reflected waves to the aorta, leading to increased afterload, decrements in coronary blood flow, and abnormalities in cardiac perfusion [100].

Differences in aortic length have also been associated with PPA among healthy populations [131]. The further the sites of reflection are from the start of the ascending aorta, the later the reflected waves will return, decreasing the augmentation pressure seen centrally. A longer aorta will thus result in lower central systolic pressures, and thus a greater PPA [131].

As the heart rate increases, there is a reduction in cardiac cycle time. This dramatically impacts the morphology of the aortic and brachial pulse waves. Similar to what is noted with increased arterial stiffness, increased HR will cause a more dramatic increase in peripheral SBP compared to aortic SBP. With exercise, HR increases, and leads to significant increases in PPA as perSBP increases disproportionately to aorSBP [131].

The CAFE trial, a branch of the ASCOT, was able to highlight the importance of measuring not only peripheral pressures, but also central pressures. In this study they noted significant differences in the central and peripheral blood pressure response to an ACE inhibitor and a calcium channel blockers (perindopril + amlodipine) as compared with a β-blocker and a diuretic (atenolol + thiazide). Aortic SBP and aortic PP were significantly decreased in the group taking ACE inhibitors and calcium channel blockers, while peripheral SBP remained unchanged. There were fewer cardiovascular events in those taking perindopril + amlodipine when compared to atenolol + thiazide, and the authors attribute this to the benefits provided at the central level with the former combination. This demonstrates the added value of evaluating not only peripheral pressures, but also those at the central level [131].

There are, however, some potential limitations when considering PPA as a parameter of arterial health. Aortic blood pressure values are obtained indirectly using applanation tonometry at the radial artery, and calibrated with sphygmanometric brachial blood pressures. As there has been sparse evidence of PPA between the brachial and radial arterial sites, this method of calibrating the *radial* pressure waveform with *brachial* pressures has been accepted by Sphygmocor, the manufacturer of applanation tonometry equipment used to conduct this study. The Asklepios Study, however, demonstrated that there may in fact be differences in PP and SBP between the brachial and radial sites, which casts some doubt on the applicability of values derived from the system software algorithms using brachial pressures as calibrates for the radial waveform [132].

There is a body of evidence describing the impact of acute and chronic smoking on blood pressure and PPA in a variety of populations. A history of cigarette smoking has been clearly associated with increased basal SBP, MAP, and PP, compared to non-smokers across a broad range of age groups [16, 133-135]. Acute smoking has been shown to further increase blood pressure and reduce PPA over and above that already present as a result of chronic smoking. The myocardium of a smoker must therefore increase its workload to match the increased intravascular pressures, leading to increased risk for CVD events, and poorer outcomes [136].

Previous studies, both older and more current, have also demonstrated that there is a clear increase in PPA with exercise [74, 125, 137]. This results primarily from increases in HR via the mechanism previously described. The net effect will be an attenuation of aortic SBP with increasing heart rate, while perSBP continues to rise, widening PPA [124]. It still, however, remains somewhat unclear if there is a dose-response to exercise. Preliminary findings from a parallel study in our laboratory (termed the "Relative Workload" study) have revealed that PPA immediately post-exercise is further increased following more strenuous bouts of aerobic exercise (data not shown).

### 2.3 Aerobic Exercise as a Physical Stressor

Physical activity and exercise play a crucial role in the maintenance of healthy living. With approximately 30% of adults being insufficiently active, reduced physical activity is

a contributing factor to more than 3.2 million deaths globally each year [138]. Current guidelines recommend at least 150 minutes of moderate-intensity aerobic exercise per week [138], as many studies have shown that regular exercise reduces the incidence of cardiovascular complications, such as obesity, diabetes, hypertension and atherosclerosis, in both men and women [139-141].

### *2.3.1 Acute Exercise and the Cardiovascular System*

Under conditions of *acute* physical stress (such as running or swimming), segments of the arterial tree are differentially affected and respond/recover in a time-dependent manner following stress cessation. This response is owing to the complex nature of the cardiovascular system under stress.

Aerobic exercise requires that the cardiorespiratory system respond to increased demands placed on the body by the large working muscles. In the face of physical stress, working muscles require, among a multitude of other factors, vital nutrients, increased oxygen delivery and increased elimination of metabolites. In order to achieve such requirements the central cardiorespiratory control mechanisms located in the higher brain centers are responsible for directing a change in systemic blood flow. Blood is thus shunted away from less active muscles and organs in order to meet increased demands of the working muscles. This is done in the face of maintaining critical delivery of oxygen to the brain and heart so an ischemic event may be avoided [142, 143].

To achieve the sensitive balance of blood redistribution there are not only central control mechanisms from the autonomic nervous system, but also chemical and mechanical factors that are produced by the working muscles in an attempt to maintain appropriate perfusion pressure. If blood pressure is left uncontrolled, so will be the redistribution of blood during and after physical stress [142, 143].

During aerobic exercise there is not only need to alter blood distribution, but the cardiac output may also need to be increased to five times upwards the resting values [144]. The rate of oxygen delivery to the large working muscles must be sufficient to maintain exercise workload. This is the direct response to an increase in sympathetic nervous system firing and decreased parasympathetic stimulation, which are both products of

central control. This results in an increase in the heart rate, alongside increased cardiac contractility and vasoconstriction to inactive and low-activity organs (such as kidneys and spleen) [143, 145]. Ultimately, the MAP also increases as a result of the aforementioned. As the active muscles produce metabolites and endothelial factors (such as nitric oxide), there is vasodilation of the vessels supplying the working muscles to allow for increased blood flow. The vasoconstriction response seen in vessels supplying less crucial organs will also force additional blood into the central circulation and increases the venous return to the heart, and thus also stroke volume. As such, cardiac output is increased [142]. There is, however, a greater degree of contribution from heart rate than from stroke volume in the increase of cardiac output at high workloads. Heart rate increases linearly with workload, whereas stroke volume may only continue increasing until 50% of maximal achievable workload is attained [142] thus final cardiac output values are heavily influenced by maximal heart rate.

Throughout acute aerobic exercise the skeletal muscles themselves play a large role in vascular dynamics, as the mechanical action of skeletal muscle pumps drive blood against gravity through the venous system. As such, there is a concomitant increase in venous return and in end-diastolic volume and pressure, which act to increase cardiac output [146]. Similarly, as exercise progresses a local buildup of metabolites ($CO_2$, potassium, lactic acid etc.) forms within skeletal muscle intracellular fluid, causing tissue pH to drop with resulting local arteriolar dilation to the working muscles [143, 147]. In tandem, vascular endothelial tissues release potent vasoactive molecules, such as prostaglandins and nitric oxide that enhance the already increased vascular conductance to working muscles [148]. While there are local vascular changes occurring throughout the body during physical stress, the MAP is tightly controlled through a series of baroreceptor feedback loops in order to ensure that both cardiac and cerebral perfusion are appropriately maintained. As there is increased demands placed on the cardiovascular system under stress, the ‘set-point’ for the carotid and aortic baroreceptors is increased to higher pressure values, which allows appropriate perfusion to crucial organs while continuing to provide the working skeletal muscles with sufficient blood supply [142, 146].

At very high workloads (>75% $VO_2$peak), the sympathetic nervous system (SNS) may also release a surge of catecholamines, further constricting vessels supplying inactive musculature and organs. This occurs in the face of aforementioned vasodilation of vessels supplying active muscles. Since the latter is greater than the former, there is a net increase in systemic vascular conductance [149], which persists into the early recovery period following exercise cessation.

The impact of acute and chronic cigarette smoking on the metabolic/respiratory response to exercise has been fairly well characterized. Multiple studies have consistently demonstrated that chronic smoking impairs oxygen delivery and availability at the tissue level, decreases $VO_2$peak, and leads to a mismatch in ventilation/perfusion of the myocardium - although not to levels of myocardial ischemia [150, 151]. Acute smoking conditions also lead to an earlier anaerobic threshold during progressive exercise, and increased respiratory exchange ratios at peak exercise capacity [150, 152]. Multiple studies have demonstrated that maximal exercise capacity (as judged by time to exhaustion on maximal exercise testing), and $VO_2$peak were non-significantly different in smokers after abstinence from smoking compared to smokers after acute smoking conditions [152, 153], while others demonstrated significantly impaired exercise capacity following acute smoking in smokers [154]. Not surprisingly, multiple older and more recent studies have demonstrated significant decreases in duration of maximal exercise among smokers when compared to non-smokers, although baseline physical activity levels were rarely reported [152, 155]. It has similarly been shown that endurance is inversely proportional to both the number of cigarettes smoked each day and to duration of smoking history [156].

The overall impact of resistance-type exercises on the modulation of arterial stiffness has also been well characterized. Studies have consistently shown increased arterial stiffening with both chronic and acute resistance training [157-162]. The effects of chronic physical activity on arterial stiffening have been similarly well documented, and studies consistently demonstrate improved arterial stiffness in aerobically trained individuals [161, 163-168]. However, controversy still abounds as to the impact of acute aerobic exercise (as an acute physical stressor) on immediate changes in arterial stiffness.

To begin answering this question the first known systematic review aimed at synthesizing evidence pertaining to acute changes in arterial stiffness shortly following aerobic exercise was performed by our group. Included in this review were all relevant clinical studies assessing the effect of acute aerobic exercise on parameters of arterial stiffness; measurements of arterial stiffness were taken before and after acute aerobic exercise in healthy human subjects. A total of 24 studies were identified, confirmed by all authors, and included in the review [37, 57-79]. All included studies were observational in nature and involved only healthy individuals. Altogether these studies evaluated 428 men and 198 women, for a total of 626 adults. All studies enrolled participants between the ages of 20-35, except Aizawa et al. [57], Nickel et al. [69], and Tabara et al. [78] who enrolled only older healthy adults. Mean participant BMI in each study was below 30 $kg/m^2$.

All included studies used a form of cycling, running, or leg extensor exercise as their primary aerobic physical stressor with the following exceptions; Aizawa et al. [57] and Ranadive et al. [73] incorporated graded arm-cycling protocols, and Tabara et al. [78] used dancing/hopping as the acute physical stressor.

Differential effects of acute aerobic exercise on arterial stiffness, depending on the anatomical segment analyzed and the time of measurements post-exercise, were noted. Therefore, summarized findings were separated into time intervals *post-exercise*; those measured in the first 5 minutes, and those measured >5 minutes after exercise.

**Arterial stiffness measurements immediately post-exercise (0-5 minutes)**

Fifteen studies were identified that characterized changes in arterial stiffness immediately (0-5 minutes) after exercise [60, 63, 70, 169-180]. Findings pertaining to changes in arterial stiffness immediately following exercise are somewhat discordant. Some groups have demonstrated non-significant changes in arterial stiffness parameters *immediately* following acute aerobic exercise [63, 70, 169, 171-180], while significant but diverging results have also been reported [60, 63, 70, 170-172, 174-178, 180].

Included studies assessing *central and upper body peripher*al arterial segments in the first 5 minutes post-exercise demonstrated non-significant changes [63, 70, 169, 171-174, 176, 179, 180] or *increased* arterial stiffness [37, 60, 63, 70, 170-172, 175, 176, 180].

Specifically, Rakobowchuk et al. demonstrated significantly increased heart-femoral PWV 2 minutes post-exercise, which remained elevated for 20 minutes [72], while Naka et al. found brPWV to be 35% higher 3 minutes post-exercise [68]. Lydakis et al. also found a decrease in *Ti* (the transit time of the pulse wave between the heart and reflection sites in the periphery) immediately following exercise to exhaustion [65]. Similarly, Doonan et al. found cfPWV to be significantly elevated 2 and 5 minutes following exhaustive treadmill exercise [37]. The AIx75 was shown to be elevated in the first 5 minutes following exercise under multiple conditions [170, 180], while diastolic AIx (which is negatively correlated to AIx) was shown to be decreased at multiple sites immediately following cycling exercise and running exercises of various intensities [67]. Further, Tordi et al. saw an elevated crPWV 2 minutes following both intermittent and constant aerobic exercise [79], while Rakobowchuk et al. also noted a trend for increased femoral B-stiffness index 2 minutes post-exercise [72], although neither aforementioned result reached statistical significance.

Conversely, all studies investigating arterial stiffness in *lower limb arterial segments*, at or near the primary exercising muscle groups, observed non-significant [68, 79] or *decreased* arterial stiffness *immediately* following exercise [72, 76, 77, 79]. Specifically, Sugawara et al. found significant decreases in femoral-ankle PWV [77], and femoral-posterior tibial PWV [75] in the exercising limb 2 minutes after light cycling, while non-significant differences were found in the non-exercising limbs. Femoral-dorsalis pedis PWV (fdpPWV) was shown to be significantly decreased 2 minutes following cycling exercises of varying intensities [72] while Tordi et al. also demonstrated decreases to carotid-dorsalis pedis PWV (a composite measure of central and peripheral arterial stiffness) as early as 4 minutes post-cycling exercise [79].

**Arterial stiffness measurements > 5 minutes post-exercise**

As time from exercise cessation increases, there is a consistent trend towards a decrease in parameters of arterial stiffness towards or below resting values, independent of the arterial segment being analyzed [170-172, 174, 175, 180-185]. However, a small number of studies also noted no changes to various parameters of arterial stiffness following exercise [57, 58, 74, 78]. Specifically, Naka et al. demonstrated a significant 6% decrease

vs. resting values in brPWV 10 minutes post-exercise, reaching a steady state of 10% below resting values by 60 minutes [68]. As previously noted, this same measure had been increased 35% at the 3 minute post-exercise time point in the same study. Ranadive et al. also found significantly decreased crPWV 10 minutes after maximal arm-ergometry in healthy volunteers [73]. At 20 and 30 minutes after cycle exercise, AIx was shown to be significantly reduced compared to resting levels in resistance and non-resistance trained men [61], while Munir et al. demonstrated significantly decreased central AIx 15-60 minutes post-exercise [67]. Diastolic AIx was also shown to be increased at multiple upper body peripheral sites 15-45 minutes following running exercises of various intensities [59]. One study did, however, demonstrate an AIx75 that remained significantly elevated for 15 minutes post-exercise [37]. Similar results were found in measurements of central stiffness; Heffernan et al. [61] and Kingwell et al. [64] found cfPWV to be significantly reduced compared to rest at 20 and 30 minutes after cycling exercise, respectively. By 10 and 20 minutes post-exercise, significantly elevated cfPWVs had also returned to resting values in studies by Doonan et al. [60] and Rakobowchuk et al. [72], respectively.

Changes in lower limb arterial stiffness measures >5 minutes post-exercise are similar to those of the upper limbs and larger central arterial segments. At 15 and 30 minutes post-exercise, Heffernan et al. demonstrated a significant decrease in fdpPWV in white men, but not in African American men. No significant change in cfPWV was noted in this same group [62]. Similarly, fdpPWV was found to be significantly decreased 10-30 minutes after cycling exercise in both resistance trained, and age-matched non-resistance trained controls [61]. These findings are further confirmed by others who showed decreased fdpPWV for 44 minutes post-exercise [72], 4-28 minutes post-exercise [79], at 10 minutes after maximal leg-ergometry [73], and at 30 minutes after cycling exercise [64]. Measures of the less conventional midthigh-ankle PWV, were also shown to be significantly decreased by 23% at 10 minutes after exercise, and reached a steady state of 10% below resting values by 60 minutes post-exercise [68].

To our knowledge, this was the first systematic review investigating the effect of acute aerobic exercise on arterial stiffness. Acute aerobic exercise was shown to have varying

effects on arterial stiffness, according to the arterial segment being assessed and the time at which the measure was taken post-exercise. Arterial stiffness of the *central* and *upper body peripheral* arterial segments is increased relative to resting values immediately post-exercise (0-5 minutes), and thereafter (>5 minutes) decreases to a level at, or below resting values. In the *lower limbs* there is a decrease in arterial stiffness immediately post-exercise (0-5 minutes), which persists well into the recovery period post-exercise (>5 minutes). Owing to differences in methodologies and protocols of included studies, a meta-analysis on the effect of acute aerobic exercise on arterial stiffness was not feasible.

## 3.0 Research Objectives:

1. To estimate differences in arterial stiffness parameters at rest among young, healthy non-smokers and current smokers under the following conditions;

    a) After 12hours abstinence from smoking

    b) Immediately after smoking one cigarette

2. To estimate differences in arterial stiffness parameters immediately after acute aerobic exercise and during the recovery period between non-smokers and smokers under the above conditions

3. To estimate differences in maximal exercise capacity and metabolic cardiorespiratory variables in response to acute aerobic exercise between non-smokers and smokers under the above conditions

### 3.1 Hypotheses:

1. Young, healthy smokers after 12 hours abstinence will demonstrate increased arterial stiffness when compared to non-smokers. This difference will be more exaggerated immediately after smoking one cigarette.

2. When compared to non-smokers, chronic and acute smoking will decrease the ability of the arteries to respond to physical stress.

    •This effect will be most pronounced in the acute smoking condition.

•Abstinence from smoking will provide results that are most closely associated to those of the non-smokers.

3. Smokers will have reduced exercise capacity and increased cardiorespiratory stress in response to acute aerobic exercise compared to non-smokers.

•Greatest impairment will be noted immediately after smoking one cigarette, and least impaired following abstinence, compared to non-smokers.

## 4.0 Methods

The McGill University Health Centre Research Ethics Board fully approved the protocol for this study (Appendix Figure 3). This study was funded through the CIHR operating grant of Dr. Stella Daskalopoulou (my primary supervisor) under the title of "Quantification of the effect of Smoking on Arterial Stiffness" (*SMOKELESS).*

### 4.1 Participants

#### *4.1.1 Inclusion criteria*

Prior to be enrolled in the study, potential subjects were given a short eligibility questionnaire to fill out and return to the research staff (Appendix Figure 1). It queried all factors relevant for the determination of subject study eligibility. All participants had to be between the ages of 18 and 45 years and be a current smoker or non-smoker. According to Health Canada, a *current daily smoker* is an individual who has smoked 100 or more cigarettes in his/her lifetime, and who has smoked at least one cigarette each day for the 30 days preceding the assessment. A minimum smoking history of 3 pack-years was a requirement for eligibility among smokers upon recruitment.

pack-years = # years smoking * # packs smoked per day  [assuming 20 cigarettes/pack]

#### *4.1.2 Exclusion criteria*

To maintain a relatively 'clean' population of participants, strict exclusion criteria were enforced; obesity (defined as a body mass index $\geq$ 30 kg/m$^2$) [138]; a previous diagnosis of cardiovascular disease (this includes acute coronary syndrome, valve diseases,

congenital heart diseases, heart arrhythmias, peripheral arterial disease, and cerebrovascular disease); specific cardiovascular risk factors (such as hypertension, diabetes mellitus, and dyslipidemia); pregnancy; menopause; and physical inactivity. Additionally, all subjects reported feeling well on the days of testing, and were not regularly taking any medications (including oral contraceptives, antidepressants, or cardio-protective agents).

### *4.1.3 Recruitment*

The study was extensively advertised for through various means, including notices on Montreal area university websites, e-mails communications, and posted information sheets in public spaces (such as within cafes, restaurants etc.), advertisement within the McGill University Health Centre, and its environs. The variety of recruitment vectors allowed us to achieve a fairly comprehensive subject pool within the confines of our eligibility criteria. Public advertisements created are included in Appendix (Figure 2).

The research team provided our Smoking Study Eligibility Questionnaire (Appendix Figure 1) and International Physical Activity Questionnaire (IPAQ) (Appendix11) once initial contact was made with participants. This was done either via telephone or email, depending on the preference of the individual. Eligible participants were provided convenient appointment dates (1-2 sessions) in accordance with their availabilities.

The IPAQ is a widely used and extensively validated tool for quantifying leisure time physical activity in individuals aged 15-69 [186-188]. It is often used in clinical research and public health planning settings. For this study we implemented the short version of the IPAQ, which queries the individual's weekly participation in various levels of physical activity, and generates a tangible number for the persons' median weekly energy expenditure.

## 4.2 Procedures

### *4.2.1 Pre-Stress Test*

There were two distinct groups of participants enrolled in this study: a cohort of never smokers forming the non-smoking group, and a cohort of current-smokers. Participants provided written informed consent (Appendix Figure 4).

Prior to arriving at the lab, all subjects were provided with an information sheet pertaining to the study (Appendix Fig 5). To reduce variability in data collection, subjects were asked to abstain from: 1) consuming caffeine and/or ethanol containing substances for at least 12 hours before the assessment; 2) Flavonoid-containing foods (like berries, grapes, apples, green tea, etc.) for at least 24 hours before the assessment; 3) any type of vigorous exercise (running, swimming, biking, weightlifting, etc.) for at least 24 hours before the assessment, and: 4) smoking for at least 12 hours before the assessment (to prevent the short-term effects of tobacco smoking). At the beginning of each assessment, participants provided saliva samples in order to confirm smoking status through the measurement of cotinine levels (these samples are to be processed at a later date, and thus not included in this analysis).

All women included in the SMOKELESS study were examined during the follicular phase of their menstrual cycles. This was done to minimize the potential impact of different phases of the cycle on arterial stiffness [189]. However, as prior investigation by our group has revealed sex differences in the evaluation of arterial stiffness under various conditions [60] the analysis presented for the purposes of the thesis included only successive male participants to reduce the impact of sex on results. Cardiovascular diseases affect men and women differently, with prevalence rates of 15.9% in men and 7.8% in women aged 20-39 [190]. Several parameters of arterial stiffness have been shown to change similarly with age between sexes [81], while others show distinct sex differences in their progression [191, 192]. It is clear that focusing on a male-only analysis is the first step towards uncovering the effects of cigarette smoking and acute physical stress on arterial stiffness.

Upon their first visit to the research laboratory, all participants completed a detailed questionnaire that included the following: sociodemographic information, family history, past medical history, current medication use, history of smoking, current diet, level of physical activity, and reproductive history and menstrual cycle for women. To calculate the body mass index, and for use with the Metabolic Cart, we obtained measures of height and weight along with waist and hip circumference measurements.

With each assessment, all participants provided a small sample of blood (10 ml) prior to having measurements of arterial stiffness and blood pressure performed pre- and post-exercise. Although blood samples collected during this process were not analyzed for the purpose of this thesis, it is still of importance as it impacted the timeline of measurements during the arterial stress test.

### *4.2.2 Arterial Stress Test*

Smokers came in for two separate arterial stress tests (Figure 1, below) on two separate days, while non-smokers came for only one assessment. The smokers performed their assessments under the following conditions:

a) After 12 hour abstinence from smoking, to capture the *chronic* effects of smoking

b) Immediately after smoking one standardized cigarette (du Maurier signature, 1.1-2.4 mg nicotine, Les Marques Imperial Ltee, Montreal, Quebec, Canada), to capture the *acute* effects of smoking

For smokers, assessments were conducted in a *randomized order fashion*, each at the same time of the day to avoid any circadian variations [193], and conducted at least 48 hours apart from one another to "allow for full recovery of exercise capacity" [194]. Non-smokers followed the same protocol as the smokers on their *chronic smoking* day.

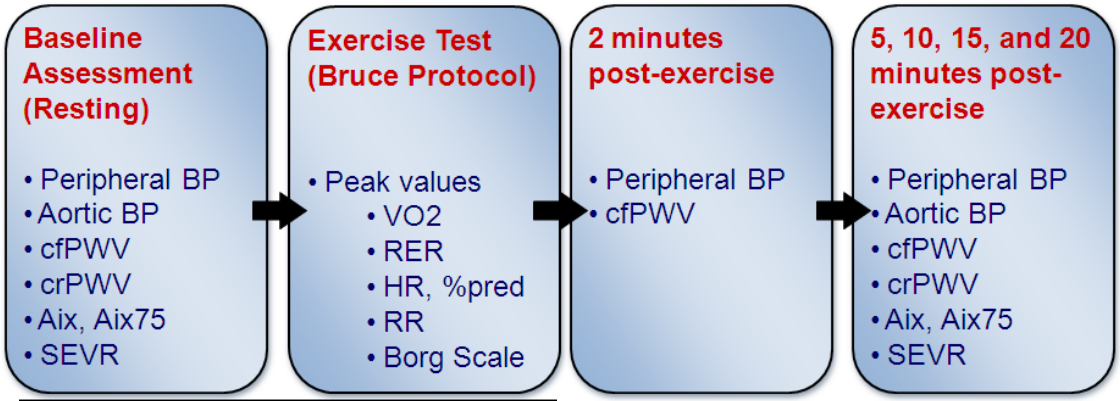



**Figure 1 – Arterial Stress Test Protocol**

Next, subjects underwent the arterial stress test (Figure 1). Resting (pre-exercise) measurements of peripheral blood pressure, PWV, and PWA were performed *in duplicate*. All measurements were conducted by the same trained technician (myself) using the technique of applanation tonometry. Following resting measures, participants underwent an incremental treadmill test (modified Bruce Protocol, Appendix Figure 7) to volitional exhaustion. Immediately post-exercise, blood was again drawn from the catheter by the research nurse for future post-exercise blood analysis, while blood pressure, PWV, and PWA measures were taken at specific time points (2, 5, 10, 15, and 20 minutes post-exercise) to estimate various associations in vascular stress and recovery among smokers and non-smokers.

### 4.3 Measurements

All measurements and testing were performed at the Vascular Health Unit of the Montreal General Hospital (Room B2.252), where the environment is controlled (ambient temperature 22±1°C and humidity 60 ± 5%, quiet).

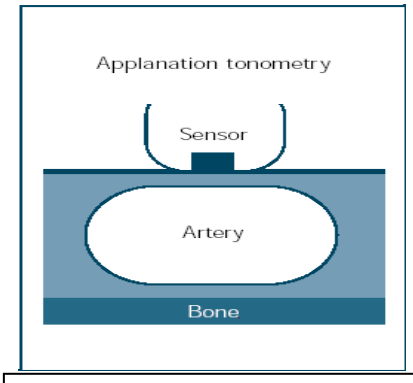



**Figure 2 – Applanation Tonometry Technique**

As described above, patients lay in a supine resting state for at least 10 minutes prior to pre-exercise measurements. Following this rest period, measurements of blood pressure, PWV, and PWA were performed in duplicate. During all hemodynamic measurements, participants were asked to refrain from

talking, falling asleep, and moving unnecessarily. These simple instructions allow for measurements with the highest level of reproducibility.

### *4.3.1 Brachial Blood Pressure*

Peripheral (Brachial) BP was taken manually with cuff sphymomatometer (Adult 11 Blood Pressure Cuff, Welch Allyn) and stethoscope (Cardiology Stethoscope T403, Aglaia Healthcare) in duplicate according to the Hypertension Canada 2013 CHEP guidelines [195].

### *4.3.2 Carotid-femoral PWV*

Measures of cfPWV and PWA were taken by applanation tonometry (Figure 2) applied to various arterial sites, namely, the carotid, femoral, and radial arteries. The commercially available pulse contour system - termed SphygmoCor (SphygmoCor, AtCor Medical Sydney, Australia) in conjunction with a highly sensitive "micromanometer" (SPC-301; Millar Instruments, Houston, TX, USA), on the tip of a pen-shaped hand-held device, is used in the recording of all measures pre- and post-exercise. Recording a signal every 8 milliseconds (sampling rate of 128 Hz), the instrument is first placed on the skin directly overtop of the artery in such a way that the artery is slightly flattened by the perpendicular downwards pressure of the probe, but not so hard as to completely occlude the artery. In this manner the pulse contour system is able to accurately record pulse waves at the arterial site. By comparing the timing of the 'foot' of the pulse wave relative to the R wave of ECG trace the SphygmoCor software calculates the pulse transit time from a) the heart (as indicated by the R wave of ECG trace) to b) the artery, for example the femoral artery (as

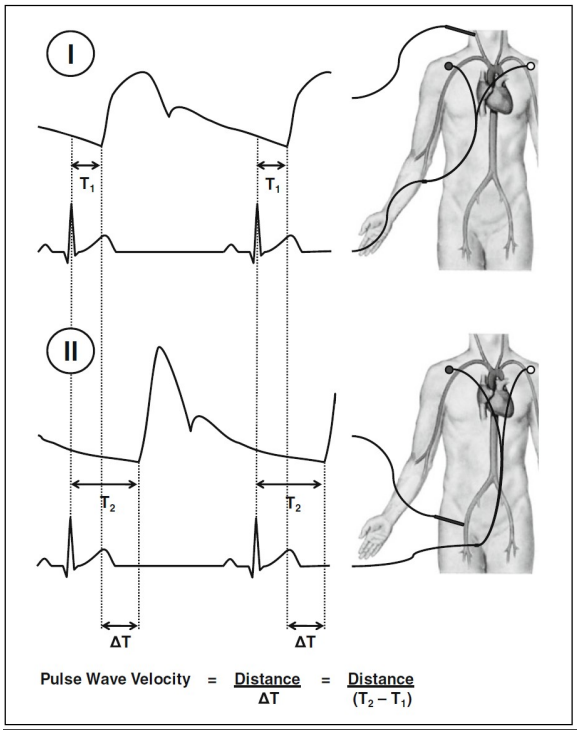



**Figure 3 – SphygmoCor technique for measurement of carotid-femoral PWV.**

indicated by the 'foot' of the pressure wave at the femoral site) (Figure 3). Once the femoral measurement is captured, the same technique is then used at the carotid arterial site, such that the transit time between left ventricular ejection to the carotid site is calculated. The estimated length of pulse wave travel is calculated from the distance between the carotid and femoral arterial sites using a tape measure and the 'subtraction method' described in Background 2.2.

Thus, PWV is calculated as [PWV = arterial length(m) / transit time(s)] (shown in Figure 4). Appendix Figure 9 is a software screenshot following a cfPWV measurement on the SphygmoCor system.

### *4.3.3 Pulse Wave Analysis*

When capturing the radial arterial waveform (using the same method described at the femoral and carotid sites), the shape of the pulse pressure waves is very important, as these waveforms will be used in the calculation of variables derived from pulse wave analysis (PWA). Measures of PWA (such as the SEVR, AIx75, and PPA), are derived from 10 sequential seconds of radial artery waveforms that are then overlaid on top of one another. The SphygmoCor software produces an average radial pressure waveform from this overlay. These radial (peripheral) waveforms are then calibrated with brachial systolic and diastolic blood pressure, taken manually via sphygmomanometry, and using a previously validated 'generalized transfer function' the software calculates a *derived central aortic pressure waveform [44]*. A multitude of variables are then calculated based on this central waveform, including the SEVR, AIx, AIx75, and central pressures, as indicated in Background sections 2.2.2, 2.2.3, and 2.2.4. The SphygmoCor 'generalized transfer function' has been internally validated by the manufacturer by simultaneously recording central waveforms both directly, via invasive recordings of aortic pressure, and indirectly, using the SphygmoCor system. A screenshot following PWA capture on the SphymoCor system is depicted in Appendix Figure 10.

### *4.3.4 Exercise protocol*

Following pre-exercise hemodynamic measurements, all participants underwent an incremental exercise test to volitional exhaustion on a treadmill (Trackmaster, FullVision

Inc., Newton, Kansas, USA). More specifically, each performed a modified Bruce Protocol to volitional exhaustion (Appendix Figure 7) under the supervision of an experienced technician (AM) and medical professional (SSD or DB). The Bruce Protocol involves 3 minute 'steps', whereby the speed and incline are changed every 3 minutes to increase the difficulty of exercise with time. Subjects begin exercise at a speed roughly equivalent to a medium paced walk, and proceed to alter their kinematic strategy as the speed and incline change with each progressing stage. This particular protocol has been previously validated and is extensively used for maximal exercise testing in young healthy individuals [196-198]. The protocol used in our laboratory also includes a 3-minute ‘warm-up’ stage to accommodate individuals across all fitness spectrums and to allow the participant to familiarize themselves with the often unfamiliar movement of the treadmill.

During each stage of exercise we ascertained subjects' feelings of perceived exertion using a standard 6-20 Borg Rating of Perceived Exertion Scale (RPE) [197] (Appendix Figure 8). Subjects were asked to point to their feeling of perceived exertion in their a) lungs, and b) legs. This allowed the research team to gain a measure of the participants’ subjective feelings at each stage, which increased safety, directed encouragement by the staff, and provided data for future analyses.

### *4.3.5 Metabolic Measurements*

Multiple measures were collected breath-by-breath during exercise with an automated metabolic cart (Medisoft Ergocard, Roxon Medi-Tech Ltd., Montreal, Quebec, Canada). Prior to each exercise test the gas analyzers and flowmeter were calibrated using a gas of known composition (4.99% $CO_2$, 11.9% $O_2$), and a volumetric cylinder of defined volume (3L, product no. 192703, Roxon Medi-Tech Ltd., Montreal, Quebec, Canada). Metabolic endpoint parameters (VO2peak, HRmax, %pred HRmax, RERmax, exercise time) were recorded from raw data extracted for each exercise test. During exercise, heart rate was continuously monitored via a 3-lead ECG (Medcard, Medisoft, Roxon Medi-Tech) connected to the Metabolic Cart, with output displayed adjacently on monitor.

Metabolic endpoint measurements were primarily utilized to ensure that participants reached their VO2peak during each assessment (since we were performing maximal exercise *stress* tests). For an arterial stress test to be considered successful, and for patient data to have been included in analysis, subjects had to meet at least 2 of the following 3 criteria:

1) RER $\geq$ 1.10
2) %HRpred $\geq$ 90%
3) Borg RPE $\geq$ 18.

If 2 of the 3 criteria were not met, the data for that assessment date was excluded from analysis. These criteria were chosen as appropriate measures of reaching maximal exercise capacity (exhaustion) based on previous studies investigating cardiorespiratory fitness in young, healthy populations [199, 200].

### *4.3.6 Acute Smoking Condition*

When randomized to complete an 'acute smoking condition' assessment, smokers were given a standardized cigarette (du Maurier signature, 1.1-2.4 mg nicotine, Les Marques Imperial Ltee, Montreal, Quebec, Canada). Following catheterization and production of a saliva sample, the participant was accompanied to the smoking area in front of the Montreal General Hospital. Subjects were given a maximum of 5 minutes to smoke the whole cigarette, and were allowed to inhale as was most natural for them. After the cigarette was finished, subjects were immediately directed back to the exercise lab where the arterial stress test was performed to characterize the acute effect of cigarette smoking on the vascular system at rest and in response to acute physical stress.

### *4.3.7 Saliva Collection*

Upon arrival to each assessment all participants were asked to rinse the mouth with water before producing a saliva sample, which was then stored at -80 degrees Celcius in collection tube (Sarstedt, Newton, North Carolina, USA). Although not analyzed in the current project, this sample will be used to verify the smoking status, or other pertinent quantifiable measures contained in saliva. Cut-off levels have previously been used to

discriminate smokers from non-smokers, and thus may help validate self-reported smoking status of participants in future analyses.

### 4.4 Statistical Analysis

All statistics were completed using SAS version 9.2 software. Demographic characteristics (Age, BMI, waist:hip circumference, IPAQ score) of smokers and non-smokers were compared using independent t-tests. Between group comparisons (smokers and non-smokers) of resting parameters were performed using general linear models with and without adjustment for age, BMI and mean arterial pressure (except BP parameters) using ANCOVA. BP parameters were adjusted for age, and BMI. Post-exercise between-group comparisons for all parameters, except MAP and PPA, were performed in similar manner using general linear models but adjusted for age, BMI, resting MAP, exercise time, and the resting value of the parameter. BPs (peripheral and aortic) were adjusted for age, BMI, exercise time and the resting value of the parameter only. Metabolic endpoint parameters (exercise time, peakMETs, Max HR, % of predicted HRmax, $VO_2$peak, and RERpeak) of smokers and non-smoker were compared using independent t-tests. All result tables and graphs indicate relevant adjustments. The graphical technique of using normal probability plots was applied to ascertain that the data were normally distributed and that the statistical analysis was appropriate.

## 5.0 Results

### 5.1 Subject Characteristics

The subject characteristics of smokers (n=34) and non-smokers (n=28) are presented in Table 1. There were no significant differences with respect to multiple variables, including; age, BMI, waist:hip ratio, and IPAQ score (3427 ± 3031 vs. 3693 ± 3305) for smokers vs. non-smokers, respectively. Among smokers, smoking exposure (as measured by pack-years) was non-normally distributed, reporting a median 5.5 [IQR 3.4-10.1].

## 5.2 Objectives 1 & 2 -Vascular Hemodynamic Parameters

### *5.2.1 Non-smokers vs. Chronic Condition*

Tables 2-7 and Graphs 1-4 describe arterial stiffness and hemodynamic parameters at rest and post-exercise in smokers- chronic condition and non-smokers.

**cfPWV** was significantly elevated in chronic condition at rest compared to non-smokers ($p=0.03$), and at 5 and 10 minutes post-exercise after adjustment. As well, prior to adjustment, the cfPWV remained significantly elevated in smokers- chronic condition at 20 minutes post-exercise compared to non-smokers, yet this relationship was lost after adjustment.

**SEVR** was non-significantly different between non-smokers and chronic condition at rest. Post-exercise, however, smokers – chronic condition demonstrated significantly higher SEVR at all times prior to adjustment ($P<0.01$ for all), and this remained significantly elevated at 15 ($p=0.003$) and 20 minutes ($p=0.02$) post-exercise following adjustments when compared to non-smokers.

**AIx75** was significantly higher in the chronic smoking condition at rest ($p=0.0009$), and at 15 and 20 minutes post-exercise after adjustment compared to non-smokers.

**PPA** was significantly lower in chronic condition at rest ($p=0.006$) and at 2, 5, and 10 minutes post-exercise after adjustment ($p<0.05$ for all) compared to non-smokers. Additionally, PPA was also significantly lower in chronic smoking condition at 15 and 20 minutes post-exercise before adjustment.

**HR** was not statistically different between non-smokers and smokers - chronic condition at rest. Post-exercise, non-smokers demonstrated significantly higher HR at 5, 10, and 15 minutes compared to smokers – chronic condition ($P<0.04$ for all).

### *5.2.2 Non-smokers vs. Acute Condition*

Tables 2-7 and Graphs 1-4 describe arterial stiffness and hemodynamic parameters at rest and post-exercise in smokers - acute condition and non-smokers.

**cfPWV** was significantly elevated in acute condition at rest (P=0.002), and at 10,15, and 20 minutes post-exercise after adjustment (P=0.02, 0.004, and 0.01 respectively) compared to non-smokers.

**SEVR** was significantly decreased at rest in acute smoking condition compared to non-smokers, before and after adjustment (P<0.0001). Post-exercise, SEVR was significantly decreased in non-smokers at 15 and 20 minutes following adjustment (P=0.03 and 0.02 respectively), while also being significantly decreased at 10 minutes prior to adjustment (P=0.04) compared to smokers – acute condition

**AIx75** was significantly higher in smokers acute condition at rest compared to non-smokers (P=0.0009), and at 15 minutes post-exercise after adjustment (P=0.04). Before adjustment, AIx75 was also significantly higher in acute smoking condition at 5 and 20 minutes post-exercise compared to non-smokers.

There were no significant differences in **PPA** at rest or post-exercise between acute smoking condition and non-smokers.

**HR** was significantly elevated at rest in acute smoking condition compared to non-smokers (P<0.0001). Following exercise there were no significant differences at any time points between non-smokers and acute smoking condition.

### *5.2.3 Chronic Condition vs. Acute Condition*

Tables 2-7 and Graphs 1-4 describe arterial stiffness and hemodynamic parameters at rest and post-exercise in smokers-chronic and acute conditions.

**cfPWV** was non-significantly elevated in acute condition at rest compared to chronic condition, however, at 5 and 15 minutes post-exercise acute smoking condition led to significantly elevated cfPWV compared to chronic condition (P=0.04 and 0.01 respectively).

Acute smoking also led to a significantly decreased **SEVR** at rest compared to the chronic condition (P<0.0001). The SEVR was significantly lower at 5 minutes post-exercise on the acute smoking day compared to chronic condition (P=0.03), but significance was lost after adjustment.

There were no baseline resting differences between chronic and acute smoking conditions on the **AIx75**. However, immediately post-exercise (5 minutes) the AIx75 was significantly higher in acute smoking condition compared to chronic smoking (P=0.02), yet this lost significance following adjustment.

The **PPA** was increased in acute smoking condition at rest (P=0.02), and at 10 minutes post-exercise (P=0.03) following adjustments compared to chronic condition. Prior to adjustments, the PPA was significantly elevated in acute smoking condition at all time points post-exercise compared to chronic smoking condition (P<0.05 for all).

**HR** was significantly elevated at rest, and at 2,5,10,15, and 20 minutes post-exercise in acute smoking condition compared to chronic condition, before adjustment (P<0.04 for all). Following adjustments, however, the relationships became non-significant.

### *5.2.4 Vascular Hemodynamic Recovery*

The **cfPWV** remained significantly elevated in non-smokers at 2, 5, 15, and 20 minutes post-exercise when compare to resting values. In the smokers–chronic condition, the cfPWV was significantly elevated for the entire 20 minutes post-exercise. Interestingly, the cfPWV was significantly elevated compared to baseline for only the first 5 minutes following exercise for the smokers – acute condition, where it then returned to resting values.

The **SEVR** was found to be significantly below resting baseline values at all time point post-exercise (5, 10, 15, and 20 minutes) under all groups and conditions (non-smokers, and both smokers conditions) ($p<0.05$ for all)

The **AIx75** was significantly elevated in non-smokers at 5, 10, 15, and 20 minutes post-exercise when compared to resting values ($P<0.05$ for all). Similarly, in both the chronic smoking and acute smoking conditions, AIx75 remained elevated 5 and 10 minutes ($p<0.001$ for all) post-exercise compared to resting values, where they then returned towards baseline.

The **PPA** was significantly elevated compared to baseline at all time points post-exercise in both chronic smoking condition and in non-smokers ($p<0.005$ for all). For acute smoking condition, the PPA was not significantly elevated 5 minutes post-exercise, but was significantly elevated compared to baseline at 10, 15, and 20 minutes post-exercise ($p<0.05$ for all).

At 2, 5, 10, 15, and 20 minutes post-exercise the **HR** remained significantly above baseline resting values in all groups, under all conditions ($P<0.01$ for all).

### 5.3 Objective 3 - Metabolic Parameters

Table 8 contains metabolic endpoint data obtained during maximal exercise testing. Three subjects (two non-smokers and one smoker) did not reach criteria considered for maximal exercise and were thus excluded from further analysis. Maximal exercise time did not differ significantly between non-smokers and smokers under both conditions. However, the peakMET, VO2peak, HRpeak and %predHRmax achieved were all significantly higher in non-smokers compared to smokers under both conditions ($P<0.05$ for all). Non-smokers also had a significantly lower RERpeak compared to chronic condition ($p=0.008$). Among smokers, there were no significant differences in any metabolic endpoint parameter between chronic and acute smoking conditions.

# TABLES

**Table 1 - Subject Characteristics**

| | Non-Smokers | Current Smokers | p-value |
|---|---|---|---|
| n | 34 | 28 | |
| Age (years) | 26.7 ± 6.3 | 29.4 ± 7.1 | NS |
| BMI ($kg/m^2$) | 23.6 ± 2.9 | 23.6 ± 2.7 | NS |
| Waist:Hip Circumference | 0.98 ± 0.04 | 0.97 ± 0.04 | NS |
| IPAQ Score (mean ± stdev) | 3693 ± 3305 | 3427 ± 3031 | NS |
| IPAQ (median [IQR]) | 2206.5 [1437-5940] | 2772 [1554-3786] | |
| Pack-years (median [IQR]) | | 5.5 [3.4-10.1] | |

BMI, body mass index; IPAQ, International Physical Activity Questionnaire
All values are mean ± standard deviation. P-values are unadjusted

**Table 2 - cfPWV (m/s)**

| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | **p-value** | | |
|---|---|---|---|---|---|---|
| | | *Chronic* | *Acute* | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| Baseline | *6.10 ± 0.57* | *6.70 ± 1.05* | *7.05 ± 0.97* | **0.031** | **0.0002** | NS |
| 2 minutes | *9.29 ± 1.31*[&] | *10.19 ± 1.92*[&] | *10.16 ± 2.15*[&] | *NS* | *NS* | NS |
| 5 minutes | *6.93 ± 0.81*[&] | *7.58 ± 1.41*[&] | *7.78 ± 1.43*[&] | *NS* | *NS* | **0.045** |
| 10 minutes | *6.37 ± 0.88* | *7.04 ± 1.10*[&] | *7.16 ± 1.22* | **0.044** | **0.023** | NS |
| 15 minutes | *6.41 ± 0.68*[&] | *7.02 ± 1.22*[&] | *7.13 ± 1.08* | **0.039** | **0.004** | **0.01** |
| 20 minutes | *6.47 ± 0.72*[&] | *7.15 ± 1.24*[&] | *7.15 ± 1.02* | NS* | **0.01** | NS |

cfPWV, carotid-femoral pulse wave velocity

*P<0.05 before adjustment.

[&]P<0.05 against baseline value (ie. This parameter has not recovered to resting levels).

Baseline cfPWV is adjusted for age, BMI and baseline MAP. Post-exercise, these variables are adjusted for age, BMI, exercise time, baseline MAP, and the corresponding baseline cfPWV.

**Table 3 - SEVR (%)**

| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | **p-value** | | |
|---|---|---|---|---|---|---|
| | | *Chronic* | *Acute* | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| Baseline | *186.81 ± 37.73* | *175.13 ± 21.06* | *141.07 ± 26.01* | NS | **<0.0001** | NS* |
| 5 minutes | *77.50 ± 19.81*[&] | *91.60 ± 21.28*[&] | *82.78 ± 16.45*[&] | NS* | NS | NS* |
| 10 minutes | *80.55 ± 23.57*[&] | *96.23 ± 24.19*[&] | *89.41 ± 16.68*[&] | NS* | NS* | NS |
| 15 minutes | *87.30 ± 16.78*[&] | *107.15 ± 24.11*[&] | *100.04 ± 16.79*[&] | **0.003** | **0.033** | NS |
| 20 minutes | *94.18 ± 20.97*[&] | *115.54 ± 24.54*[&] | *108.65 ± 17.14*[&] | **0.02** | **0.021** | NS |

SEVR, subendocardial viability ratio

*P<0.05 before adjustment.

[&]P<0.05 against baseline value (ie. This parameter has not recovered to resting levels).

Baseline SEVR is adjusted for age, BMI and baseline MAP. Post-exercise, these variables are adjusted for age, BMI, exercise time, baseline MAP, and the corresponding baseline SEVR.

**Table 4 - AIx75 (%)**

| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | **p-value** | | |
|---|---|---|---|---|---|---|
| | | *Chronic* | *Acute* | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| Baseline | *-11.47 ± 11.08* | *0.15 ± 9.15* | *0.04 ± 8.78* | **0.0009** | **0.003** | NS |
| 5 minutes | *10.13 ± 8.16*[&] | *10.32 ± 7.89*[&] | *13.61 ± 4.67*[&] | NS | NS* | NS* |
| 10 minutes | *5.61 ± 7.96*[&] | *7.46 ± 7.09*[&] | *6.63 ± 6.91*[&] | NS | NS | NS |
| 15 minutes | *1.00 ± 7.72*[&] | *3.54 ± 8.40* | *2.56 ± 6.70* | **0.012** | **0.041** | NS |
| 20 minutes | *-0.53 ± 6.34*[&] | *1.54 ± 9.32* | *1.37 ± 8.31* | **0.044** | NS* | NS |

Aix75, augmentation index adjusted to heart rate of 75 beats per minute

*P<0.05 before adjustment.

[&]P<0.05 against baseline value (ie. This parameter has not recovered to resting levels).

Baseline Aix75 is adjusted for age, BMI and baseline MAP. Post-exercise, these variables are adjusted for age, BMI, exercise time, baseline MAP, and the corresponding baseline Aix75.

**Table 5 - PPA**

| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | **p-value** | | |
|---|---|---|---|---|---|---|
| | | *Chronic* | *Acute* | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| Baseline | *1.66 ± 0.09* | *1.56 ± 0.12* | *1.66 ± 0.14* | **0.006** | NS | **0.021** |
| 5 minutes | *1.71 ± 0.12*[&] | *1.64 ± 0.14*[&] | *1.70 ± 0.11* | **0.032** | NS | NS* |
| 10 minutes | *1.74 ± 0.11*[&] | *1.68 ± 0.12*[&] | *1.75 ± 0.12*[&] | **0.041** | NS | **0.0328** |
| 15 minutes | *1.77 ± 0.10*[&] | *1.70 ± 0.12*[&] | *1.77 ± 0.13*[&] | NS* | NS | NS* |
| 20 minutes | *1.77 ± 0.10*[&] | *1.70 ± 0.10*[&] | *1.76 ± 0.13*[&] | NS* | NS | NS* |

PPA, pulse pressure amplification

*P<0.05 before adjustment.

[&]P<0.05 against baseline value (ie. This parameter has not recovered to resting levels).

Baseline PPA is adjusted for age and BMI. Post-exercise, these variables are adjusted for age, BMI, exercise time, and the corresponding baseline PPA.

**Table 6 - HR (bpm)**

| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | **p-value** | | |
|---|---|---|---|---|---|---|
| | | *Chronic* | *Acute* | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| Baseline | *58.86 ± 8.20* | *60.99 ± 5.98* | *70.60 ± 10.34* | NS | **<0.0001** | NS* |
| 2 minutes | *107.74 ± 12.06*[&] | *102.36 ± 15.37*[&] | *107.61 ± 12.55*[&] | *NS** | *NS* | NS* |
| 5 minutes | *99.22 ± 8.56*[&] | *91.42 ± 10.55*[&] | *97.33 ± 8.92*[&] | **0.02** | NS | NS* |
| 10 minutes | *95.43 ± 7.75*[&] | *86.85 ± 9.19*[&] | *93.91 ± 9.56*[&] | **0.019** | NS | NS* |
| 15 minutes | *92.44 ± 7.60*[&] | *84.56 ± 9.38*[&] | *90.93 ± 9.68*[&] | **0.04** | NS | NS* |
| 20 minutes | *89.64 ± 8.32*[&] | *82.33 ± 8.95*[&] | *87.96 ± 9.45*[&] | NS* | NS | NS* |

HR, heart rate

*P<0.05 before adjustment.

[&]P<0.05 against baseline value (ie. This parameter has not recovered to resting levels).

Baseline HR is adjusted for age, BMI, and baseline MAP. Post-exercise, these variables are adjust for age, BMI, exercise time, baseline MAP, and the corresponding baseline HR.

**Table 7 - MAP (mmHg)**

| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | **p-value** | | |
|---|---|---|---|---|---|---|
| | | *Chronic* | *Acute* | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| Baseline | *79.78 ± 5.92* | *81.58 ± 6.16* | *84.22 ± 7.97* | NS | NS | NS |
| 5 minutes | *80.03 ± 8.28* | *81.32 ± 8.04* | *85.00 ± 9.49* | NS | **0.026** | NS |
| 10 minutes | *79.06 ± 6.67* | *79.15 ± 7.09*[&] | *80.96 ± 6.90*[&] | NS | NS | NS |
| 15 minutes | *78.88 ± 6.61* | *80.58 ± 7.65* | *82.26 ± 7.63*[&] | NS* | **0.04** | NS |
| 20 minutes | *79.63 ± 5.68* | *81.31 ± 5.87* | *82.63 ± 7.23* | NS | NS | NS |

MAP, mean arterial pressure

*P<0.05 before adjustment.

[&]P<0.05 against baseline value (ie. This parameter has not recovered to resting levels).

Baseline MAP is adjusted for age and BMI. Post-exercise, these variables are adjusted for age, BMI, exercise time, and the corresponding baseline MAP.

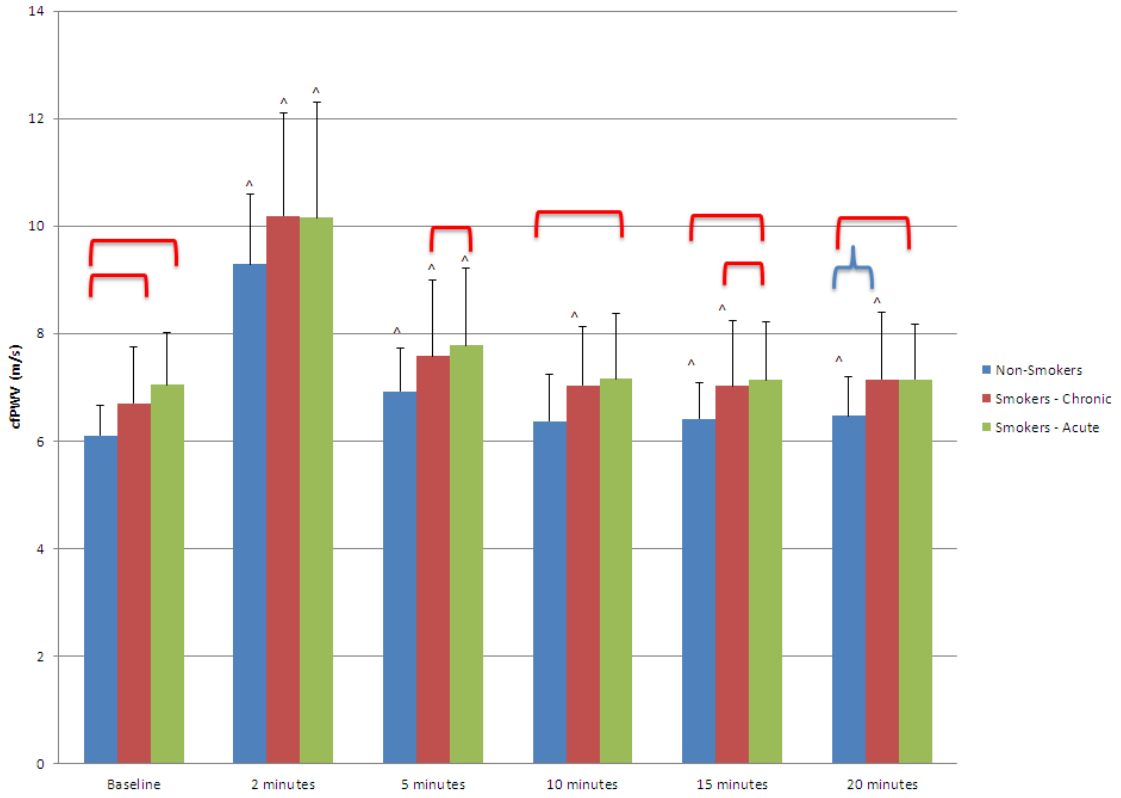
| **Table 8 - Metabolic Parameters** | **1** | **2** | **3** | **p-value** | | |
|---|---|---|---|---|---|---|
| | *Non-Smokers (n=34)* | *Smokers (n=28)* | | 1 vs. 2 | 1 vs. 3 | 2 vs. 3 |
| | | *Chronic* | *Acute* | | | |
| Exercise Time (minutes) | 16.84 ± 2.92 | 16.06 ± 2.14 | 16.18 ± 2.05 | NS | NS | NS |
| peakMETs | 16.04 ± 2.93 | 14.17 ± 2.19 | 14.36 ± 1.93 | **0.0084** | **0.0127** | NS |
| Max HR (bpm) | 193.38 ± 9.49 | 183.12 ± 14.68 | 186.19 ± 10.65 | **0.0018** | **0.0072** | NS |
| %predHRmax (%) | 100.05 ± 3.86 | 96.14 ± 6.69 | 97.78 ± 3.73 | **0.006** | **0.0242** | NS |
| $VO_2$peak (ml/kg/min) | 54.23 ± 10.94 | 47.92 ± 8.52 | 47.95 ± 7.63 | **0.0183** | **0.0129** | NS |
| RERpeak | 1.19 ± 0.08 | 1.24 ± 0.07 | 1.22 ± 0.07 | **0.0079** | NS | NS |

peakMETS, peak workload expressed as metabolic equivalents; Max HR, maximal heart rate achieved; %predHRmax, percentage of predicted maximal heart rate achieved; $VO_2$peak, maximal volume of oxygen consumption; RERpeak, peak respiratory exchange ratio

All values are mean ± standard deviation. P-values are unadjusted.

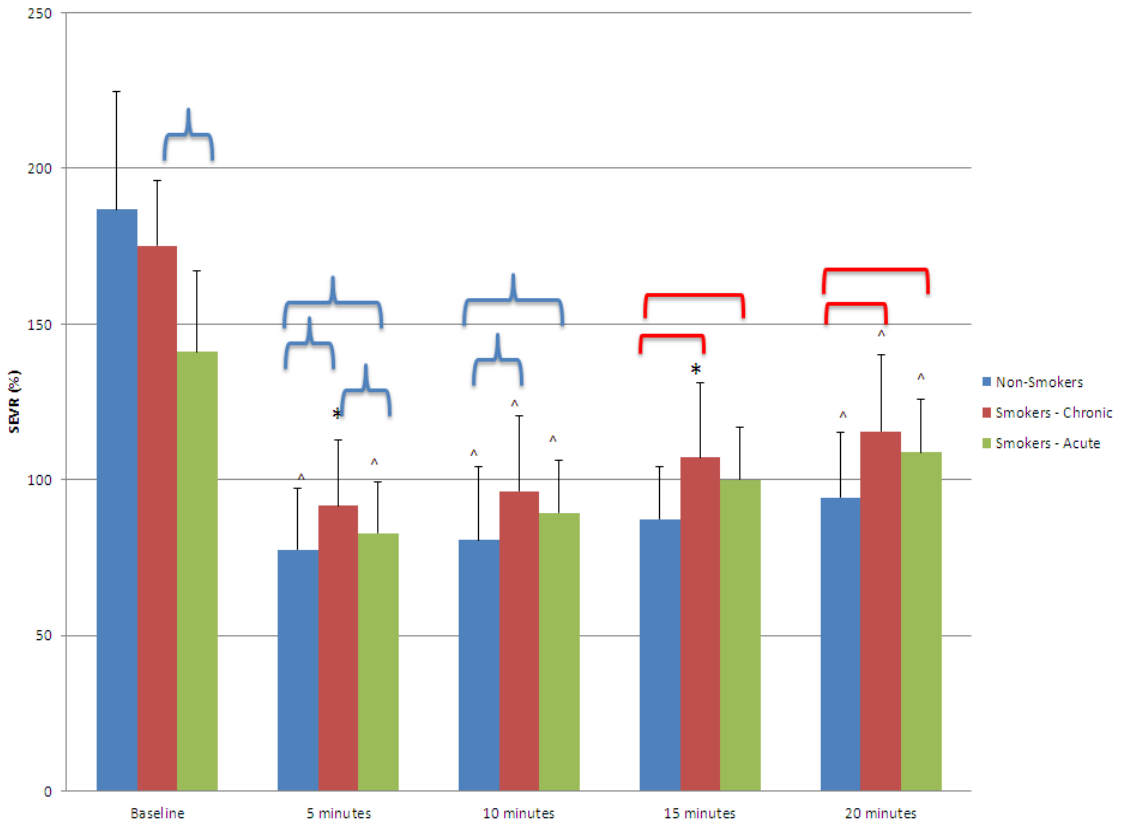
# FIGURES

**Graph 1 – Exercise induced changes in carotid-femoral Pulse Wave Velocity**



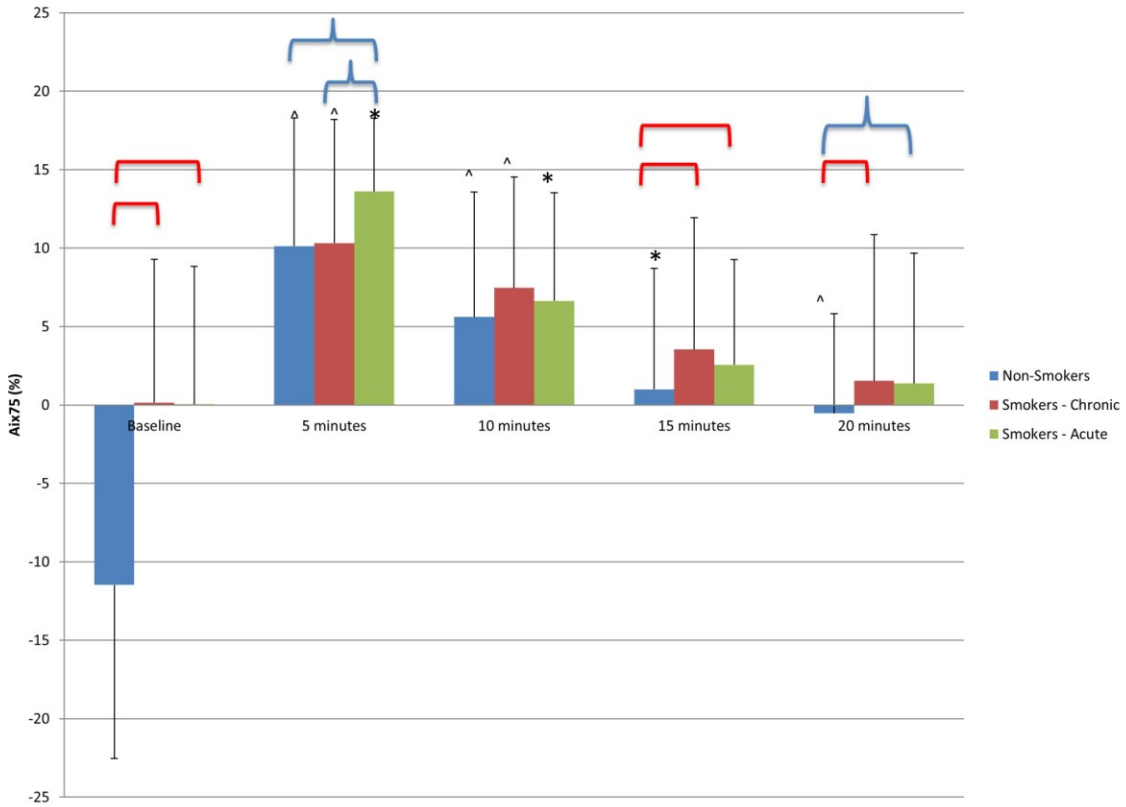

^ p<0.05 vs. Baseline, before adjustment
[ p<0.05, with adjustment
{ p<0.05, before adjustment for Age, BMI, restingMAP, and resting value of the parameter

**Graph 2 – Exercise induced changes in Subendocardial Viability Ratio**



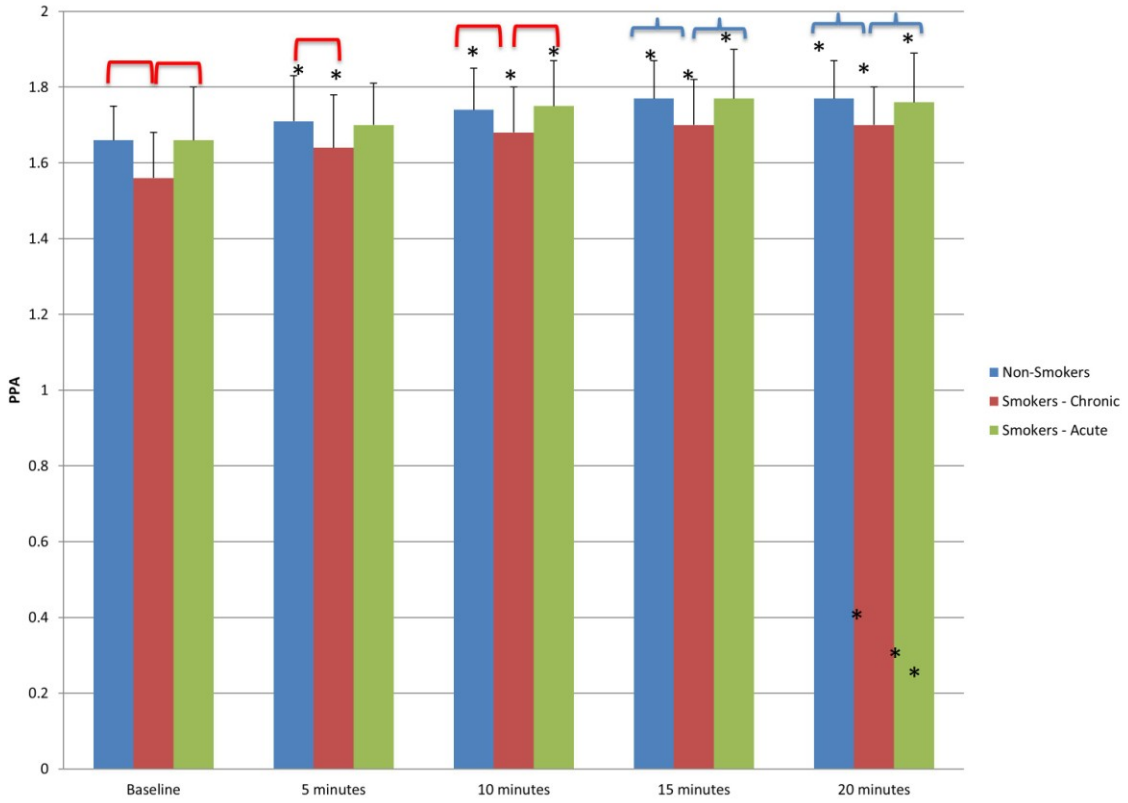

* p<0.05 vs. Baseline, adjusted for Age, BMI, restingMAP, and resting value of the parameter
^ p<0.05 vs. Baseline, before adjustment
[ p<0.05, with adjustment
{ p<0.05, before adjustment for Age, BMI, restingMAP, and resting cfPWV

**Graph 3 – Exercise induced changes in Augmentation Index @ 75 bpm**




* p<0.05 vs. Baseline, adjusted for Age, BMI, restingMAP, and resting value of the parameter
^ p<0.05 vs. Baseline, before adjustment
[ p<0.05, with adjustment
{ p<0.05, before adjustment for Age, BMI, restingMAP, and resting cfPWV

**Graph 4 – Exercise induced changes in Pulse Pressure Amplification**




* p<0.05 vs. Baseline, adjusted for Age, BMI, restingMAP, and resting value of the parameter
^ p<0.05 vs. Baseline, before adjustment
[ p<0.05, with adjustment
{ p<0.05, before adjustment for Age, BMI, restingMAP, and resting cfPWV

## 5.0 Discussion

The nature of cardiovascular system function following acute stress is a complex matter, which is only confounded by the inclusion of the lifestyle choice to smoke cigarettes. Since humans are by nature dynamic beings, we encounter stressors in many forms through our daily lives and must adapt to such external variables. It is thus important to begin to quantify and qualify the impact that stress places on the body, particularly the vascular system, to better understand how we may improve and maximize our health in such a shifting living environment. For this study, we enrolled young, healthy light-moderate smokers and non-smokers to perform acute aerobic exercise in order to estimate the impact of chronic and acute smoking on the vascular system at rest and in response to increased demands. We compared cfPWV, SEVR, AIx75 and PPA at rest and post-exercise between healthy light-moderate smokers (median 5.5 pack-years smoking) after 12 hours abstinence from smoking (chronic condition) and immediately after smoking 1 standardized cigarette (acute smoking) and non-smokers using the 'arterial stress test'. We noted marked differences between smokers and non-smokers on vascular hemodynamic parameters both at rest and in response to physical stress.

At rest, the cfPWV was significantly elevated in chronic condition when compared to non-smokers, with a further significant increase observed following acute smoking. During the recovery period following exercise cfPWV in the acute smoking condition remained significantly elevated in comparison to the non-smokers at various time points. Increased cfPWV is very important given that a 1m/s increase in cfPWV has been associated with a 14% increase in total cardiovascular events [53]. This is extremely worrisome owing to the fact that our smokers demonstrated a cfPWV that was greater than our non-smokers by 0.6m/s in the chronic condition, while acute smoking further exacerbated this gap to 0.95m/s. Thus, we have uncovered that even otherwise healthy, young, active, light-moderate smokers have permanent damage found at rest when compared to equally active non-smokers, which is only further amplified by acute stress. This increased cfPWV observed in smokers may be the result of various potential mechanisms:

a) *Increased oxidative stress*. Both acute and chronic smoking have been associated with increased oxidative stress [6, 10]. Increased levels of reactive oxygen species associated with cigarettes decreases the activity of nitric oxide synthase, and limits bioavailability of the vasodilator nitric oxide, produced by the endothelium [201, 202]. Chronic smoking also leads to decreased circulating antioxidant levels such as nitric oxide and peroxynitrite, limiting the body's ability to attenuate the harmful impact of oxidants, increasing endothelial dysfunction and potentially augmenting stiffness of the central arteries at rest and in response to exercise [201, 203, 204].

b) *Altered lipid metabolism.* Lipid metabolism can be impacted both acutely and chronically with cigarette smoking. Acute smoking impacts catecholamine release by the adrenal medulla into the bloodstream and the activity of lipoprotein lipase (LPL) [205]. This results in poor clearance of triglycerides from the circulation, and abnormal release of free fatty acids. As such, total circulating triglycerides and the LDL:HDL ratio are substantially increased in smokers, leading to structural alterations in the arterial wall [205]. Increased lipid deposition in the arterial wall causes intima-media thickening and leads to atherosclerosis with progressing age, both of which have been associated with increases in arterial stiffness [8]. Conversely, treatment with lipid-lowering medications (as with statins) has shown to improve measures of arterial stiffness [206, 207]. As our smoking participants have a median 5.5 pack-years smoking history and are an average age of 29.4, this change in arterial wall properties had likely already begun and may thus increase stiffening in our smokers

c) *Pro-inflammatory state.* Smoking increases pro-inflammatory cytokines, while decreasing anti-inflammatory cytokines [9]. Several inflammatory markers have previous been correlated with increased arterial stiffening, including C-reactive protein, lipoprotein-associated phospholipid-A2, interleukin 6, and osteoprotegrin [12, 208-212]. Vlachopoulos et al. demonstrated significant associations between increased cfPWV and levels of such biochemical markers of inflammation as C-reactive protein and interleukin-6 [212]. Chronic inflammation, as in chronic smoking, also leads to increased levels of matrix metalloproteinases in the arterial wall, which act to degrade proteins in the extracellular matrix and can lead to significant alterations in vascular wall structure

and response to stress [213, 214]. The cfPWV in chronic smokers may thus be influenced by this pro-inflammatory milieu and can act to also directly increase cfPWV and AIx75, as there is a faster return of reflected waves off peripheral arterial sites.

d) *Insulin resistance.* Acute and chronic smoking are both associated with increased insulin release leading to insulin resistance [205, 215] and stiffening of the arteries [216, 217]. The aortic PWV has previously been correlated with mean daily glucose in diabetic subjects [217], and has been shown to increase even in early insulin-resistance states [216]. Similarly, smoking is associated with decreased physical activity and a poor diet, which may further increase lipid levels and insulin-resistance [215]. However, this was not necessarily the case for our participants since our smokers and non-smokers alike had similar median IPAQ scores. Diet was not assessed in our participants, yet several indicators of food intake were queried on the Questionnaire issued upon first arrival to the laboratory and may be further evaluated at a later date if deemed necessary. We did not, however, directly assess lipid or glucose metabolism in the included study, although we excluded subjects with history of dyslipidemia or glucose abnormalities.

e) *Impaired kidney function.* Cigarette smoking has been associated with albuminuria in healthy populations, and is the result of decreased filtration rates and impaired kidney function [218]. It has been demonstrated that even mildly impaired kidneys can cause arterial calcification and collagen accumulation in arterial walls through a reduction in glomerular filtration rate. Arterial stiffness may thus be increased through alterations in vascular wall structure resulting from such dysfunction [218].

f) *High Blood Pressure.* Cigarette smoking acutely and chronically increases central and peripheral blood pressures [15, 49, 219-221], and the risk for developing chronic hypertension [49, 222, 223]. Hypertensive individuals display increased arterial stiffness compared to age-matched controls. Not only has increased MAP been correlated with higher incidence of cardiovascular events [224], but it also leads to increased vessel wall tension and an induced increase in mechanical wall damage and vessel remodelling [121, 225, 226]. Such remodeling may include increases in collagen and calcium deposition (as in renal insufficiency), changes in smooth muscle cell activity, and altered extracellular matrix protein deposition, which all lead to increased arterial [209, 227-230], as

demonstrated by both chronic and acute smoking conditions. With progressing age these effects compound, leading to progressive worsening of arterial stiffness [191]. Even when treated with anti-hypertensive therapy, vascular abnormalities present as a result of prior structural changes to the arterial wall linger and arterial stiffness remains above age-predicted levels [45]. The Atherosclerosis Risk in Communities Study (ARIC) has similarly demonstrated that a 1 standard deviation decrease in arterial elasticity was associated with a 15% greater risk of developing hypertension, independent of other established hypertension risk factors [231]. This notion has been recently substantiated by Kaess et al., who demonstrated that increased aortic stiffness and augmentation index (AIx) were positively associated with increased incidence of developing hypertension [232].

In direct accordance with summarized findings of our systematic literature review (Section 3.1), cfPWV was significantly elevated in all groups and conditions at 2 and 5 minutes post-exercise compared to baseline resting values. These relationships were, however, lost after adjustment. Most previous studies have taken a less conservative approach, having not made statistical adjustments, yet we were able to find significant results even after appropriate adjustments. Thus, the general trend still remains important, demonstrating that immediately following exercise there is an increase in the stiffness of central arteries, which recover towards baseline resting values through time.

One of the primary mechanisms responsible for increases in arterial stiffness is a change in the properties of arterial wall structure components with exercise. Acutely, as the MAP changes with increasing stress, as in aerobic exercise, there is an alteration in the way collagen and elastin fibres sustain pressure loads on the vessel wall. When MAP is low, as in the resting state in a healthy individual, elastin fibres sustain most of the pressure load and slow the progression of pulse waves through the arterial system. As central pressures increase, as with acute physical stress, collagen fibres are progressively recruited and lead to increased stiffening of the large central arteries, increasing cfPWV [233]. A concomitant constriction of vascular smooth muscle cells alongside the aforementioned shift could begin to account for immediately increased central arterial stiffness observed following exercise [45, 233-235]. There is also an associated increase

in blood flow and resultant shear stress in vessels supplying blood to working muscles during exercise, causing vasodilation and increases to vessel diameter. As the arterial diameter increases there may again be a resultant shift from elastin to collagen fibres, and could account for early increases in arterial stiffness of upper body arterial segments immediately following exercise [64, 233]. Alongside vasodilation, exercise leads to reduced total peripheral resistance and an increase in whole body arterial compliance [64, 236, 237]. It has been proposed that a relaxation of vascular smooth muscle could work in the opposite fashion to increased vascular tone by moving from primarily collagen fibres to more compliant elastin, and could account for progressive decreases in arterial stiffness seen after exercise in portions of the arterial tree [238].

Following acute aerobic exercise there is also a reduction in muscle sympathetic nerve activity [64, 239, 240]. In response to stretching, baroreceptors located in the aortic arch and carotid artery send afferent signals to the brainstem that act to inhibit sympathetic activity to the periphery, resulting in vessel vasodilation and associated alterations to arterial stiffness as discussed above, and in section 2.3.1 [240]. Baroreflex sensitivity was shown to be significantly decreased 30 minutes following cycling exercise, independent from changes to arterial wall components and in the presence of reduced cfPWV and femoral-dorsalis pedis PWV [241]. It is, however, possible that decreases in arterial stiffness may also be seen concomitantly with sympathoexcitation, as vasodilation has previously been shown to occur alongside sympathetic activation [242, 243].

With exercise, increased intravascular shear stress, and amplitude and frequency of pulsatile flow cause the production and release into the blood of multiple modulators of vascular tone derived by the endothelium. A number of these blood markers may be implicated in the mechanisms governing changes in arterial stiffness following aerobic exercise. There is an alteration in circulating levels of such vasorelaxing factors as nitric oxide [244-247], atrial natriuretic peptide [248], prostaglandins [249, 250], and potential vasoconstrictors such as endothelin-1 [251-253], angiotensin-II [224], and epinephrine [254] that help mediate vascular smooth muscle tone with exercise. There is current evidence supporting a relationship between arterial stiffness and various blood markers in resting conditions [248], but further research needs to be conducted investigating the

relationship between blood markers and changes in arterial stiffness with exercise. Blood samples collected during the SMOKELESS study are to be used in such future analyses.

Chronic smoking leads to increased AIx in both younger and older [15, 29, 32, 38], while acute smoking acts to immediately decrease the AIx, primarily through an increase in heart rate [11, 14, 15].Wilkinson et al. determined that there was a 3.9% decrease in AIx for every 10 beats per minutes increase in heart rate [114], which corroborates the results found in this study. Our study demonstrated that non-smokers had significantly lower resting AIx75 when compared to both smokers' conditions, yet there were non-significant differences between smoking conditions on resting AIx75, likely because measures were corrected to a heart rate of 75 bpm, while the actual heart rate was significantly elevated in the acute smoking condition. Acute physical stress led to significantly elevated AIx75 in smokers-acute condition immediately after exercise cessation compared to both chronic condition and to non-smokers. It was noted that by 15 minutes post-exercise AIx75 remained significantly elevated for both smoking conditions compared to non-smokers. As aforementioned, increased MAP observed in acute smoking condition leads to vascular remodelling that will decrease transit time of reflected pulse waves from peripheral sites because of a reduced buffering capacity, increasing the AIx75 [91, 213]. For the first 10 minutes post-exercise, AIx75 remained significantly elevated compared to baseline values in all groups and under all conditions. These results are in line with previous studies demonstrating increased AIx75 following aerobic exercise [68, 69]. Smokers under both conditions recovered towards their baseline resting values of AIx75 faster than non-smokers. This is largely due to the very low resting values of AIx75 observed in non-smokers and relatively increased resting AIx75 values in both smoking conditions. Smokers and non-smokers both demonstrated similar maximal stress response in AIx75 immediately post exercise, yet because of resting differences a more prolonged recovery to their baseline value is expected in non-smokers.

There were no significant differences in resting SEVR (an indication of myocardial oxygen supply:demand) between non-smokers and smokers in the chronic condition. Both the non-smokers and smokers alike demonstrated very high resting values of SEVR (above 175%), indicating appropriate ratios of myocardial oxygen supply:demand at rest,

In smokers, acute smoking was shown to significantly decrease the SEVR by 35%, although not to levels associated with cardiac ischemia [100]. Blood flow in the subendocardium during diastole is dependent on several factors, including; coronary artery diastolic pressure [106]; the driving pressure between coronary arteries and small coronary vessel; time in diastole [108]; and blood viscosity [107], all of which have been shown to be acutely altered with smoking, and under conditions of acute stress. Abnormal alterations in any factor may result in decreased oxygen supply to the myocardium, acutely impacting exercise capacity and vessel hemodynamics. For example, increasing blood viscosity will increase the resistance within small coronary vessels, reducing the driving pressure gradient for perfusion [99]. This is very significant because low resting SEVR has been associated with a decrease in coronary flow reserve, which may be necessary to achieve higher workloads [103]. Immediately following exercise, non-smokers demonstrated significantly lower SEVR than smokers under both conditions, indicating notable group differences in myocardial perfusion following stress. These findings are extremely interesting, and confirm our previous work [37] that the greatest decrease in oxygen supply:demand following physical stress is in non-smokers. The lowest SEVR at 5 minutes post-exercise was noted in non-smokers and was 78% (significantly lower than both smoking conditions). Non-smokers thus demonstrated a potentially increased ability to positively stress their cardiovascular system in order to achieve higher workloads ($VO_2$peak) and increased rates of oxygen consumption. This occurred despite exercising for the same average length of time as smokers (all post-exercise measures were also corrected for maximal exercise time). Although this decrease may seem alarming, myocardial ischemia is not said to occur until 40-50% SEVR, thus non-smokers may have a greater ability to positively push into their coronary flow reserve towards ischemic levels without actually becoming ischemic [48, 100]. This argument is substantiated by a previous study correlating low resting SEVR with coronary flow reserve ($r=0.651$, $P<0.001$) [82]. Hoffman et al. have proposed that if there is reason for coronary artery resistance to be elevated, as in left ventricular hypertrophy related to increased afterload often seen in smokers, then the critical value for myocardial ischemia may be increased [99]. It is possible that the significantly higher values of SEVR noted in smokers immediately following exercise may be the result of a

*higher* critical oxygen supply:demand ratio and therefore they cannot safely stress their cardiovascular system to the same degree. Acute smoking also not only increases the oxygen demands placed on the heart, as heart rate increases significantly, but also decreases the supply of oxygen through production of reactive oxygen species. Of note is the lack of significant differences between smokers acute condition and smokers chronic condition on the post-exercise SEVR at 15 and 20 minutes. There remains to be an agreement on when the acute effects of cigarette smoking on the cardiovascular system disappears or is significantly eliminated. A recently conducted systematic review revealed that some studies indicate an effect lasting only 15 minutes, while others indicate an effect lasting upwards of 2 hours [37]. It is thus possible that the immediate impact of cigarettes was attenuated by the time measurements of arterial stiffness were taken after exercise, which may begin to explain a lack of significant intra- and inter-group differences post-exercise in the SEVR, AIx75, PPA, and cfPWV.

Our results have demonstrated that chronic smoking has a significant impact on depressing the PPA at rest when compared to non-smokers. This relationship remained significant at all time points post-exercise, and is in accordance with previous work [15, 74, 130]. Acute smoking caused a significantly elevated PPA compared to the chronic condition, both at rest, and at 10, 15, and 20 minutes following exercise, before adjustment. As chronic smokers generally demonstrate increased arterial stiffness at rest and in response to stress, the pulse waves reflecting off of peripheral sites return earlier in the cardiac cycle, disproportionately augmenting the central SBP over peripheral BP, narrowing the PPA [124]. Also, PPA may be influence by a variety of other factors, with one such being heart rate. As HR increases, there is a reduction in cardiac cycle time that dramatically impacts the morphology of the aortic and brachial pulse waves. As both acute smoking and physical stress lead to increased heart rates, our data are in agreement with previous studies demonstrating a relation between increased heart rate and PPA [124]. Therefore, in healthy individuals who have low arterial stiffness and resting heart rates, reflected waves superimpose during diastolic portions of the central pressure wave, resulting in improved cardiac perfusion, left ventricular filling, and a widened PPA. [100, 124] On the other hand, reduced PPA is strongly associated with cardiovascular disease, and has been shown to be a predictor of total mortality [92, 120]. Since central pressures

most often increase disproportionately to those taken peripherally, the PPA tends to decrease with age. Chronic cigarette smoking acts much like aging on the depression of PPA, whereby increases in central and peripheral blood pressures occur, more markedly increasing centrally, giving credence to the lower PPA seen in chronic smokers [15, 49, 219-221]. Smokers with decreased PPA are more prone to resulting morbidity and mortality as increased central blood pressures have been correlated with increased intima-media thickness and left ventricular workload, both of which are independent predictors of mortality [128, 129]. These results are concordant with a previous study having demonstrated decreasing PPA during exercise with increasing age [74]. Acute smoking significantly increased PPA at rest and post-exercise in smokers up to levels displayed by non-smokers. The PPA remained significantly elevated post-exercise, as was HR, compared to resting values in all groups, under all conditions. At rest, smokers displayed significantly elevated cfPWV despite similar resting MAP compared to non-smokers. Thus, before the acute smoking of a cigarette, smokers had an already elevated *central* arterial stiffness but no significant difference was noted in brachial blood pressure. Acute smoking likely caused increases to both central and peripheral artery stiffness, but this may have been more marked peripherally because of the already present increased central stiffness. These results also substantiate various previous studies describing significantly increased PPA during periods of increased vascular stress [74, 125, 137].

Metabolic cardiorespiratory endpoint data obtained following maximal exercise were primarily gathered to ensure that all participants exercised to exhaustion during each assessment. The results of such investigation, however, are quite interesting and are in strong accordance with previously conducted studies investigating the impact of acute and chronic smoking cardiorespiratory response to stress [150, 151]. Although there were non-significant differences in maximal exercise time achieved between all groups, non-smokers achieved significantly higher maximal heart rates and VO2peak, with significantly lower peak RER values. Arterial stiffness has been linked with higher cardiorespiratory fitness, as $VO_2$peak was shown to be significantly and inversely associated with both central and peripheral PWV, independent of lifestyle factors [255, 256]. Our smokers demonstrated increased resting cfPWV and significantly decreased $VO_2$peak. There were no group differences in leisure time physical activity levels, as

judged by IPAQ scores, between smokers and non-smokers. Although several studies have indicated an inverse relationship between smoking frequency and physical activity, we specifically recruited physically active smokers so that we may investigate the relation between smoking and changes in hemodynamic parameters without the confounding variable of leisure time physical activity level. Thus, we have an 'active' cohort of smokers, despite their current smoking status (median 5.5 pack-years).

The reduced $VO_2$peak noted in smokers under both conditions may represent a significantly impaired ability of the cardiovascular system to deliver oxygen to the working muscles. Multiple mechanisms may play a role in this impaired delivery, including; reduced transport of oxygen in the blood due to lowered maximal cardiac outputs (as may be indicated by significantly lower HR in smokers at all time points post-exercise in both smoking conditions compared to non-smokers) [257]; impaired redistribution of blood flow to the working muscles because of vasoactive components that are present in cigarettes [258]; defective transfer of oxygen from the lungs to the arterial blood; increased levels of carboxyhemoglobin (HbCO), which reduces the ability of hemoglobin to carry oxygen in the vasculature by dramatically shifting the oxy-hemoglobin dissociation curve to the left [259]; impaired uptake of oxygen by the working muscles at the tissue level.

It has been demonstrated that chronic smokers also have a reduced oxygen pulse (representing the amount of oxygen extracted at the tissue level with each successive myocardial contraction), which is further exacerbated by acute smoking [150]. This decrease in oxygen extraction at the tissue will directly impact the RER, causing a dramatic decrease in compromised smoking subjects and may explain increased peakRER values in response to stress in the smoker cohort. If the ability of vessels, bound for the working muscles, to vasodilate is compromised during physical stress through one of the mechanisms previously described, this will also impede delivery of oxygen causing an increase in RER.

In cases of increased HbCO, the delivery of oxygen to muscle mitochondria is reduced due to a reduction in the number of possible oxygen binding sites. The net result is a

decrease in capillary $PO_2$ for a given blood oxygen content. Not only would this result in depressed $VO_2$peak, but also increased RER [259].

There have previously been discordant findings pertaining to the relationships between some arterial stiffness parameters and acute exercise, and between arterial stiffness and smoking. This variance provided the impetus to attempt to better clarify these relationships. Previously, no study had yet investigated the impact of smoking on changes in arterial stiffness following acute physical stress in young, healthy individuals. Using the arterial stress test we demonstrated significant differences at rest, a) between young, healthy smokers and non-smokers, and b) in smokers after acute smoking and following abstinence. Most importantly, we were able to elicit vascular abnormalities in smokers that were not present at rest by subjecting them to acute physical stress and measuring vascular hemodynamic responses. By identifying vascular abnormalities at an early stage it provides us with an opportunity to intervene and reverse or prevent further damage before cardiovascular disease takes hold.

### 6.1 Clinical Relevance

Increased cfPWV, AIx75, and PPA have all been shown to be independent risk factors for increased cardiovascular morbidity and mortality [48, 49, 52, 120], while demonstrating strong correlations to the development of atherosclerosis with age in various arterial sites [260]. The measurement of arterial stiffness is thus a very useful clinical tool in estimating the progression of disease, and to monitor the impact of treatment on vascular health. The arterial stress test may therefore be applied to various cohorts of patients to assess damage done to the vascular system induced by various endothelial stressors, as in cigarette smoke. Although smoking rates have been on the decline in recent years, they still remain quite high, particularly in younger age groups. Over 24% of young adults aged 20-24 are current smokers in Quebec [3], while still averaging nearly 13 cigarettes smoked per day. According to the INTERHEART survey, smoking a mere five cigarettes per day significantly increases risk for cardiovascular events in both young and older populations, so these statistics are not to be taken lightly [5]. Smoking therefore still represents a staggering public health issue, and monitoring the progression of vascular alterations through the arterial stress test may prove to be a

highly valuable tool in the clinical setting. By quantifying and qualifying damage done to the vascular system over time we may now be able to gauge the effect that smoking cessation therapies and medications have on arterial health. The Framingham Heart Study demonstrated there was a 48% increase in arterial disease risk associated with a 1 standard deviation increase in cfPWV. If, through the use of the arterial stress test, we may stem the progression of arterial stiffness by educating those at risk, then we may take large leaps forward in curtailing the individual and societal burdens associated with cigarettes. Also, we may now begin to dissect and directly quantify the relationship between smoking and endothelial dysfunction rather than inferring damage through smoking exposure. Through vascular characterization we may begin to classify individuals who are at increased risk for cardiovascular disease and complications (risk stratifications), and direct treatment to those at higher risk, independent of their smoking status.

### 6.2 Limitations and Future Work

There are several limitations to this study. Since we enrolled consecutive male participants who responded to our advertisements, we did not match for age or BMI. There were, however, non-significant differences in these parameters between group and we also adjusted for these covariates in all analyses. As sex differences in the arterial response to exercise have previously been described [60], the analysis herein was performed only on male subjects. Thus, this study may act as a foundation for future work investigating arterial response in more comprehensive populations. An inherent limitation to this study was the small sample size (n=62), but despite this we were able to demonstrate significant differences in vessel hemodynamic parameters between smokers and non-smokers at rest and following acute physical stress. Owing to technical limitations, applanation tonometry was not performed during the exercise stress test, nor were metabolic cardiorespiratory variables captured following exercise cessation. Additionally, as there were time constraints immediately after exercise, PWA measures were not performed 2 minutes following exercise. For the most part, our study did not enrol heavy smokers, but rather we evaluated a cohort of light-moderate smokers. Additionally, our smokers were very physically active. This enrollment was to achieve a

relatively ‘clean’ study population with smoking as the only risk factor. Further studies need to be conducted to evaluate the impact that heavy smoking and physical inactivity may have on the ability of the arteries to respond to acute physical stress. Of importance to note again is that there continues to be disagreement on the length of time acute smoking impacts the cardiovascular system. Thus, one must be aware of this when evaluating post-exercise measures of arterial stiffness following acute smoking condition. Much of the data collected through the SMOKELESS study are to be analyzed by future members of the Vascular Health Unit team, and was thus not incorporated into this thesis (including that from saliva and blood samples, metabolic data captured during exercise, subject data from questionnaire responses, etc.). Future students may continue to elucidate the complex relationships between smoking, physical stress, and arterial stiffness as analysis continues to develop.

## 7.0 Conclusions

Herein, we have investigated arterial stiffness, hemodynamic parameters, and cardiorespiratory response in young, healthy smokers and non-smokers at rest, and following acute physical stress. At rest, smokers displayed significantly elevated cfPWV, AIx75, and decreased PPA compared to non-smokers. Acute smoking also caused significant depression of the SEVR. Taken together this indicates that vascular alterations are present even at rest in young, healthy light-moderate smokers, which are only exacerbated by acute smoking. Smokers demonstrated a blunted ability to respond to increased demands, as cfPWV, SEVR, and AIx75 were all significantly elevated compared to non-smokers following exercise. Thus, even in otherwise healthy, young smokers there is a drastic alteration in the ability of the arterial system to respond to the increased demands of physical stress. The arterial stress test has proven to be a very useful tool in the evaluation of vascular hemodynamics. Future clinicians may use this tool in the assessment of risk for cardiovascular complications and disease, whereby they may better identify those in jeopardy of poor vascular outcomes. In this way we may better direct the clinical approach to treatment with the hope of diminishing the individual and societal burdens associated with cigarette smoking.

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# Appendix

Figure 1 – Eligibility Questionnaire used for the study

**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.

**Smoker**s

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 | Posters (Streets, Cafes etc) | Craigslist | 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?
   If **YES**, when and for how long (provide dates if you can)? _____

| YES | NO |
|---|---|
| | |

4. If you are a non-smoker have you ever smoked **Cigarette or Sheehsa** in your life ?
   - 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)? _____

| YES | NO |
|---|---|
| | |

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? XX / XX / XXXX
   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)
    - If **YES**, Explain: _____

| YES | NO |
|---|---|
| | |

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 you are a student, 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?

| DAY | Sunday | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday |
|---|---|---|---|---|---|---|---|
| AM | | | | | | | |
| PM | | | | | | | |

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

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

18. Are you a coffee drinker?

| YES | NO |
|---|---|
| | |

19. Part 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:
    a. Excellent ___
    b. Very good ___
    c. Good ___
    d. Poor ___
    e. 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?
If yes, when? _____

| YES | NO |
|---|---|
| | |

24. Has a doctor ever told you that you have high cholesterol?
If yes, when? _____

| YES | NO |
|---|---|
| | |

25. Has a doctor ever told you that you have diabetes?
If yes, when? _____

| YES | NO |
|---|---|
| | |

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**:
    f. Do you have regular periods? _____
    g. When is the start of your next period (please provide a date)?_____
    h. How long is your cycle (i.e. how many days between the start of your periods)? _____
    i. 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.

Figure 2 – Public Advertisement used for the study

Figure 3 – McGill University Health Center Research Ethics Board Approval

Provided on Request as per e-Thesis submission regulations and instructions.

Figure 4 – SMOKELESS Study Consent Form

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

### *RESEARCHERS*

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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 **two** appointments on **two** different days:

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

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 _____

Figure 5 – Pre-Assessment Information Sheet given to participants

Subject Instructions

Please follow these instructions carefully when preparing for your assessment.

- **Do not** consume alcohol for 12 hr before your scheduled assessment.
- **Do not** consume any caffeine for 12 h before your scheduled assessment (this includes coffee, tea, caffeine pills, soft drinks, energy drinks or chocolate).
- **Do not** perform any kind of exercise/sports for 24 h before your scheduled assessment.
    - Please **DO NOT** bike into the lab on the morning of your assessment.
- If you are a smoker, **do NOT** smoke for 12h before your scheduled assessment. This is extremely important.
- You must bring a piece of photo identification with you on the day of your assessment.
- Please arrive on-time for your scheduled appointment!

It is also very important that you wear **proper exercise attire**. This means **running shoes, a t-shirt, and running shorts.**

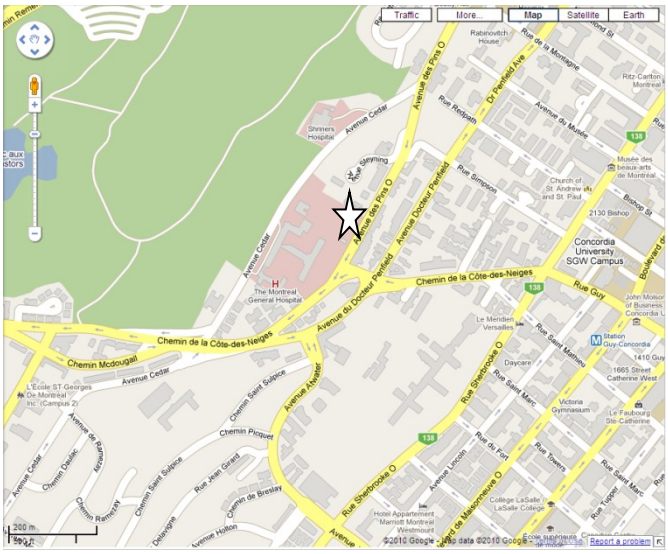
The assessment will be conducted in our lab, located at the following address:

Vascular Research Laboratory
The Montreal General Hospital
1650 Cedar Avenue
Montreal, Quebec H3G 1A4
**Office: B2.252**

**DIRECTIONS:**

**From CEDAR ->** Enter from Cedar Avenue -> Walk straight ahead to the end of the Main hallway to the second set of elevators -> Take elevator down to Floor 2 ->Follow signs for room B2.252

**From PINS ->**Enter from Pins Ave. (at Cote-des-Neiges) ->Take first set of elevators to Floor 2 ->Follow signs for room B2.252




Your participation in this study is very much appreciated. If you have any questions, or you need to reach a laboratory member please e-mail: cigarette.smoking.study@gmail.com or call 514-934-1934 ext. 42478.

Figure 6 – SphygmoCor technique for the measurement of carotid-femoral PWV

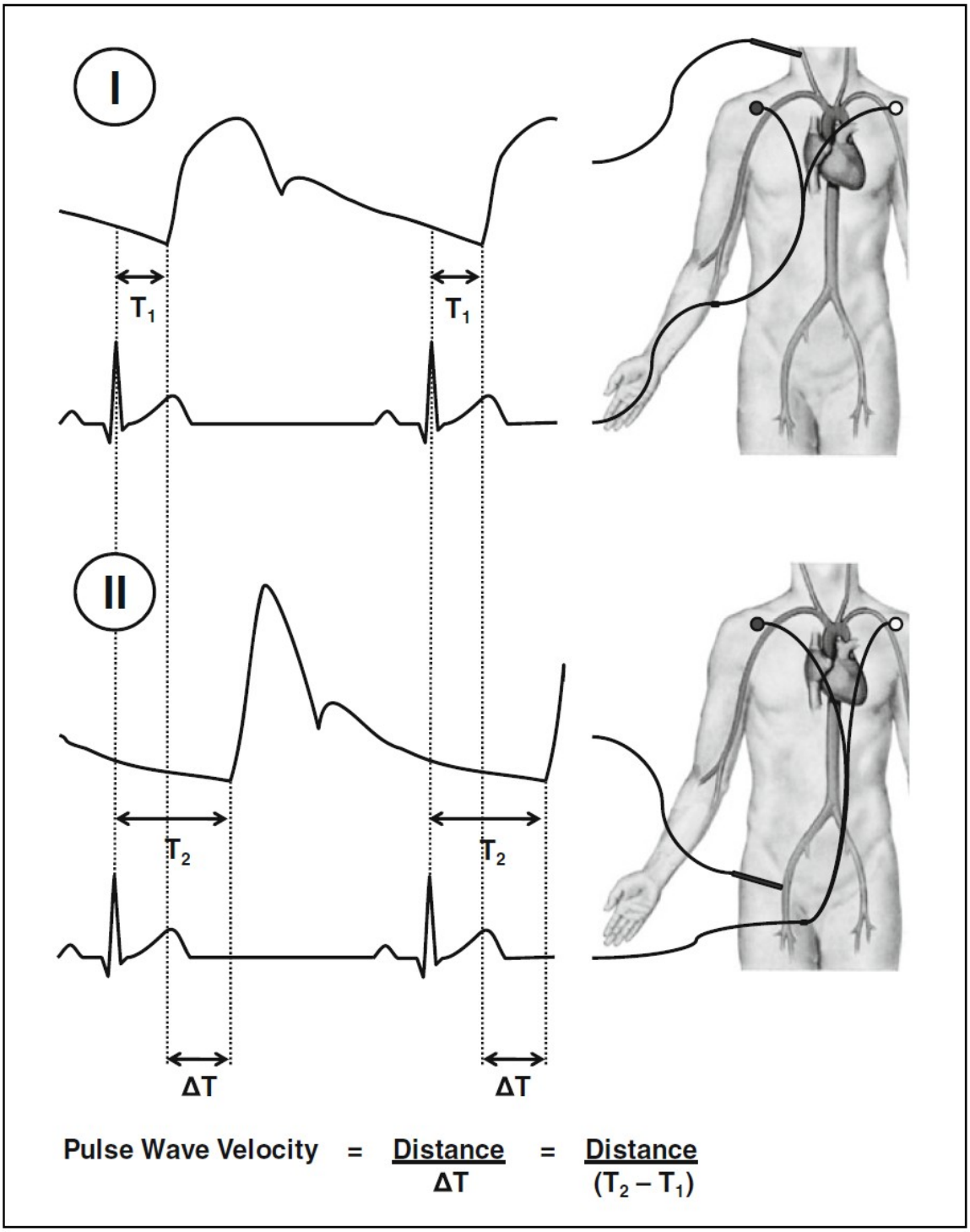


Figure 7 – Modified Bruce Protocol

| Modified Bruce Treadmill Protocol | | | |
|---|---|---|---|
| Stage | Speed (mph) | Incline (%) | Duration (minutes) |
| 0 | 0 | 0 | 1 |
| 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 |

Figure 8 – Borg Scale Rating of Perceived Exertion

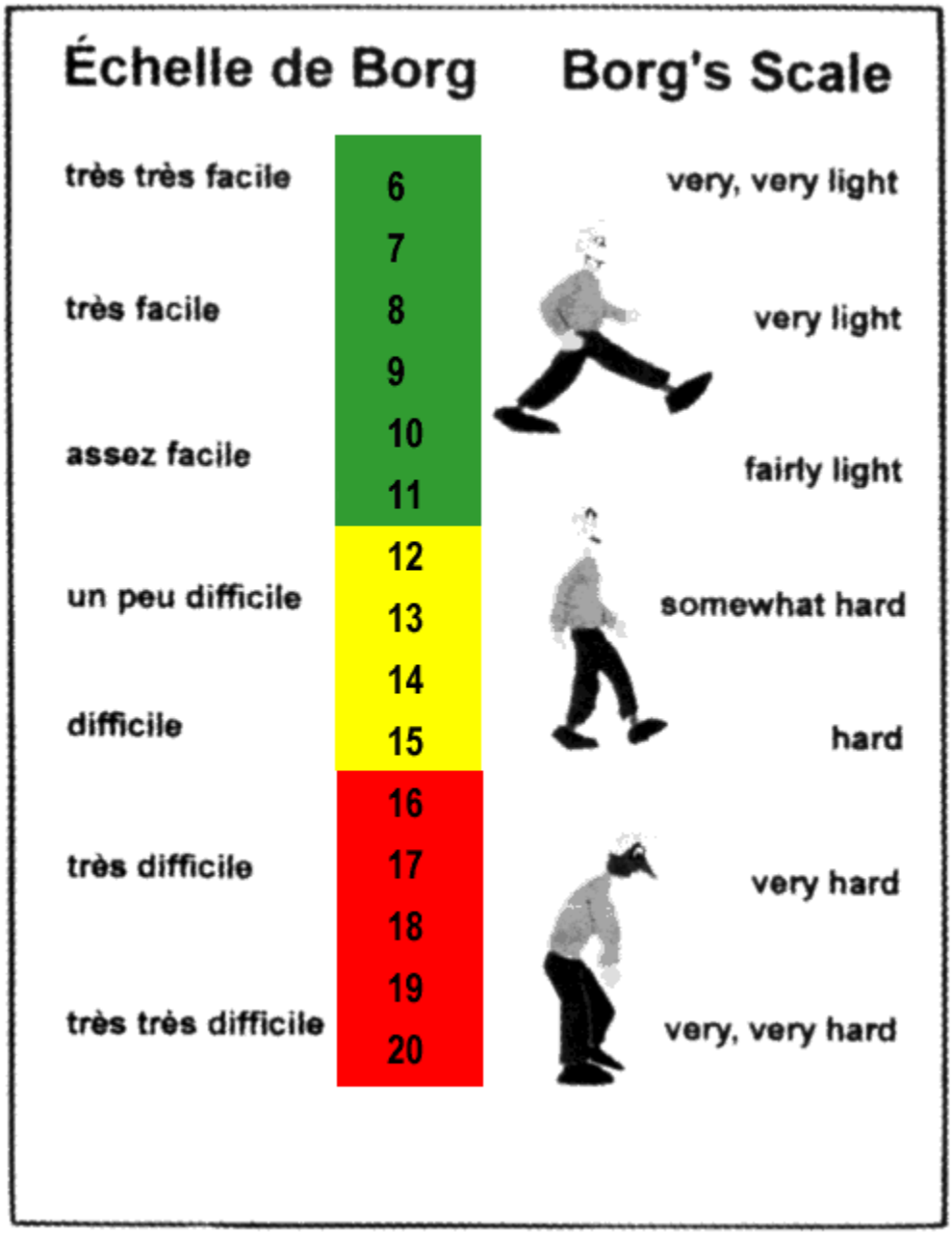


Figure 9 – SphygmoCor Screen Capture following PWV measurement


Figure 10 – SphygmoCor Screen Capture following PWA measurement

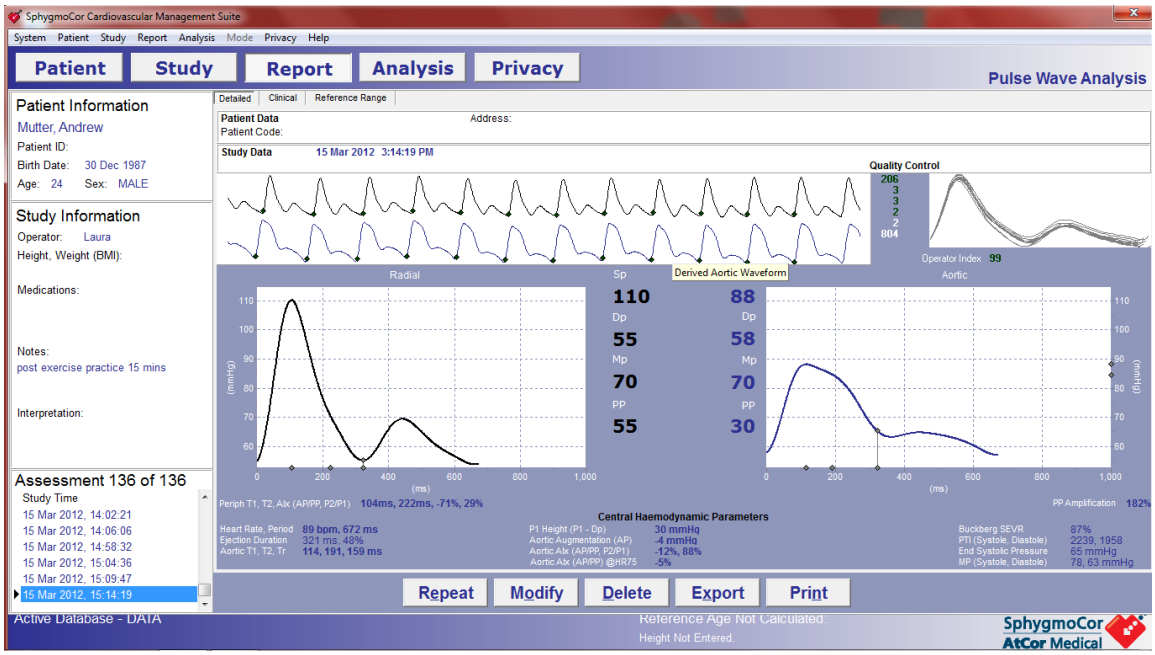


Figure 11 – International Physical Activity Questionnaire (short English version)

## 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 a **normal 7 day period**. 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 do in a normal **7 day period**. **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 a normal **7 day period**, 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 in a normal **7 day period**. **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 a normal **7 day period**, 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 a normal **7 day period**. . 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 a normal **7 day period**, 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 in a normal **7 day period**. 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 a normal **7 day period**, 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!