Full Title: Cerebral oxygenation and sympathetic responses to smoking in young and middle-aged smokers.

Short Title: Hemodynamic and sympathetic responses to smoking.

Abstract

This study examined the effects of acute tobacco smoking on cerebral oxygenation and autonomic function in 28 male, habitual smokers of shorter (YSM) or longer (MSM) smoking history. Following baseline testing, participants undertook a smoking protocol involving the consumption of two cigarettes within 15 min. Measures of cerebral oxygenation and autonomic function were collected before, during and 0 min, 30 min, 1 h, 4 h post-smoking. Tissue saturation index (TSI) for MSM was greater than YSM during cigarette consumption ($p < 0.05$). Moreover, MSM observed significant within-group changes for TSI during and post cigarette consumption ($p < 0.05$). Further, MSM observed an increase in low frequency (LF) band from 30 min to 1 h post-consumption, followed by a decline; whereas, an elevations above MSM were observed in YSM at 4 h ($p < 0.05$). Both MSM and YSM showed a decrease in high frequency (HF) band post-cigarette, while increased LF/HF ratio post-consumption was observed in YSM. A decline in the standard deviation of RR intervals post-cigarette consumption was evident in MSM ($p < 0.05$). Moreover, the root mean square of R-R interval in both groups similarly decreased following cigarette consumption ($p < 0.05$). Acute smoking affects heart rate variability, suggestive of vagal withdrawal and may indicate an effect of smoking history. Additionally, prolonged smoking history alters cerebral microcirculatory responses to acute tobacco exposure in MSM.

Introduction

Active or passive exposure to tobacco smoke exposes multiple organs, particularly those of the pulmonary and cardiovascular systems to repeated chemical insult,[1] whereby profound effects on many biological systems can ensue.[2,3] As a result underlying pathogenesis of chronic diseases including cardiovascular disease (CVD), cancers, pulmonary diseases and diabetes can develop.[4,5] Systemic diseases resulting from regular exposure to tobacco smoke are thought to occur via increased vascular permeability, reduced endothelium-dependent vasodilatation,[3-6] prolonged increases in blood pressure and heart rate, and changes in cerebral and peripheral hemodynamics.[7-9] These cardiovascular outcomes are accompanied by an imbalance between oxidant stress and anti-oxidant defence, increased platelet aggregation, increased cellular adhesion molecules, inflammation and sheer stress.[3,10] Collectively, the above mentioned factors can increase the risk for cardiovascular events.[11] While the role of chronic tobacco smoking on endothelial dysfunction and autonomic control have been well documented,[12-14] it is not well known whether the mechanisms precipitating such changes can be attributed to age and smoker history or whether these changes are a result of the acute responses to repeated smoking exposure.

Amongst notable physiological changes from tobacco smoking is the disruption of normal autonomic nervous system (ANS) balance, characterised by sympathetic hyperactivity and attenuated parasympathetic activity.[12] This autonomic imbalance may be attributed to nicotine exposure,[15] which stimulates the release of catecholamines, epinephrine and norepinephrine,[7,15] subsequently stimulating cardiovascular events.[15,16] In turn, this sympathetic stimulation also increases myocardial contractility, cardiac output, stroke volume and peripheral vasoconstriction.[17] Although the autonomic effects of chronic tobacco

smoking have been studied extensively, the acute effects are less well known.[12,16,18] Given the unfavourable effects of tobacco smoking on cardiovascular health, particularly autonomic regulation, it seems important to determine the acute microcirculatory and sympathetic responses to smoking. Moreover, given the absence of longitudinal data, a comparison of acute autonomic responses to smoking in those with a longer compared to shorter smoking history may provide insight into the pathophysiological pathways contributing to cardiopulmonary disease.

In addition to nicotine, tobacco smokers are exposed to a myriad of chemical compounds, which may impose many deleterious effects on cardiovascular function.[4] The presence of compounds such as carbon dioxide and nitric oxide exert vasodilatory effects, whereas compounds such as nicotine act as a vasoconstrictor.[19] Consequently smoke exposure is suggested to alter cerebral hemodynamics, increasing the risk of cerebrovascular disease.[9] Previous literature from transcranial Doppler ultrasound,[20] near-infrared spectroscopy (NIRS)[9] and positron emission tomography[19] suggest that regional cerebral blood flow velocity is increased in response to tobacco smoking [19,20] and temporarily reduces vasomotor reactivity.[20] Additionally, tobacco smoke has been reported to reduce peripheral microcirculation.[8] Such changes in cerebral and peripheral hemodynamics may present as a precursor for early endothelial injury, ultimately predisposing to the development of atherosclerosis.[8] However, despite the abundance of literature concerning the many deleterious effects of tobacco smoking on aspects of cardiovascular physiology, the effects of tobacco smoking on cerebral oxygenation are less understood and could prove pivotal in advancing knowledge about smoking and the progression of systemic disease.

The acute sympathetic and hemodynamic responses to tobacco smoking are associated with adverse cardiovascular events.[11,21] Currently there is limited literature describing the acute effects of tobacco smoking on autonomic regulation, particularly in regards to the length of smoking history.[12] Additionally, the acute effects of tobacco smoking on microcirculation as determined by NIRS are less understood. Collectively, it is unknown whether the acute changes in sympathetic activity and microcirculation are influenced by the length of smoking history as a surrogate for the lack of longitudinal data. Therefore, the aim of this study was to determine the effect of acute tobacco smoke inhalation on measures of microcirculation and autonomic function. A further aim was to determine whether a longer smoking history alters the aforementioned responses compared to a relatively shorter smoking history. It was hypothesised that smokers with a longer smoking history would present with greater autonomic imbalance and reduced microcirculation compared to those with a shorter smoking history.

Methods

Participants

The study cohort consisted of 14 smokers with a relatively short smoking history (YSM; 22.0 ± 1.6y of age; 2.86 ± 1.91 pack-years) and 14 smokers with a longer smoking history (MSM; 33.3 ± 7.7y of age; 12.15 ± 9.61 pack-years). The delimited age ensured groups would have differences in smoking history, but would still be of a similar state of health to ensure differences in fitness and other variables of aging were not confounding factors. Participants reported as apparently healthy and free from any known metabolic, cardiovascular or pulmonary disease, immunological irregularities or other conditions. Any participant that was confirmed as having any of these conditions or taking potentially

confounding medications was excluded from the study. The self-reported smoking history for the YSM and MSM populations were 5.2 ± 1.7 and 14.6 ± 6.5y of smoking and 12.3 ± 6.8 and 15.8 ± 7.3 cigarettes per day, respectively. Prior to the commencement of the study all participants were required to provide written and verbal consent following an outline of all procedures and measures. This study conformed to the Declaration of Helsinki and was approved by the Research in Human Ethics Committee at Charles Sturt University.

Study Overview

Prior to involvement in the study, participants were required to undergo a medical screening, completed an adult pre-exercise screening system (APSS) healthy history questionnaire and the Fagerstrom test for nicotine dependence.[22] If participants satisfied the study inclusion criteria, they were then enrolled into the study. Participants completed an initial familiarization prior to a baseline testing session, which included anthropometry, a graded exercise text (GXT), spirometry, and a dual-energy x-ray absorptiometry (DXA) scan. Following approximately 7d rest, participants returned to the laboratory and were required to partake in a single testing session. Participants were instructed to successively smoke two cigarettes of the same brand (Winfield Blue, 12mg tar 1mg nicotine) within 15 min; with heart rate, blood pressure and cerebral oxygenation measured throughout the smoking period and before and after (0min, 30min, 1h, 4h) cigarette consumption. To ensure standardised responses, following the smoking protocol participants remained in the laboratory with the researchers until 4h post-measures in a fasted and rested state, with no additional exposure to tobacco smoke (i.e. environmental).

Baseline Testing

Following a prior familiarisation with all procedures, participants reported to the laboratory between 0530 h and 0800 h, rested and fasted for a baseline testing session. Stature (Stadiometer: Customised, Bathurst, Australia), body mass (HW 150 K, A & D, Bradford, MA, USA), and waist and hip circumferences (steel tape, EC P3 metric graduation, Australia) were obtained for analysis of body composition based on standardized techniques. Body mass index (BMI) was calculated from mass and stature, whilst waist and hip circumferences provided a waist to hip ratio. In addition, a supine dual-energy x-ray absorptiometry (DXA) scan was conducted for the determination of body composition (XR800, Norland, Cooper Surgical Company, Trumbull, CT, USA). Scanning resolution and speed were set at 6.5 x 13.0mm and 130 mm s^{-1}, respectively. Whole body scans were analyzed (Illuminatus DXA, ver. 4.2.0, USA) for total body lean mass and total body fat mass and are reported in absolute and relative terms.

Resting blood pressure was measured through a commonly used indirect technique involving the use of an aneroid sphygmomanometer and stethoscope (Welch-Allyn, Arden, North Carolina), while participants were also fitted with a heart rate (HR) monitor (Rs800cx,Vantage NV, Polar, Finland) to obtain a measure of resting heart rate. Additionally, a baseline blood sample was collected to determine fasting glucose and total cholesterol. Participants then underwent spirometry procedures. Participants were instructed to perform a maximal inhalation followed by a maximal exhalation for the duration of 6 seconds, data collected provided force vital capacity (FVC), forced expiratory volume in one second ($FEV_{1.0}$) and $FEV_{1.0}$ as a percentage ($FEV_{1.0\%}$) at baseline (Spirometer 20.600, Vitalograph Ltd. Buckingham, England).

Participants then performed a GXT on an electronically-braked cycle ergometer (LODE Excalibur Sport, LODE BV, Groningen, The Netherlands) for the determination of $VO_{2\,peak}$. The younger population began the incremental GXT at 100 W and increase by 25 W every minute until volitional exhaustion, whereas, the middle-aged population began the GXT at 25W and increase 25W every minute. A measure of HR was obtained every minute until the completion of the GXT. Pulmonary gas exchange was measured by determining O_2 and CO_2 concentrations and ventilation to calculate VO_2 using a metabolic gas analysis system (Parvo-Medics, True2400, East Sandy, UT, USA). The system was calibrated according to the manufacturer's instructions. This involved the pneumotachometer calibration using a 3L syringe. The gas analyzers were calibrated using a two-point fully automated process involving room air and gas calibration for fractional gas concentration with a gravimetric gas mixture of known concentrations (CO_2, 4.1 (0.1)%; O_2, 15.7 (0.2)%).

Experimental Protocol: Cigarette Consumption

Participants reported to the laboratory between 0530 h and 0800 h in a fasted and rested state for the completion of the smoking protocol. Participants were instructed to smoke two filtered cigarettes (Winfield Blue, 12mg tar 1mg nicotine) within 15 min in a private but open area near the laboratory. Participants remained seated throughout the protocol with no or minimal movements to ensure standardised measurements. Normal smoking behaviour was encouraged during the consumption of the two cigarettes, adequacy of smoking ensured by visual observation by the research team. The selection of the acute smoking protocol was chosen based upon previous research by Van der Vaart et al.[23] who administered two cigarettes of the same brand within 30min. Given the lack of acute smoking research, this was the guideline for selection of the smoking protocol used here. Further, selection of the

brand of brand of cigarette was also based upon research published by Van der Vaart et al.[23], involving two cigarettes of 12mg tar, 1mg nicotine. The selected brand in the current study is considered average in terms of nicotine dose, and prior questioning regarding smoking habits deemed this brand an appropriate brand and nicotine content amongst the selected group.

Near-infrared Spectroscopy (NIRS)

A continuous wave NIRS instrument was used as a non-invasive tool for measuring microcirculatory changes in oxygenated ([HbO_2]), deoxygenated ([HHb]) and total cerebral haemoglobin ([THb]) concentrations (Artinis Medical System, Oxymon MK III, Zetten, the Netherlands). NIRS data were recorded at 10 Hz for the duration of the smoking protocol; a further 3min recording was obtained at 30min, 1h and 4h post cigarette consumption. During all NIRS sampling participants were required to be seated in an upright position and following a 5min stabilisation period, normalised breathing patterns were ensured. NIRS data collected during the acute smoking protocol was normalized against approximately 120s of baseline data, collected prior to each measurement in a rested state, seated in an upright position. For each time point, the NIRS probe was placed over the left prefrontal cortex between Fp1 and F3 (international EEG 10-20 system) and placement was adjusted approximately < 5mm for individual variance. The NIRS probe was affixed with double-sided adhesives and the inter-optode distance was fixed at 3.5cm via a black plastic spacer. A modified Beer-Lambert law was applied to determine oxygenated and deoxygenated haem concentration, based on the absorption coefficient of continuous wavelength infrared light (856 & 794nm) and age-dependent differential path-length factors. Total haemoglobin was calculated via the sum of oxygenated and deoxygenated haemoglobin concentrations to give an indication of regional blood volume. Further, tissue saturation index (TSI) was calculated

as a ratio of oxygenated to total haemoglobin concentrations.

Heart Rate and Blood Pressure

Participants wore a HR monitor (Rs800cx, Vantage NV, Polar, Finland) for the attainment of HR and heart rate variability (HRV) during the testing protocol. The collection of HRV was paralleled with the collection and timing of NIRS variables. HRV was collected throughout the smoking protocol and for 3min at each subsequent post-measure, following a 5min stabilization period. Following recording, HR files were downloaded to Polar software (Polar Protrainer 5, Polar Electro Oy, Professorintie 5, 90440 Kempele, Finland) via infra-red; after visual inspection, occasional ectopic beats were identified and replaced with interpolated (linear) adjacent R–R interval values. HRV analysis was performed using HRV software (Kubios 2.1, Biosignal Analysis and Medical Imaging Group, Finland). Both time and frequency-domain analyses were performed. The mean RR interval, the standard deviation of RR interval (SDNN), and the root mean square of R-R interval differences (rMSSD) were analysed. A power spectral analysis using Welch's periodgram provided frequency-domain parameters (Kubios 2.1, Biosignal Analysis and Medical Imaging Group, Finland). Components of power spectrum were computed with the following bandwidths: high frequency (HF) (0.15 to 0.4 Hz), low frequency (LF) (0.04 to 0.15 Hz), thus providing the LF/HF ratio. Data are expressed as raw values for both frequency and time domain parameters.

Blood pressure was obtained through a commonly used indirect technique involving the use of an aneroid sphygmomanometer and stethoscope (Welch-Allyn, Arden, North

Carolina). The cuff was placed on the upper arm over the brachial artery, and above the antecubital fossa. The head of the stethoscope was placed over the antecubital fossa. The cuff was inflated to occlude the brachial artery then gradually deflated while the assessor listens for the appearance of Korotkoff sounds, using the first and fifth stages as systole and diastole. The measurement was repeated following sufficient rest, and the two readings averaged to provide an individual's blood pressure.[24]

Statistical Analysis

Normal distribution was determined by Shapiro-Wilk's test and non-normally (rMSSD, LF & HF) distributed data was logarithmically transformed prior to analysis All data are reported as mean ± standard deviation (SD). Repeated measures analysis of variance (ANOVA) (condition x time) was used to determine within- and between-group differences. Where a group interaction was noted, one-way ANOVA tests were applied to determine the source of significance. Significance was set at $p < 0.05$. All statistical procedures were performed using Predictive Analytic Software (PASW) (Statistical Package for the Social Sciences for Windows version 18.0, Chicago, IL, USA).

Results

Baseline variables for anthropometric variables and smoking history are reported in Table 1. The MSM group had significantly ($p = 0.001$) greater smoking history in terms of years of smoking and pack-years than YSM. However, the dependence level (based upon the Fagerstrom Test for Nicotine Dependence)[22] and volume of cigarette smoke did not differ between groups ($p = 0.19$). There were no differences between the groups for VO_{2peak}

(p=0.26), though the MSM group demonstrated greater absolute and relative fat mass than YSM (p=0.00). Further, YSM had higher FVC and $FEV_{1.0}$ at baseline compared to MSM (p=0.007; p=0.027).

There were no observed differences between groups for HR or BP at rest or across the protocol (Fig. 1). Additionally, there were no within-condition changes in SBP for YSM or MSM. However, an increase in DBP from pre- to post for YSM (p=0.043) was observed, that was not present in MSM, despite a decline in DBP from 1h to 4h in MSM (p=0.041). No between group differences were observed for HR. However, both groups showed a within-condition increase for HR from pre- to post cigarette consumption followed by a decline at 30min, which continued only for YSM to 1h post (p=0.00-p=0.022).

The time-domain parameters for heart rate variability are presented in Fig 2. There were no baseline differences in rMSSD or SDNN between YSM and MSM. MSM showed a significant decline in SDNN post-cigarette consumption (p=0.009), which was not significant in YSM. Both groups showed elevations in SDNN at 30min in post-consumption (p=0.04; p=0.004). Further, rMSSD in both groups decreased following cigarette consumption (p=0.04; p=0.01). The frequency-domain parameters are presented in Fig 3. Despite no significant baseline differences for HF, LF or LF/HF, only MSM observed a decrease immediately post cigarette consumption followed by an increase LF from 30min to 1h post-consumption, followed by a decline thereafter (p=0.04; p=0.02); conversely, YSM observed higher values for the raw power of LF at 4h compared to MSM (p=0.02). Both groups observed within-group changes for HF, with a decrease from pre to immediately post (p=0.02; p<=0.05), followed by an increase at 30min (p=0.01; p=0.00) and only a decline

observed for MSM (p=0.04). For LF/HF, an increase in YSM was observed from pre to post (p=0.00), which was not observed in MSM (p=0.54), although both groups observed a decline from immediately post to 30min (p=0.00; p=0.01). Further, MSM observed a significant increase from 30min to 1h post, (p=0.03) which occurred later for YSM (1h-4h). Values for YSM remained above pre at 4h (p=0.01).

In regards to NIRS responses, there were no significant differences in [HbO_2], [HHb] or [THb] between groups at baseline or throughout the protocol (Fig 4). However, for MSM TSI was greater than YSM during consumption of the second cigarette (p= 0.048; p= 0.01). While YSM showed within-group changes from pre to start of first cigarette in TSI (p=0.05), this was not different to MSM (p=0.25. Further, while YSM showed no within-group change for [HHb], values for MSM increased immediately from pre to start of cigarette consumption followed by a decrease to post cigarette consumption (p= 0.023; p=0.002; p=0.002). Following cigarette consumption, elevations in [HHb] to baseline were observed to 1h in MSM (p=0.021), however were not observed in YSM. Finally, no within-group changes were observed for either group for [HbO_2].

Discussion

This study aimed to elucidate what effects acute tobacco smoking had on autonomic and hemodyamic changes in smokers, with particular respect to comparison of shorter versus longer smoking histories. The findings revealed that acute cigarette smoking may result in prolonged vagal withdrawal which may be indicative of sympathetic hyperactivity this was evidenced in YSM by decreases in HF and an increase in the LF/HF ratio. A further finding

indicates that acute tobacco smoking increases TSI and [HHb] during the cigarette consumption, followed by declines post cigarette consumption amongst in [HHb] amongst MSM, but not YSM, which also suggests an effect of smoking history on cerebral hemodynamics (Fig. 4).

While the adverse effects of tobacco smoking are many, changes in autonomic control and hemodynamics may be amongst the most important for the determination of cardiovascular risk. Chronic tobacco smoking is known to augment sympathetic dominance[18] and reduce vagal modulation;[25] consequently such alterations in autonomic balance have been associated with adverse clinical outcomes.[16] However, despite the abundance of literature concerning the chronic effects of smoking on heart rate variability, only a few studies have reported on the acute effects of tobacco smoke on autonomic control.[16,18] Mendonca, et al.[18], reported both raw high and low frequency power to decrease following smoking, with the LF/HF ratio increased, suggesting sympathetic dominance. Findings from the current study reveal that even brief exposure to tobacco smoke produces notable changes in sympatho-vagal balance, as reflected by decreases high and low frequency power and elevated LF/HF ratio in YSM following cigarette consumption. Further, the sympathetic response was delayed, or inhibition of parasympathetic tone was present in YSM following acute smoke exposure (as represented by the delayed LF/HF peak when compared to MSM). Our study observed similar findings to that of Karakaya et al.[16] who reported acute smoking increased the LF/HF ratio and reduced mean RR intervals, SDNN and rMSSD within 5 min of smoking a cigarette. Further, time-domain parameters suggest reduced vagal modulation, particularly amongst MSM, who similarly to Karakaya et al.[16] observed a reduction in SDNN post-cigarette consumption. Additionally, rMSSD decreased following cigarette consumption in both groups, which is indicative of reductions in the parasympathic component of autonomic

control.[26] Moreover, in a study by Hayano et al.[27] concerning HRV parameters in heavy, moderate and non-smokers, taking into account the respiratory component, suggested that heavy smoking (~12 years and >25 cigarettes per day) results in acute and transient decreases in vagal cardiac control and that heavy smoking results in long term reductions in vagal cardiac control in habitual smokers with a shorter smoking history.

Tobacco smoke has powerful excitatory effects of the SNS,[11] which may be a direct effect of nicotine via the stimulation and blocking of autonomic ganglia or direct impairment of baroreflex function.[27,28] Increased sympathetic activity has pro-arrhythmic, atherosclerotic and thrombotic actions which may be involved in the elevated cerebrovascular risk observed in smokers.[25] Consequently, results from the current study are reflective of acute vagal withdrawal which may be suggestive of sympathetic hyperactivity. While the precise mechanism behind sympathetic dominance remains elusive, acute responses may be useful in understanding how tobacco smoking results in the disruption of autonomic balance.

Smoking is an important risk factor for cerebrovascular diseases.[29] While chronic tobacco smoking is associated with reduced cerebral blood flow, literature concerning the acute responses to smoking is inconsistent.[9,30] Previous studies have reported that tobacco smoking decreases regional cerebral blood flow (rCBF), as measured by transcranial Doppler sonography;[9,20] however, the effects of tobacco smoking on cerebral microcirculation as determined by NIRS remain less well known - although this methodology could assist in elucidating microcirculatory changes associated with the heightened cerebrovascular risk in smokers. While Terborg et al.[9] reported no change in deoxygenated hemoglobin with smoking, Pucci, Stepanov and Toronov,[30] reported deoxygenated hemoglobin to increase after 5 min of smoking, and additionally observed concomitant increases in oxygenated

haemoglobin. The current study revealed that despite the initial increase in TSI in YSM it was comparatively greater during cigarette consumption in smokers with a longer smoking history (MSM). Furthermore, smoking induced increases in [HHb] in MSM during the cigarettes followed by declines, but no notable changes in YSM. In contrast to the present study Siafaka et al.[8] reported no change in peripheral haemoglobin saturation as a result of smoking. However, we found that [HbO_2] remained unchanged, whilst [HHb] was increased during the consumption of cigarettes followed by a decrease [HHb] in MSM. Such a finding would indicate that smoking induces a transient desaturation in the MSM. If this is the case, an increase in [HHb] in the prefrontal cortex without simultaneous increases in [HbO_2] might compromise neuronal activity in this group of MSM. In the present study, the observed increase in TSI and [HHb] in MSM during cigarette consumption may be attributed to carbon monoxide (CO) exposure, which due to the high affinity of CO to haemoglobin, causes a leftward shift in the haemoglobin-oxygen dissociation curve.[8] Moreover, during cigarette smoking approximately 5-22mg of CO are emitted,[31] consequently given that the absorption of carboxyhemoglobin by red light is of a similar wavelength to [HbO_2], the presence of CO may interfere with readings of [HbO_2]. While the acute effects of smoking report conflicting findings, previous literature did not define groups based upon smoking history, which could be a limiting factor. The younger smoker group in the present study observed no significant changes in cerebral hemodynamic parameters, suggesting when isolated by age, long-term smoking may alter cerebral oxygenation and the associated hemodynamics.

While previous studies have reported tobacco smoking to increase cerebral blood flow velocity and reduce vasomotor reactivity[20] as demonstrated by trancrainial Doppler sonography[9] given the changes to autonomic control, it seems imperative to determine whether the changes to autonomic control are reflected in changes to cerebral

microcirculation. While an increase in sympathetic dominance was resultant from acute cigarette smoking, these effects were not reflected in cerebral oxygenation of either YSM or MSM. Previous vasomotor studies using NIRS methodology suggest that changes in cerebral oxygenation are representative of changes in cerebral blood flow.[32] However, in the present study, despite the observed increase in sympathetic drive, elevations in cerebral oxygenation did not occur, which may be attributed to compounds such as CO or the reduction of bioactive nitric oxide that act as a vasodilator, and is reduced by chronic smoking.[33,34] Granted the effect of tobacco smoking on the vasculature can be influenced by multiple biochemical and hemodynamic factors, the determination of microcirculatory and autonomic age-based responses to smoking may further our understanding, particularly in regards to the negative longitudinal effects of smoking.

Despite these findings, certain limitations must be acknowledged. The respiratory rate of smokers may present as a limitation to the current study as there is an established relationship between breathing frequency and heart rate variability parameters. Brown et al.[35] reported a reduction in the absolute power of LF and HF components, suggesting that breathing may have an effect on parameters of HRV. As a means to control this, participants were seated in an upright position, where breathing patterns were normalised before the collection of data. Further, while the authors acknowledge the LF/HF ratio data can indicate higher coefficient of variation[36] it may provide indications and directions for future research in the area. A further limitation to the study is the age of the MSM, while an older population is desirable, many presented with pre-existing medical conditions, thus the MSM on average maybe younger than desired. However, despite age being a limitation, the MSM had comparable health status to YSM, thus eliminating any age-related constraints.

In conclusion, the present study indicates an acute effect of tobacco smoking on both the frequency and time-domain parameters of HRV, suggestive of sympathetic dominance, and may further indicate an effect of smoking history, particularly in regards to LF/HF in YSM. A further finding is the observed changes in [HHb] and TSI in MSM which may suggest that prolonged tobacco smoking alters cerebral microcirculatory responses to smoke, which ultimately may increase the risk of cerebrovascular disease and suppressed neuronal activity at specific instances. While the measures are novel and represent a small portion of the physiological responses to tobacco smoking, these findings may provide future direction into the acute microcirculatory and sympathetic effects of smokers who present with different smoking histories.

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Table 1. Mean ± SD Baseline descriptive, anthropometric, dual-energy x-ray absorptiometry (DXA), biochemistry, aerobic fitness and smoking variables within the young smoker (n=14) and middle-aged smoker (n=14) populations.

Anthropometric & Descriptive Data	*YSM*	*OSM*
Age (years)	*22.0 ± 1.57*	*33.27 ± 7.75**
Height (m)	*1.82 ± 0.07*	*1.77 ± 0.07*
Weight (kg)	*81.78 ± 12.07*	*81.22 ±12.87*
VO_2 peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	*36.67 ± 3.06*	*33.93 ± 8.74*
Final stage Watts (GXT)	*275 ± 36.69*	*230 ± 46.48*
Waist Circumference (cm)	*84.46 ± 8.44*	*87.67 ± 8.92*
Hip Circumference (cm)	*98.22 ± 5.95*	*101.54 ± 8.34*
Waist to hip ratio	*0.86 ± 0 .05*	*0.86 ± 0.06*
% Fat mass	*15.62 ± 5.78*	*24.75 ± 6.76**
Lean Mass (kg)	*63.03 ± 9.05*	*59.02 ±6.61*
Fat Mass (kg)	*12.37 ± 5.32*	*20.68 ± 6.83**
Smoking Variables		
Years of smoking	*5.21 ± 1.72*	*14.62 ± 6.55**
Cigarettes per day	*12.31 ± 6.81*	*15.79 ± 7.34*
Pack years	*2.86 ± 1.91*	*12.15 ± 9.61**
Fagerstrom score	*2.31 ± 1.38*	*2.48 ± 1.28*

*Denotes significantly different ($p < 0.05$) to YSM.