



**EFFECTS OF SMOKING ON ARTERIAL STIFFNESS AND
HAEMODYNAMICS IN MALE ADOLESCENTS IN LUSAKA**

By

THERESA CHIKOPELA

**A dissertation submitted to the University of Zambia in partial fulfilment of the
requirements for the degree of Master of Science in Human Physiology**

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DECLARATION

I declare that this report represents my own work. It has not previously been submitted for a degree or any award at this University or any other Institution. All published work or material from other sources incorporated in this report have been specifically acknowledged.

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Signed by Student; Theresa Chikopela

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Signed by Supervisor; Fastone M. Goma (Dr.)

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Signed by Head of Department; Dr Gibson Sijumbila

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ABSTRACT

Background: Cigarette smoking causes changes in the cardiovascular system including changes resulting from alterations in peripheral resistance such as increase in blood pressure. Increase in Pulse Wave Velocity (PWV), suggesting an increased arterial stiffness has been demonstrated in white smokers. An increase in plasma catecholamines and an impaired Nitric Oxide production are the likely cause of the endothelial dysfunction. The aim of this study was to determine the effects of smoking on arterial stiffness and haemodynamics in black male adolescents using PWV and Arterial Stiffness Index (ASI) and haemodynamic measurements.

Methodology: Twenty-two black, male adolescents; age range 19-25 years, who were active-smokers were included in this observational study. Complior Analyze Unit protocol was used to obtain the carotid-femoral PWV (cfPWV) and the carotid-radial PWV (crPWV) 15 minutes before smoking, and an hour after smoking. The Diasys Ambulatory machine was used to determine blood pressure and heart rate for 15 minutes before, 15 minutes during and the hour after smoking.

Results: Smoking caused a significant increase in mean PWV and ASI from their baseline values (cfPWV - 7.9 ± 1.94 m/s, cfASI - 26.1 ± 6.0 m/s, crPWV - 11.0 ± 1.62 m/s and crASI - 22.9 ± 3.52 m/s) to cfPWV- 8.5 ± 1.87 m/s, cfASI - 28.6 ± 6.19 m/s, crPWV- 11.5 ± 1.75 m/s and crASI- 24.3 ± 3.53 m/s. cfPWV and cfASI reverted to baseline 15 minutes post smoking while it took over 45 minutes for crPWV and crASI to return to baseline. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) significantly increased during smoking. SBP increased from 113.5 ± 13.15 mmHg to 127.9 ± 13.80 mmHg and returned to baseline after 30 minutes while DBP increased from 79.5 ± 8.79 mmHg to 85.6 ± 10.92 mmHg, returning to baseline immediately after smoking. HR had a significant increase during smoking from a baseline of 74.3 ± 13.75 bpm to 95.2 ± 16.72 bpm taking 15 minutes to return to baseline.

Conclusion: Smoking causes an acute increase in PWV and ASI in African, male adolescents signifying an increase in arterial stiffness in both the elastic and muscular arteries. Smoking also causes a significant rise in haemodynamics (SBP, DBP and HR), which are indices for risk factors for cardiovascular complications such as stroke and heart failure.

To Chikusela, Zanga and Kuzipa Sikazwe

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ABBREVIATIONS AND ACRONYMS

ASI	Arterial Stiffness Index
baPWV	Brachial-ankle Pulse Wave Velocity
BBC	British Broadcasting Corporation
CAD	Coronary Artery Disease
cfASI	Carotid-femoral Arterial Stiffness Index
cfPWV	Carotid-femoral Pulse Wave Velocity
CHD	Chronic Heart Diseases
CO	Carbon Monoxide
crASI	Carotid-radial Arterial Stiffness Index
crPWV	Carotid-radial Pulse Wave Velocity
CVD	Cardiovascular diseases
CYP	Cytochrome P450-dependent monooxygenase
DBP	Diastolic Blood pressure
EDV	Endothelium-dependent vasodilation
ETS	Environmental tobacco smoke
GYTS	Global Youth Tobacco survey
HR	Heart Rate
MI	Myocardial infarction
NO	Nitric oxide
NOS	Nitric oxide synthase
Pb-210	Lead-210
PM	Particulate matter
Po-210	Polonium-210
PTT	Pulse transit time
PWV	Pulse Wave Velocity
SBP	Systolic Blood Pressure
UNZA	University of Zambia
US	United States
USDHEW	<i>The Health Consequences of Smoking</i> , U.S. Department of Health, Education, and Welfare
UZABREC	University of Zambia Biomedical Research Ethics Committee

CHAPTER 1

INTRODUCTION

Tobacco use, including cigarette smoking, is said to be the single most common cause of preventable morbidity and mortality globally. At present, tobacco use is responsible for 10% of all global deaths from cardiovascular diseases and is the second leading cause of cardiovascular diseases (CVD), after high blood pressure (Mendis et al. 2011). In the year 2004, 15% of cardiovascular deaths were attributable to tobacco use in Europe, 9% were attributable to tobacco use in South-East Asia, and 6% were attributable to tobacco use in the Western Pacific (World Health Organization. 2012). It is estimated that by 2020, tobacco-related deaths will be the leading cause of deaths in developing countries (Goma et al. 2011). According to the 2014 Zambia Demographic and Health Survey (ZDHS), 19.8% of men age 15-49 years reported smoking cigarettes, a pipe, or using other tobacco products. Most of these men smoked cigarettes (94.4%). Goma et al. (2011) showed a daily prevalence of 17.5% cigarette smoking in men in Lusaka City. Since 2007, there has been an increase in cigarette smoking among males in Lusaka of over 8% (ZDHS, 2014).

Adolescents are said to be an emerging priority population for the tobacco industry. This age has been defined as the period in human growth and development that occurs after childhood and before adulthood. This period is between the ages 12 to 25 years (Woollaston, 2013) and includes three stages; early adolescence ranging from 12 to 14 years, middle adolescence from 15 to 17 years and 18 to 25 years as late adolescence (BBC report, 2013).

The Global Youth Tobacco Survey (GYTS, 2011) Collaborating Group reported a prevalence of ever-smoked cigarettes of 19.3% overall among 3,377 respondents. They reported that the ever-smoked cigarettes population included 20.2% among boys and 17.7% among girls of ages 13 to 15 years in Zambia. ZDHS 2014, has recently recorded an increase in smoking prevalence among adolescents age 20 – 24 years of over 3% from 2007.

The data noted on adolescents in Zambia has been limited to the primary and secondary school-going age bracket (9 to 15 years old). This has limited analysis of smoking behaviour in adolescents to only a portion of the adolescent age bracket. Many smoking prevention programmes have been focused almost exclusively on youth, mainly within the school setting. However, research on contemporary patterns of smoking among young-adults is beginning to challenge the wisdom of this strategy, along with traditional assumptions about patterns of

smoking in adulthood. In recent survey data, as many as one fifth of smokers reported starting smoking after the age of 18 years which is a substantial increase over historical norms (Giovino 1999; US Department of Health and Human Services 1994). According to Backinger et al. (2003), smoking behaviour is reported to be largely fixed by the age of 18 years. Even among smokers who first try smoking in their youth, “regular” or daily smoking may not develop until much later, typically between the ages of 20–21 years (Lantz 2003). There also appears to be a relatively higher proportion of occasional smokers among late adolescence, as compared to older smokers, with considerable shifting between daily and non-daily use (US Department of Health and Human Services, 1998).

There is a growing interest in targeting tobacco control efforts at this population segment because though a high rate of smoking is reported among young adult men, there are still very significant gaps in data pertaining to this age group. This is despite the fact that smoking is a major independent risk factor for cardiovascular morbidity and mortality in adults (Stefanadis et al. 1997; Kannel et al. 1990; Peto et al. 1992). Knowledge of behaviours, attitudes and effective interventions is lacking; and young-adults are a key target group for tobacco industry promotional efforts. This report endeavours to enhance the knowledge base on effects of smoking on this age group in relation to cardiovascular risks that may result from smoking.

Chemicals noted in cigarettes include highly toxic/heavy metals that in very small amounts may potentially be harmful and for some of these metals that support life, when taken in large amounts, can become toxic. Examples include arsenic and cadmium (toxic heavy metal that is used in batteries) with smokers typically having twice as much cadmium in their bodies as non-smokers (Martin 2014). Smoke carries an increased danger for anyone breathing it mainly due to the toxic metals such as Lead-210 (Pb-210) and polonium-210 (Po-210), which are poisonous and radioactive heavy metals. Other harmful substances include ammonia compounds, which are commonly used in cleaning products and fertilizers and are mainly used to boost the impact of nicotine in manufactured cigarettes. Carbon monoxide, also present in car exhaust, is lethal in very large amounts and cigarette smoke can contain considerably high levels of carbon monoxide. Nicotine is a poison used in pesticides and is the addictive element in cigarettes (Martin 2014). Over 4000 chemical compounds are created by burning a cigarette – 69 of which are chemicals known to cause cancer.

There are two phases of cigarette combustion product that can be distinguished: the gas-phase and the solid particulate phase (also known as tar phase). Most of the particulate matter is the material retained on a filter or smouldering in the side stream smoke, whereas most gas-phase smoke passes through the filter (mainstream smoke). It has been noted that smoking one cigarette exposes the human respiratory tract to between 10,000 and 40,000 μg particulate matter (PM) (National Research Council, 1986). Both the particulate and gas-phase smoke are very rich sources of highly pathogenic free-radicals (Halliwell and Gutteridge, 2007; Jordan and Daley, 1997).

Free-radicals are molecules, usually of oxygen, that have lost an electron, making them unstable. Among other things, they can attack enzymes and proteins, disrupting normal cell activities, or cell membranes, producing a chain reaction of destruction. Such membrane damage in the cells that line blood vessels can lead to hardening and thickening of the arteries. Free-radical attacks on collagen can cause cross-linking of protein molecules, resulting in stiffness in the tissue. The radicals associated with the tar phase are long-lived (hours to months); whereas the radicals associated with the gas-phase have a shorter life span (seconds) (Pryor and Stone 1993; Smith and Fischer 2001; Pryor et al. 1998).

Cigarette smoke includes the portion inhaled in the smoker's mouth known as the mainstream smoke and the one emitted from the burning ends of the cigarette (side stream). Active-smokers usually inhale both phases of the cigarette smoke. The mainstream cigarette smoke comprises 8% of tar and 92% of gaseous components (Pryor and Stone 1993). An active smoker is also exposed to the side stream smoke, which contains a relatively higher concentration of the toxic gaseous component than mainstream cigarette smoke (Glantz and Parmley 1991). Passive smokers inhale the mainstream smoke when the smoker exhales and the side stream phase. Second hand smoke contains the same spectrum of toxins as mainstream smoke and is thus likely to have the same spectrum of harmful effects as active smoking (Venn and Britton, 2007).

Various mechanisms have been proposed for the hazardous effects of smoking that lead to cardiovascular morbidity and related mortality. These include changes in the haemostatic factors, the endothelial function and the blood lipids, as well as alterations in the dynamic properties of the arterial wall (Kool et al. 1993; Rhee et al. 2005).

The impairment of vasodilatory function is said to be due to impairment of nitric oxide (NO). This is an endothelial derived free-radical that is primarily responsible for the vasodilatory function of the endothelium and also helps regulate inflammation, leukocyte adhesion, platelet activation, and thrombosis (Napoli and Ignarro 2001). Therefore, an alteration in NO biosynthesis could have both primary and secondary effects on the initiation of stiffening of arteries.

Arterial stiffness describes the reduced capability of an artery to expand and contract in response to pressure changes. Increased arterial stiffness (Laurent 2001) is a determinant of cardiovascular mortality contributing to cardiovascular risk. Pulse Wave Velocity (PWV) is said to be the gold standard for measuring arterial stiffness. During systole, the contraction of the left ventricle and the ejection of blood into the ascending aorta acutely dilates the aortic wall and generates a pressure wave (pulse wave) that moves along the arterial tree. The velocity of this movement is a factor of arterial compliance. Thus, PWV is noted to increase when arteries are stiffened. It is known to be an established index of arterial stiffness in adults (Chuang et al. 2005; Blacher et al. 1999; Lee et al. 2006) and increased arterial stiffness is an independent predictor of the CVD risk and morbidity (Shiotani et al. 2005).

When two pressure waves are recorded at two different sites of the vascular tree, it is possible, owing to the propagation of the waves, to measure the time delay. This pressure can be noted from an upstream pressure point such as in the carotid artery to a downstream pressure point such as the brachial, femoral or radial arteries. Since the aorta is the major component of arterial elasticity, the carotid-femoral PWV (cfPWV) offers the simplest reproducible and non-invasive evaluation of regional stiffness. This measurement allows the recording of the pulse pressure at two different sites of the aorta and the measurement of the distance between the two pressure waves.

Acute cigarette smoking has been shown to increase the PWV, suggesting that there is increased arterial stiffness in adult smokers (Hee Sun Koo et al. 2007). Previous research has established that as a result of smoking, there is a rise in heart rate, cutaneous vasoconstriction and an elevation of systemic blood pressure. In some smokers, cardiac output is increased, while in others, it is unchanged (Thomas et al. 1956). There is limited data on the effects of smoking in adolescents with respect to haemodynamic variations and arterial stiffness.

While there is documentation of these in experimental settings in the white population, there is no study that has been done in African or in Zambian adolescents. This research details the findings on the effects of smoking on the PWV of adolescent males in Zambia with an overall aim of determining the acute changes that occur in PWV and haemodynamics in male adolescents in Zambia due to smoking.

CHAPTER 2

LITERATURE REVIEW

2.1 Cardiovascular physiological effects of cigarette smoke

Cigarette smoke is said to contain over 4,000 known components, of which only a few components have been examined in isolation. It has been noted to cause an increase in arterial stiffness (Doonan et al. 2010; Kubozono et al. 2011). Increased arterial stiffness leads to vessel wall damage. Without the shock-absorbing capacity, the stiff arterial wall may be subjected to increased intraluminal stress on impact of increased pulsatile pressure (Demer 1991).

The two substances absorbed in appreciable amounts from tobacco smoke are carbon monoxide and nicotine. Previously, many of the effects of smoking were attributed to carbon monoxide. Now, acceptable evidence indicates that carbon monoxide plays little, if any, role in producing the cardiovascular effects, and this leaves nicotine as the most important agent (Roth and Shick, 1958) that causes the notable changes in the cardiovascular system. Nicotine is an alkaloid found in the leaves of tobacco plants. Nicotine is carried proximally on the tar droplets which are inhaled. Absorption of nicotine across biological membranes depends on pH. Nicotine is a weak base with a pKa of 8.0 (Fowler 1954). In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. Once the tobacco smoke reaches the small airways and alveoli, a rapid absorption of nicotine is noted due to the basic nature (pH 7.5) of alveoli fluid (Benowitz 1990). The average cigarette contains 6 - 11 mg nicotine and delivers about 1 - 3 mg nicotine systemically to the smoker (Brunten et al. 2008). Upon absorption, it reaches a blood maximum concentration after 5 - 8 minutes (Hukkanen et al. 2005; Lunell et al. 2000). Once it is in the blood, nicotine stimulates catecholamine hormones to facilitate the secretion of adrenocortical hormones, leading to vascular contraction (Kannel 1976) which increases peripheral resistance.

In most people nicotine is 70% to 80% metabolised to cotinine by C-oxidation through hydroxylation. This is aided by an enzyme known as cytochrome P450-dependent monooxygenase (CYP). It is then converted to an aldehyde and subsequently to cotinine by a cytosolic enzyme known as CYP2A6 (Yildiz 2004; Nakajima et al. 1996; Messina et al. 1997). Its high levels of concentration in the blood have been noted to decline 20 minutes from inhalation due to tissue distribution (Hukkanen et al. 2005; Lunell et al. 2000).

It has been demonstrated that nicotine is excreted through urine, faeces, bile, saliva, gastric juice, sweat, and breast fluid (Balabanova et al. 1994; Seaton et al. 1993). Nicotine disappears rapidly from the blood, with a half-life of two to three hours in humans (Health Council of the Netherlands, 2004).

Smoking in adolescence has been associated with higher aorto-iliac PWV, as well as with inflammation and endothelial dysfunction levels, independently of other adolescent and adult lifestyles. Cigarette smoking affects regional arterial stiffness by increasing the central arterial PWV (Kim et al. 2011). Hee Sun Koo et al. (2007) investigated the effects of smoking on PWV in adolescents (14 to 16 years old) and found no significant difference in PWV in smokers and non-smokers. He did, however note higher PWV values in smokers. Hee (2007) explained that the insignificant difference was due to a reduced average smoking duration in his population. The adolescents in his study had an average smoking duration period of 12.2 months. He emphasized that though the exact duration or intensity of smoking that causes chronic changes in the arterial wall properties are unknown, adolescents generally have a relatively short exposure to smoking, which is probably too short to cause a significant difference in arterial stiffness. From their findings, the assumption was that smoking in adolescents could affect arterial stiffening in a cumulative manner, which may at some point induce significant changes in arterial stiffness. Li et al. (1994) supported this hypothesis stating a dose dependent effect on arterial stiffness. Other researchers have reported significant differences in PWV in individuals before and after smoking. The aortic (carotid-femoral) PWV showed a significant increase after smoking in reports by Kool et al. (1993), who reported that smoking caused a short-term increase in arterial wall stiffness of both the elastic common carotid and the muscular brachial arteries.

Van Popele et al. (2001) noted that impaired arterial distensibility might pave a way to the development of hypertension in the long term. Indeed, increased aortic stiffness is associated with both systemic hypertension and left ventricular hypertrophy, and reduced compliance of the aorta amplifying arterial wall impairing that accompanies ageing. Francesco et al. (2006) also noted that aortic PWV is an independent predictor of coronary heart disease and stroke in apparently healthy participants. It is imperative to note that the PWV in carotid-femoral (elastic - cfPWV) part of the arterial tree is the validated marker of arterial stiffness over the central arteries and has been established as an important predictor of future cardiovascular risk (Kullo et al. 2006; Hansen et al. 2006; Tillin et al. 2007; Sugawara et al. 2005).

However, in order to include observations in changes within the muscular part of the arteries, the carotid-radial PWV (crPWV) can be observed so as to have a reflection of the peripheral muscular arterial stiffness (Sugawara et al. 2005).

In both animal and human models, several studies have demonstrated that active cigarette smoking is associated with a decrease in vasodilatory function leading to increased blood pressure and heart rate (Celermajer et al. 1993; 1996; Barua et al. 2001; Kugiyama et al. 1996; Sumida et al. 1998; Ijzerman et al. 2003; Mayhan et al. 1996; 1997; Ota et al. 1997; Barua et al. 2003; McVeigh et al. 1996). The impaired endothelium-dependent vasodilation has been noted to occur in macro vascular beds such as brachial arteries and in microvascular beds (Celermajer et al. 1993; 1996; Barua et al. 2001; Kugiyama et al. 1996). Nicotine down-regulates the expression of endothelial nitric oxide synthase (NOS), an enzyme involved in the generation of NO, which mediates vasodilation (Zhang et al. 2001). It has also been noted to up-regulate asymmetric dimethyl arginine, which would further impair the release of NO (Jiang et al. 2006). Nicotine also increases the concentration of epinephrine and norepinephrine in blood, increasing heart rate, blood pressure, and cardiac output (Smith and Fischer 2001; Benowitz 1997; Mayhan and Sharpe 1999; Li et al. 1994; Clouse et al. 2000; Pellegrini et al. 2001). This mechanism is through the stimulation of the sympathetic nervous system and adrenal medulla by nicotine increasing norepinephrine to 324 ± 39 pg/ml from 227 ± 23 pg/ml and epinephrine from 44 pg/ml to 113 ± 27 pg/ml (Cryer et al. 1976).

Studies done to validate the effects of smoking on haemodynamics are mostly on whites. In a study by Farha et al. (2011), an increase of 8 mmHg and 1.6 mmHg Systolic blood pressure (SBP) and Diastolic blood pressure (DBP), respectively, were observed in male smokers. He also noted 9.6 bpm difference in heart rate before (64 bpm) and after smoking (73.6 bpm). In a study by Vandanah (2012), pulse rate in smokers was found to be (87 ± 16.53 bpm) and (75 ± 7.82 bpm) in non-smokers, and it was statistically significant ($p = 0.0007$). SBP and DBP readings were found to be raised to near hypertension levels in smokers (140/90mm of Hg) in comparison to the non-smokers (120/70mm of Hg). Hee Sun Koo et al. (2007) reported an increase from 70.3 bpm to 76.4 bpm in adolescents between the ages of 14 to 16 years old.

In a meta-analysis of individual data obtained from one million adults, increase of 10 mmHg in SBP or 5 mmHg in DBP was found to be associated with 40% higher risk of stroke death and 30% higher risk of death from coronary heart disease.

Even increase of 2 mmHg in SBP was associated with 10% higher stroke mortality and 7% mortality from ischaemic heart disease (Lewington et al. 2002).

Several factors have been noted to influence the effects of nicotine on vascular changes. They largely depend on the clearance of nicotine which is dependent on liver blood flow and rate of metabolism. Its metabolism is slowed down by meals consumed during a steady infusion of nicotine (Lee et al. 1989; Gries et al. 1996). Nicotine's clearance is decreased in elderly (age > 65) compared with adults (Molander et al. 2001) with no difference noted in age range of 18 to 39 years old. It can also be influenced by gender with its metabolism being higher in women (Benowitz and Jacob 1984). It has been noted that nicotine metabolism is higher in blacks than whites of similar age and body weight (Perez-Stable et al. 1998; Benowitz et al. 1999).

2.2 Mechanism behind cigarette smoke effects on arterial stiffness

Arterial stiffness is a general term for the elasticity (or compliance) of the arteries. The stiffness of arteries influences how hard the heart has to work to pump blood through the body. Arterial stiffness is emerging as the most important determinant of increased systolic and pulse pressure. It is therefore, the root cause of a host of cardiovascular complications and events, including left ventricular hypertrophy and failure, and a major contributor to atherosclerotic and small vessel disease and thus to stroke. Many mechanisms are responsible for arterial stiffness caused by cigarette exposure. Due to the superoxide anions that are produced by cigarette smoke, there is a reduced production and bioavailability of NO. It increases production and release of endothelin, causing endothelial dysfunction, and generates pro-inflammatory alterations (Rahman 2007; Yufu et al. 2009; Adamopoulos et al. 2008; Celermajer et al. 1996; Woo et al. 2000).

Arterial stiffness increases acutely after cigarette smoking as measured by augmentation index (Aix), cfPWV, brachial PWV (brPWV) or crPWV (Lemogoum et al. 2006; Mahmud et al. 2003; Berlin et al. 1990; Vlachopoulos et al. 2004). In a study comparing chronic-smokers and non-smokers, at baseline, the brachial-ankle Pulse Wave Velocity (baPWV) was not significantly different. It was however, significantly higher in chronic-smokers 5 minutes after cigarette smoking and remained higher for 30 minutes ($p < 0.05$).

The acute effects of smoking were also evaluated on aortic, carotid, radial and brachial distensibility and compliance; in all studies, distensibility and compliance decreased in all arterial beds studied (Failla et al. 1997; Giannattasio et al. 1994; Zamir et al. 2006; Kool et al. 1993; Stefanadis et al. 1997; 1998). Swampillai et al. (2006) reported a significant smoking-induced increase in stiffness index (SI) 15 minutes after smoking ($p < 0.05$), but did not observe changes in wave speed in the carotid artery.

Smoking alters lipid metabolism acutely (Barnoya et al. 2005 and Chelland et al. 2008) and changes in lipid metabolism contribute to structural changes of the arterial wall including intima-media thickening and atherogenesis (Riccioni 2009; Chambless et al. 1997) and may lead to an increase in arterial stiffness. Further increase in arterial stiffness has been shown to be because of smoke induced oxidative stress, which alters vascular tone (Rahman et al. 2007; Cacciola et al. 2007). Smoking increases the production of reactive oxygen species, which decreases the activity of NOS, inhibiting NO production by the endothelium and the platelet-derived NO production also decreases contributing to a hypercoagulable state (Peluffo et al. 2009; Valavanidis et al. 2009).

Smoking has also been noted to increase blood pressure and the risk for hypertension (Minami et al. 2009; Pardell et al. 2005; Benowitz 1988; Rempher 2006). The wall tension induces mechanical vessel wall damage, vascular hypertrophy, increased collagen and calcium deposition, smooth muscle cell restructuring and extracellular matrix deposition, which leads to increases in arterial stiffness (Asmar et al. 1995; Hasegawa et al. 1997; McEniery et al. 2007; Nichols et al. 1998; Dart et al. 2001).

Catecholamine elevation has been observed in male volunteers after smoking and was associated with an increase in peripheral resistance, blood pressure and heart rate (Cryer et al. 1976). There is an increase in both circulating and local catecholamine levels caused by nicotine stimulating the sympathetic ganglia and increasing the central nervous system sympathetic neural discharge, and impaired NO production resulting in endothelial dysfunction. Increased luminal pressure due to hypertension also stimulates more collagen (Zieman et al. 2005) than elastin, which are the two prominent proteins that stabilise vascular walls.

2.3 Reversibility of acute effects of smoking

Cryer et al. (1976) noted that plasma catecholamines peaked at the end of a 10-min period of smoking and returned to baseline levels 30 minutes after the start of smoking. As the catecholamines are a major factor in dynamics of blood flow, they may be contributory to the documented changes in PWV. PWV is influenced by endothelial dysfunction which causes reduced vascular compliance. Catecholamines are said to indirectly cause endothelial dysfunction by impairing nitric oxide (NO) production and directly by facilitating peripheral vasoconstriction. This would lead to changes in blood pressure. The catecholamine surge would also cause an increase in heart rate, further exacerbating blood pressure and may precipitate myocardial ischemia in patients of coronary artery disease. As catecholamines are the cause of impaired NO production and central nervous system sympathetic discharge leading to endothelial dysfunction, PWV would return to baseline in a similar period as the catecholamines as noted in several other studies.

The brachial-ankle Pulse Wave Velocity (baPWV) is a unique measure of systemic arterial stiffness that is measured by brachial and tibial arterial wave analyses. It has been shown to return to baseline 45 minutes after smoking two cigarettes (nicotine content 3mg) (Kim et al. 2005) and 15 minutes after smoking one cigarette (nicotine content of 0.9mg) (Rhee et al. 2005).

2.4 Justification of the study

Smoking is said to be a leading cause of cardiovascular diseases in adolescents. This study investigates the acute effects of smoking on arterial stiffness and haemodynamic parameters. Arterial stiffness is an independent predictor of cardiovascular morbidity and mortality. Appreciation of these changes may help determine the magnitude of the effects that smoking has on the dynamics of blood flow and the potential to initiate cardiovascular pathology.

CHAPTER 3

AIMS AND OBJECTIVES

3.1 General objective:

To determine the acute effects of smoking on haemodynamics and arterial stiffness in male adolescents in Lusaka.

3.2 Specific objectives:

1. To determine the acute effect of smoking on the Pulse Wave Velocity and Arterial Stiffness Index in adolescents
2. To determine the acute effect of smoking on the haemodynamic indices (blood pressure and heart rate) in adolescents.

CHAPTER 4 METHODOLOGY

4.1 Study design

This observational study involved healthy young men who were active tobacco smokers. These were recruited through advertisements placed on the student notice boards within the University of Zambia Campus and also by the use of the chain – referral (snowball) sampling.

4.1.1 Selection criteria

The target population was all young, male adolescents in Lusaka. The sample population included all tobacco smoking, male adolescents between ages 18 and 25 years who were daily smokers (someone who has smoked daily during the past month). Excluded were adolescents with hypertension, diabetes mellitus, high levels of cholesterol, and respiratory diseases such as bronchitis or asthma. The participants were required to abstain from smoking prior to the protocol and to abstain from taking alcohol and coffee beverages for at least 12 hours before the study period. Written consent was obtained from each one of the participants and they were assured that denial of participation was not consequential on their academic progression and they were free to withdraw from the study at any time. The research ethics clearance was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC).

All data was stored on a trusted, password protected computer. Identification of participants was by unique research laboratory numbers and was stored as such. All measurements involved were non-invasive and were taken in a research unit specifically designed for participants comfort. All participants recruited were smokers and none of them were subjected to abnormal levels of smoking (more than what the participant usually smokes). Any abnormalities in the results were highlighted and the participants were advised to consult a clinician as appropriate.

4.1.2 Sample size

The sample size was estimated using the following formula:

$$N = (Z_{\alpha/2})^2 s^2 / d^2$$

Which considers a single subject design approach where

- N is the sample size.
- s is the standard deviation of means of right brachial-ankle pulse wave (RbaPWV) in smokers and a control group in a study on effects of smoking on PWV by Hee Sun Koo et al. 2007 study (27.2).

- d is the accuracy of estimate or how close to the true mean, which can also be noted as the difference between the means (SBP in smokers and a control group in a study on effects of smoking on PWV by Hee Sun Koo et al. 2007 study (58)).
- $Z_{\alpha/2}$ is the normal deviate for a two- tailed alternative hypothesis at a level of significance noted as 1.96.

N calculated = 18

4.2 Arterial stiffness and haemodynamics data collection

4.2.1 Preliminary data collection and screening

4.2.1.1 Baseline measurements

All consenting participants were invited to the cardiovascular laboratory at the University of Zambia, School of Medicine. They were interviewed to note socio-demographic data and health information such as age, marital status, current medications, existing pathological conditions, age at which participants started smoking, duration of smoking to date, amount of cigarettes smoked in a day, type of cigarettes smoked, alcohol consumption and time of last meal.

4.2.1.2 Anthropometric measurements

Height and weight were measured using a Micro T3 PW-BMI Digital Physician Scale. Blood pressure was measured by OMRON HEM-757 (Omron, Kyoto, Japan) blood pressure measuring machine in both standing and lying down positions.

4.2.2 Pulse Wave Velocity (PWV) readings

The participants were instructed to lie down comfortably on a surface as flat as possible with no pillow or reclining seatback. The length from the carotid artery in the neck to the femoral artery at the groin (leg) was measured using the Finger-Finder measuring tape.

The PWV was determined by the Complior Analyze Unit (V1.9 Beta Version 2013; ALAM-Medical, France) which has predefined sensors/probes for the carotid, femoral and radial pulses. In this procedure, the carotid-femoral PWV (cfPWV) and the carotid-radial PWV (crPWV) were measured. The carotid probe was gently placed on the carotid artery on the neck at the point where the strongest carotid pulse was palpable.

The participant's groin was then exposed for access to the femoral artery. The probe was applied on the femoral artery and adjusted to obtain the best signal. In cases where the femoral artery was deep, some pressure was applied and once the probes were correctly in place, pressure signals from the sensors were displayed on the screen of the Complior Analyze Unit. The values for PWV, heart rate and transit time were displayed on the screen and were then transferred on to data collection sheets. Baseline measurements were obtained for 15 minutes at 5-minute intervals and the average reading obtained for each variable was the baseline value.

4.2.3 Haemodynamic readings

The participant's chest was cleaned with alcohol solution to ensure good attachment of the ECG electrodes and, if necessary, partially shaved to help the electrodes stick. Three electrodes were placed on the chest for the detection of the QRS signal. The electrodes were then connected to the Diasys Ambulatory Blood Pressure Monitoring system (Novacor, France). The path of the brachial artery was located about 2cm above the crease of the elbow. The inside of the arm was cleaned with a clean cloth. For hygiene reasons, an adhesive strip was applied on the inside of the cuff, lining the cuff connector up to the edge of the pouch. The cuff was wound around the arm of the participant, positioning the markings on the cuff opposite the artery. The tubing was passed behind the participant's neck and the tubings were connected to the Diasys, with the Diasys already in its pouch.

When the test measurements confirmed that the microphone was working correctly, an adhesive patch was clicked onto the stabilising flap behind the air tubing and it was placed on the patient's arm to ensure that the cuff stayed in place during the entire period of observation. The Diasys was then fastened around the participant's waist and turned on to take measurements at 5-minute intervals. The monitor was worn through the entire session – before, during and after smoking.

Following baseline measurements, the participant was allowed to smoke two cigarettes (1.4 mg nicotine content each) in fifteen minutes, during which the blood pressure and heart rate were recorded at 5-minute intervals. The PWV, blood pressure and heart rate were thereafter recorded for an hour at 15-minute intervals to note the recovery period.

4.3 Data management and analysis

4.3.1 Social demographic data analysis

The means and ranges for the baseline data were obtained using STATISTIX statistical package for Windows Version 10, 2013.

4.3.2 Pulse Wave Velocity (PWV) analysis

Using the Complior Analyse Unit, the pulse transit time was determined as recorded during PWV measurement. To correct for the size of the participant, the transit time was divided by the height of the participant. The resultant value was noted as the Arterial Stiffness Index (ASI), which was expressed in meters/second as was done by Binder and the group (2008).

The mean for each variable before smoking was noted as the baseline value. The means of ASI and PWV obtained from the Complior Analyse Unit before and after smoking were compared to note for significant differences. All data was expressed as the mean \pm standard deviation from mean. Analysis of Variance (ANOVA) of repeated measures was used for the comparison of participant's parameters before and after smoking. To determine the exact points of significant difference, the Bonferroni all pairwise comparison test was used.

4.3.3 Analysis of haemodynamic parameters

The mean haemodynamics readings obtained from the Diasys for all individuals at 15-minute intervals during and after smoking were compared with their respective baseline readings to note for any significant differences using ANOVA of repeated measures. The differences in mean haemodynamic readings over time were used to determine how long it takes for the values to normalise after smoking. All analyses was done at 0.05 error. All statistical analyses were performed using the STATISTIX statistical package for Windows Version 10, 2013.

CHAPTER 5 RESULTS

5.1 Anthropometric and baseline data

Twenty-two (22) male participants were recruited for the study. The participants were all active-smokers aged between 19 and 25 years old. The anthropometric data of the study population is shown in Table 1 and the characteristics of smoking in the group are illustrated in Table 2.

Table 1. Anthropometric and baseline data for smokers

Variable	Mean	SD	Minimum	Maximum
Age (years)	20.636	2.0827	19.000	25.000
Height (m)	1.688	0.0519	1.6000	1.8100
Weight (kilograms)	61.509	10.999	49.000	92.200
BMI (kg/m²)	18.166	2.8103	14.244	25.470
cfPWV (m/s)	7.872	1.9423	4.5000	12.200
crPWV (m/s)	10.967	1.6156	7.2000	15.300
cfASI (m/s)	26.090	5.9796	17.174	39.765
crASI (m/s)	22.942	3.5181	15.094	32.947
SBP (mmHg)	113.485	13.1525	88.500	156.00
DBP (mmHg)	79.468	8.7860	60.000	116.00
Heart Rate (bpm)	74.25	13.7525	50.000	133.50

As noted in Table 2 below, among the 22 smokers, the mean number of cigarettes smoked per day was 6.9 ± 5.05 cigarettes. The group was characterised by smokers who had a total duration of smoking ranging from 12 months to 192 months. The minimum age at which individuals started smoking was 7 years and the maximum was noted as 19 years.

Table 2. Smoking parameters in study population

Variable	Mean	SD	Minimum	Maximum
No. of cigarettes smoked per day	6.864	5.0455	1	20
Duration of smoking (months)	71.455	44.9730	12	192
Starting age of smoking (years)	14.681	3.0140	7	19

5.2 Acute effects of smoking on the Pulse Wave Velocity

5.2.1 Effects of smoking on the cfPWV

The mean baseline cfPWV was 7.9 ± 1.94 m/s. The values were noted to increase after smoking to 8.5 ± 1.87 m/s. The cfPWV rapidly decreased over time with values recorded lower than the recordings obtained at baseline. There were statistical differences in cfPWV observed at the 45th minute (7.7 ± 1.49 m/s) and that observed immediately after smoking (8.5 ± 1.87 m/s) ($p < 0.05$) as shown in figure 1. There was also a significant difference noted between the peak cfPWV value noted immediately after smoking and that observed at the 60th minute (7.7 ± 1.56 m/s). It is notable that in this study, PWV had reverted to baseline readings by the 15th minute after cessation of smoking. The means for cfPWV during the periods of observation are as summarized in Table 3.

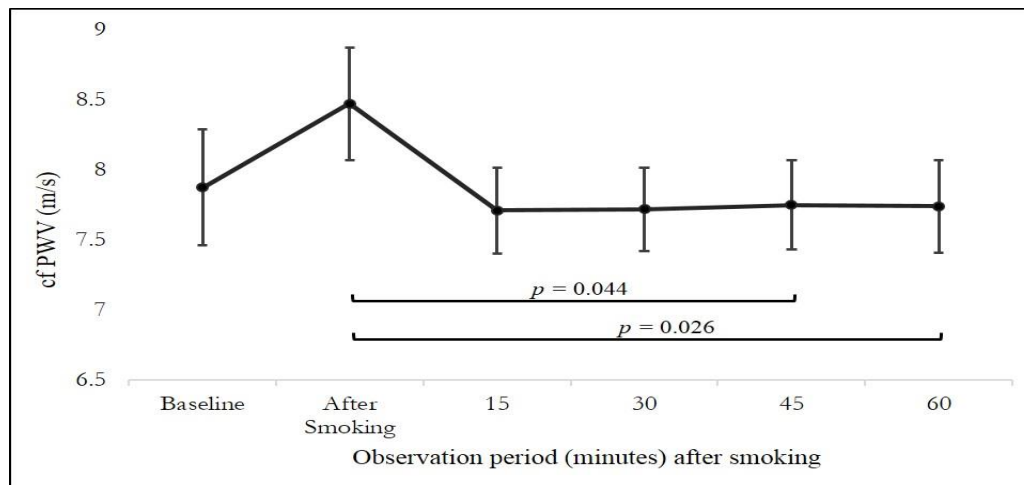


Figure 1. Mean cfPWV (m/s) before and after smoking showing statistical significance of mean differences ($p < 0.05$). Bars represent the standard deviation.

5.2.2 Effects of smoking on the crPWV

The mean baseline crPWV was noted as 11.0 ± 1.62 m/s. It increased immediately after smoking to 11.5 ± 1.75 m/s as depicted in figure 2. By the 45th minute of recovery, crPWV (10.9 ± 1.31 m/s) showed a significant difference compared to the mean crPWV noted immediately after smoking ($p < 0.05$). The crPWV noted at the 60th minute of recovery (10.3 ± 1.53 m/s) was also significantly lower than the peak mean value noted ($p < 0.05$). Other statistical differences were noted as shown in figure 2 below. The means for crPWV during the periods of observation are as summarized in Table 3.

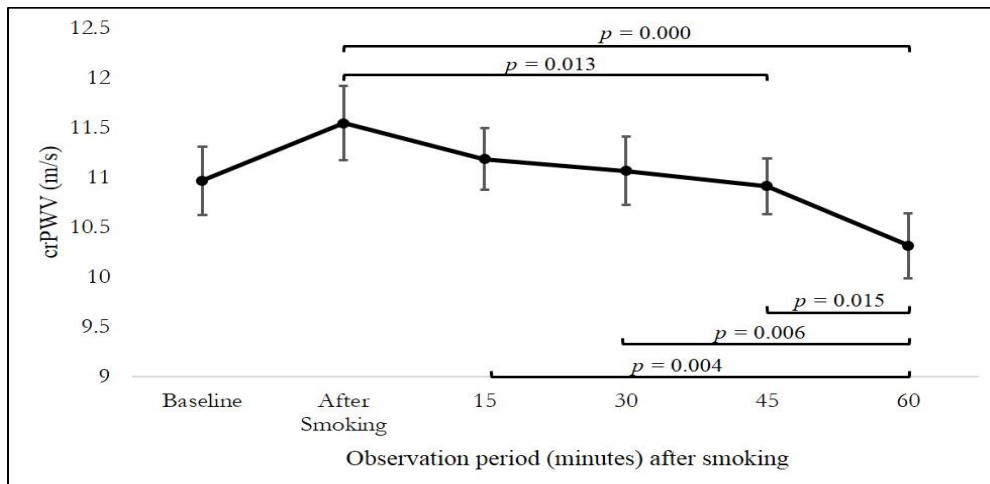


Figure 2. Mean crPWV (m/s) before and after smoking with statistical significance of mean differences ($p < 0.05$) shown. Bars represent the standard deviation.

5.3 Acute effects of smoking on the Arterial Stiffness Index

5.3.1 Effects of smoking on the cfASI

The mean baseline cfASI was 26.1 ± 6.0 m/s. This increased to 28.6 ± 6.19 m/s immediately after smoking ($p > 0.05$). The cfASI decreased significantly over time to values lower than those noted before smoking. The lowest cfASI was noted at the 45th minute (26.1 ± 3.90 m/s) post-smoking. The cfASI value had actually returned to baseline value by the 15th minute. The means for cfASI for each period of observation are summarized in Table 3.

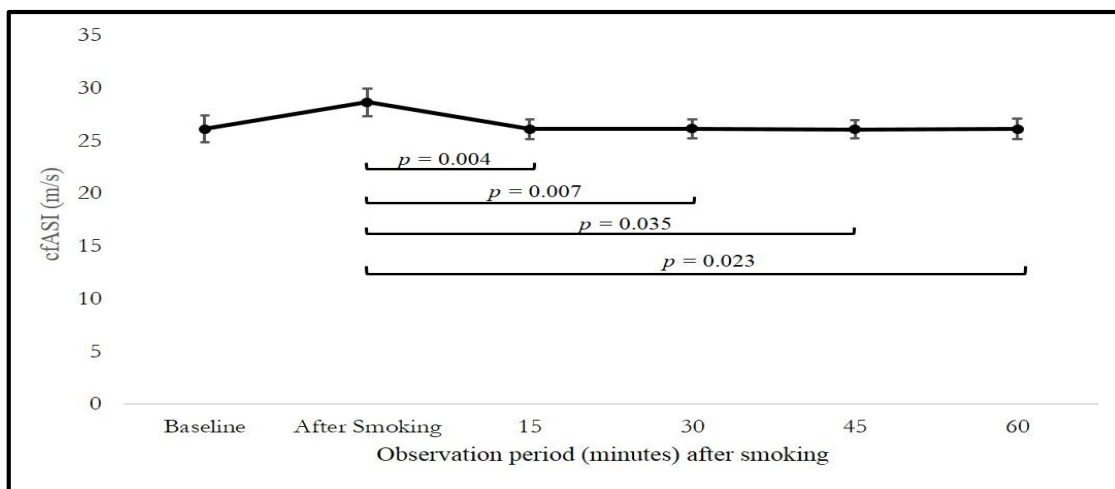


Figure 3. Mean cfASI (m/s) before and after smoking with statistical significance of mean differences ($p < 0.05$) shown. Bars represent the standard deviation.

5.3.2 Effects of smoking on the crASI

The baseline reading for crASI was 22.9 ± 3.52 m/s and it increased after smoking. The values obtained immediately after smoking of 24.3 ± 3.53 m/s, were not statistically different from those observed at baseline. A significant difference was noted between the highest crASI obtained and that noted 45 minutes (22.8 ± 2.72 m/s) ($p < 0.05$) and 60 minutes (21.7 ± 3.01 m/s) ($p < 0.05$) after smoking, as depicted in figure 4 below. The crASI values had reached baseline values 15 minutes after smoking. There were statistical differences between the lowest crASI noted at the 60th minute and that observed from the 15th to the 45th minute of recovery. The means for crASI during the periods of observation are as summarized in Table 3.

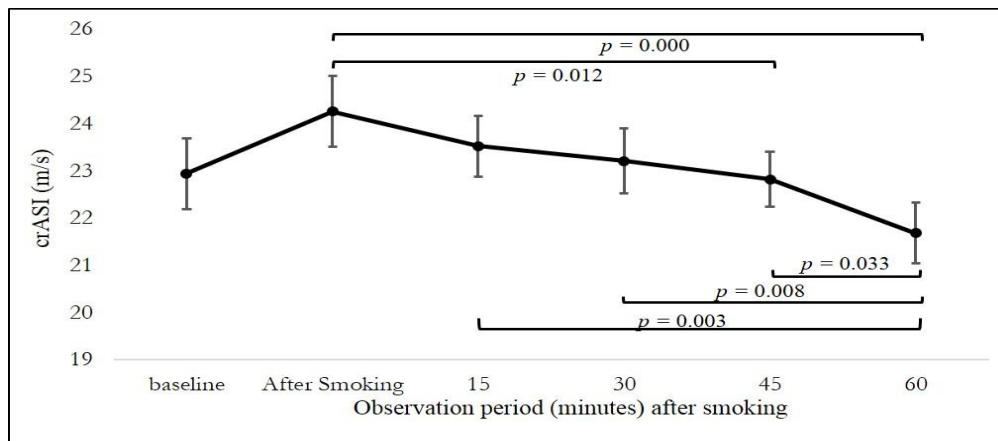


Figure 4. Mean crASI (m/s) before and after smoking with statistical significance of mean differences ($p < 0.05$) shown. Bars represent the standard deviation.

Table 3. Summary of mean PWV (m/s) and ASI (m/s) over period of observation

Variable	Baseline	0 MAS	15 MAS	30 MAS	45 MAS	60 MAS
cfPWV (m/s)	7.8718 ± 1.9423	8.4682 ± 1.8730	7.7091 ± 1.4362	7.7159 ± 1.4027	7.7455 ± 1.4902	7.7364 ± 1.5586
crPWV (m/s)	10.967 ± 1.6156	11.545 ± 1.7549	11.186 ± 1.4390	11.068 ± 1.5939	10.914 ± 1.3192	10.314 ± 1.5301
cfASI (m/s)	26.090 ± 5.9796	28.643 ± 6.1908	26.097 ± 4.4972	26.135 ± 4.1877	26.087 ± 3.8961	26.111 ± 4.5292
crASI (m/s)	22.942 ± 3.5180	24.254 ± 3.5342	23.521 ± 3.0324	23.208 ± 3.2420	22.819 ± 2.7248	21.678 ± 3.0129

*MAS – minutes after smoking

5.4 Acute effect of smoking on the haemodynamic indices (blood pressure and heart rate)

5.4.1 Effects of smoking on the systolic blood pressure

There was a significant rise in SBP (mmHg) during smoking (127.9 ± 13.80 mmHg) from baseline values (113.5 ± 13.15 mmHg) ($p < 0.05$) as shown in figure 5 below. The increased values were significantly higher than the baseline values (Bonferroni pairwise comparison test)

until 30 minutes after smoking cessation. The means for SBP (mmHg) during the periods of observation are as summarized in Table 4.

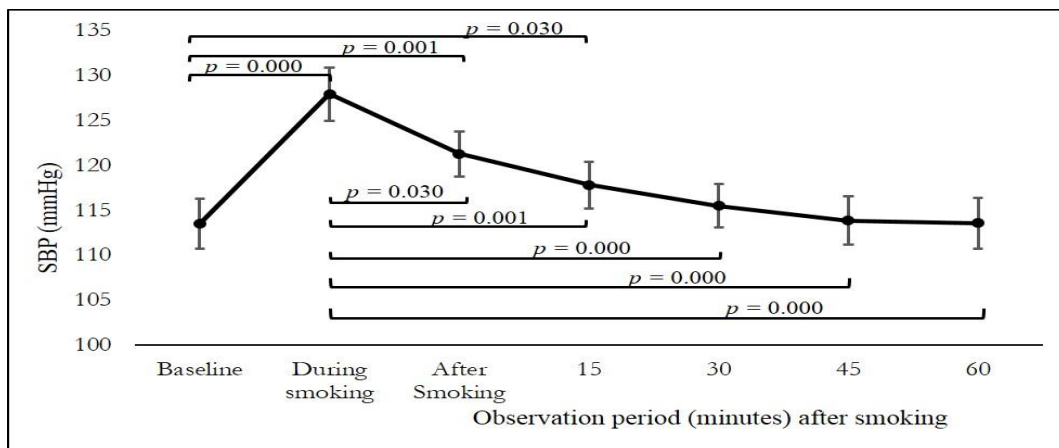


Figure 5. Mean SBP (mmHg) before during and after smoking with statistical significance of mean differences ($p < 0.05$) shown. Bars represent the standard deviation.

5.4.2 Effects of smoking on the diastolic blood pressure

DBP (mmHg) increased from baseline (79.5 ± 8.79 mmHg) to 85.6 ± 10.92 mmHg during smoking ($p < 0.05$) as shown in figure 6 below. Immediately after smoking, the means obtained were noted to be of no significant difference with those obtained at baseline when tested with the Bonferroni comparison test indicating a return to baseline. The trend of the values noted in the period of observation are as shown in figure 6 and the means for DBP (mmHg) are as summarized in Table 4.

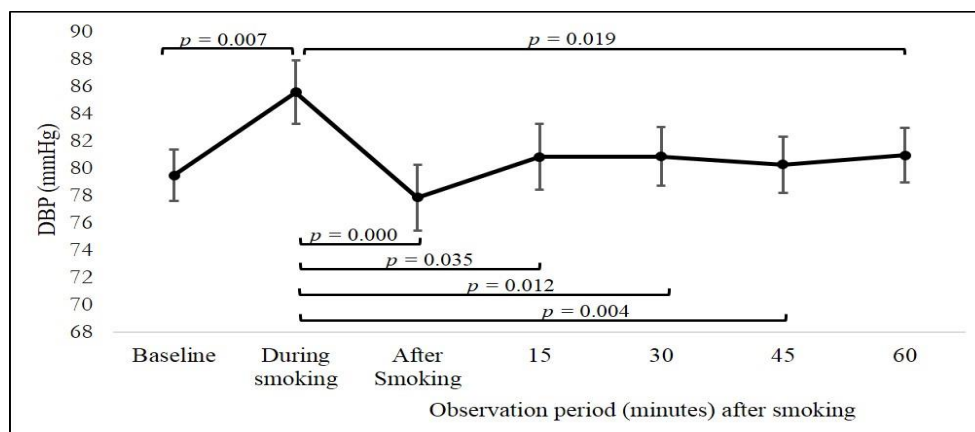


Figure 6. Mean DBP (mmHg) before, during and after smoking with statistical significance of mean differences ($p < 0.05$) shown. Bars represent the standard deviation.

5.4.3 Effects of smoking on the heart rate

Heart rate (bpm) was also noted to significantly increase during smoking (95.2 ± 16.72 bpm) from the values noted before smoking (74.3 ± 13.75 bpm) ($p < 0.05$). There was a significant drop immediately after the participants stopped smoking to 81.6 ± 15.0 bpm ($p < 0.05$) as

shown in figure 7 below. The mean value for heart rate returned to baseline value by the 15th minute of recovery. The means observed during the periods are summarized in Table 4.

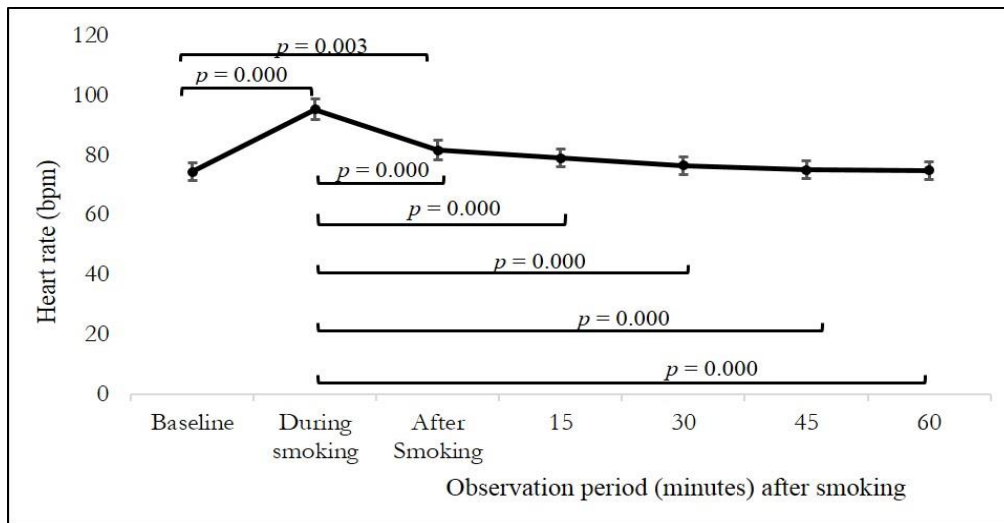


Figure 7. Mean Heart Rate (bpm) before, during and after smoking with statistical significance of mean differences ($p < 0.05$) shown. Bars represent the standard deviation.

Table 4. Summary of mean haemodynamic values over period of observation

Variable	Baseline	During smoking	0 MAS	15 MAS	30 MAS	45 MAS	60 MAS
SBP (mmHg)	113.49 ± 13.152	127.89 ± 13.800	121.23 ± 11.682	117.77 ± 12.004	115.45 ± 11.304	113.82 ± 12.648	113.55 ± 13.132
DBP (mmHg)	79.468 ± 8.7860	85.568 ± 10.9178	77.864 ± 11.3107	80.818 ± 11.2827	80.864 ± 10.1432	80.273 ± 9.6223	80.955 ± 9.3680
HR (bpm)	74.250 ± 13.7525	95.227 ± 16.7195	81.591 ± 14.9846	78.818 ± 13.8792	76.364 ± 14.1977	74.909 ± 13.7803	74.773 ± 14.2693

*MAS – minutes after smoking

CHAPTER 6 DISCUSSION

6.1 Anthropometric and baseline data

Adolescents are an emerging priority population for the tobacco industry which seems to be oblivious to the harms caused by promoting tobacco addiction. As earlier defined in this study, adolescence is the period between the ages 12 to 25 years (Woollaston, 2013) and includes three stages, early adolescence ranging from 12 to 14 years, middle adolescence from 15 to 17 years and 18 to 25 years as late adolescence. Considering the importance of late adolescence in human growth and development, this study focused on individuals aged 18 to 25 years.

A total of twenty-two (22) participants were recruited to participate in this study. All participants were active-smokers and eleven of them (50%) smoked 5 or more cigarettes per day. This high percentage of participants who smoked 5 or more cigarettes is unlike that reported by Siziya et al. (2002) that showed only 11% of adolescents smoked more than five cigarettes daily. However, Siziya conducted his study on a larger sample than in this study. Aside the statistical bias that may arise from the smaller sample size in this study, the greater prevalence of adolescents smoking more cigarettes may be attributed to socio-demographic factors, such as socioeconomic status of the selected participants and developmental challenges associated with adolescence. Environmental factors such as acceptability and availability of tobacco products, interpersonal variables and perceived environmental variables may also play a role in the increase in number of smokers who smoke more cigarettes daily.

The average age at which the participants started smoking was 14 years, with some reporting that they started smoking at 7 years of age. They were all normotensive, with a mean blood pressure of 118/81 mmHg and none of the participants were overweight or obese.

6.1.1 *Baseline values for Pulse Wave Velocity (PWV)*

At baseline, the mean cfPWV and crPWV were 7.9 ± 1.94 m/s and 11.0 ± 1.62 m/s, respectively. These values were higher than those noted by Lemogoum et al. (2006) (cfPWV of 6 ± 1.6 m/s and crPWV of 8.1 ± 1.4 m/s) in black adolescents who smoked.

Lemogoum's study population included both males and females. This could have potentially contributed to the lower PWV values observed in Lemogoum's study as females have lower

PWV compared to males (Doonan et al. 2012). This difference may also be a result of the increased frequency of smoking in this study population, causing a significant stiffening of the arteries.

Kingwell et al. (1997) observed a lower mean PWV value (cfPWV of 6.2 m/s) in healthy, non-smoking, white young-adults compared to this study (7.9 ± 1.94 m/s). A lower mean crPWV (5.69 ± 0.11 m/s) was also noted by Doonan et al. (2009) in non-smokers compared to the baseline value obtained in this study (11.0 ± 1.62 m/s). The increased mean PWV noted in the current study could be due to the elastic and muscular arteries being hardened as a result of smoking. Levenson et al. (1987) and Jatoi et al. (2007) observed that healthy non-smokers had significantly lower baseline mean PWV values compared to healthy smokers. They consequently postulated that chronic smoking increased arterial stiffness, and might be an underlying mechanism for the increased cardiovascular events observed in hypertensive patients. There is therefore an increased risk of hypertension and cardiovascular related diseases among the actively smoking adolescent population in Zambia.

Empirical evidence suggests that whites have a lower baseline PWV compared to blacks (Lemogoum et al. 2006). According to Schutte et al. (2011) and Morris et al. (2013), blacks have arterial stiffness that could already be elevated from earlier years of life, leaving them susceptible to an increased baseline PWV due to the influence of smoking. Lemogoum et al. (2006) attributed these differences to a greater presence of free-radicals noted in blacks after cigarette smoking compared to whites. This may be a contributing factor to enhancing the PWV response to smoking in blacks.

6.1.2 Baseline values for haemodynamics

Notable changes in baseline heart rate, systolic and diastolic blood pressures have been reported in candidates who smoke tobacco. The baseline SBP noted in this study was 113.5 ± 13.15 mmHg. It was lower than the SBP noted by Mushabati et al. (2015), who reported an SBP of 123 ± 9.7 mmHg. The difference could be due to the fact that Mushabati's population included smokers as well, hence giving a higher baseline SBP reading.

Mushabati's population also included older subjects (42 ± 8.7 years) compared to the current study. With age, the larger elastic arteries increase in collagen content, elastin fracture, and calcification and reduction in the elastin content. There are also changes in endothelial function,

wall thickness media to lumen ratio, and arterial stiffness with ageing. These lead to an increase in SBP (Rodriguez et al. 1994) and may explain the comparatively lower SBP values noted in this study.

The baseline DBP (79.5 ± 8.9 mmHg) in this study was similar to the values noted in Lemogoum's study (79 ± 4 mmHg) and that observed by Mushabati et al. (2015) in a normotensive Zambian population (79 ± 7.1 mmHg). Lower DBP values have been observed in healthy non-smokers by Vandanah (2012), who observed a mean value of 70 mmHg. The higher DBP noted in smokers can be mostly attributed to the nicotine mediated activation of the sympathetic nervous system with local and systemic release of catecholamines and, possibly, the release of vasopressin (Grassi et al. 1994; Cryer et al. 1976; Robertson et al. 1988). These cause vasoconstriction that increase peripheral resistance.

In the current study, the baseline heart rate was 74.3 ± 13.75 bpm. This was similar to the baseline heart rate noted in black adolescent smokers by Lemogoum (2006), who recorded a mean value of 70 ± 10 bpm. In a study by Papathanasiou et al. (2012) in male adolescents, the resting heart rate was higher in the smokers (72.8 ± 6.1 bpm) compared to non-smokers (66.3 ± 6.1 bpm). In healthy non-smokers, heart rate values observed by Farha et al. (2011) were lower (64 bpm) than those observed in this study. Smoking is associated with autonomic dysfunctions and with selective alterations in cardiac autonomic control. More specifically, smoking, acting at peripheral sympathetic sites, increases circulating levels of catecholamines, augments sympathetic outflow, and causes a long-term reduction in vagal drive (Hayano et al. 1990). This sympathetic predominance, seen even in young heavy smokers, is also associated with impaired baroreflex function, leading to a marked increase in baseline heart rate.

6.2 Acute effects of smoking on arterial stiffness

6.2.1 *Acute effects of smoking on the Pulse Wave Velocity (PWV)*

Arterial stiffness is measured by the rate of transmission of the arterial pulse pressure wave from the carotid artery, an upstream pressure point, to some defined downstream pressure points (Pulse Wave Velocity). The downstream pressure points used in this study were the femoral and radial arteries to obtain the cfPWV and crPWV, respectively.

The carotid-femoral PWV (cfPWV) offers the simplest reproducible and non-invasive evaluation of regional arterial stiffness as the aorta is the major component of arterial elasticity while the carotid-radial PWV (crPWV) reflects the stiffness noted in the muscular arteries.

Pulse Wave Velocity (PWV) is defined as the speed at which the pulse wave travels on an arterial segment, (Complior Analyse Operator Manual, 2013) and is a measure of arterial stiffness; that is, the higher the PWV, the higher the arterial stiffness. Among other factors such as haemodynamic stress and lipid abnormalities, cigarette smoke has been noted to cause injury to the endothelium, significantly affecting the arterial compliance. Endothelial damage is a central feature in the evolution of vascular disease induced by smoking. Factors that continuously injure the intima may result in the formation of fibro-fatty plaques which cause endothelial dysfunction. Furthermore, the inhalation of cigarette smoke results in the activation of the adrenergic mechanism. The nicotine contained in tobacco stimulates the sympathetic nerve terminals, with consequent systemic release of epinephrine and norepinephrine. The released catecholamines bind to α_1 -adrenergic receptors on the vascular smooth muscle to cause muscle contractions and a reduction in arterial distensibility and vasoconstriction (Mahmud et al. 2003 and Failla et al. 1997) exacerbating endothelial dysfunction and thus increasing the PWV.

In this study, smoking 2 cigarettes caused a significant rise in both the cfPWV and crPWV. The initial rise from baseline was insignificant in both PWV measurements ($p > 0.05$). The values for cfPWV significantly reduced by the 45th minute 10.3 ± 1.53 m/s. The values for crPWV significantly reduced from those noted immediately after smoking by the 60th minute (7.7 ± 1.56 m/s). The significant reduction in PWV noted during the recovery period could be due to these individuals having an exaggerated PWV at baseline as they anticipated the investigation and it returned to their true baseline after the exercise. This implies that there was indeed a significant increase due to smoking from their true baseline PWV values. It has been shown that anxiety can indeed increase PWV (Cicek et al. 2012; Yeragani et al. 2006) explaining why the baseline values were higher than those observed a while after smoking cessation. These observations were in line with the findings of Ozgur (2009), who also showed that smoking 2 cigarettes (2.8mg nicotine) caused significant changes in cfPWV in healthy smokers.

The cfPWV in this study increased by 7.6% ($p < 0.05$) from a baseline value of 7.9 ± 1.6 m/s to 8.5 ± 1.9 m/s immediately after smoking and crPWV significantly increased from 11.0 ± 1.61 m/s to 11.5 ± 1.8 m/s (5% increment, $p < 0.05$). These observations were similar to those noted by Lemogoum et al. (2006), who had an increase in both cfPWV and crPWV in adolescent smokers (blacks and whites) after smoking. He noted a significant increase in cfPWV from 5.9 ± 1.7 m/s to 6.5 ± 1.5 m/s (39% increment, $p < 0.05$) after 5 minutes of smoking and 11% increase in crPWV from 8.2 ± 1.3 m/s to 9.1 ± 1.4 m/s ($p < 0.05$). The percentage increase in PWV was lower in this study compared to the increase noted by Lemogoum et al. (2006). This could be due to the higher baseline PWV noted in the current study.

Other studies showing significant increase in PWV due to smoking include Vachapoulos et al. (2004), Mahmud et al. (2003) and Kool et al. (1993). The latter observed that smoking caused a short-term increase in arterial wall stiffness of both the elastic common carotid and the muscular brachial arteries. They postulated that such increase could be due to acute effects on the endothelial function (Kim et al. 2011). Therefore the increase in PWV observed in this study suggests that there is an increase in arterial stiffness after acute smoking in the black, male adolescents.

The mean PWV was noted to return to baseline 15 minutes after the cessation of smoking. This is similar to the findings of Cryer et al. (1976), who also reported a return to baseline of the PWV in 15 minutes. They also reported that plasma catecholamines were maximal at the end of a 10-minute period of smoking after which there was a decline. Catecholamines are said to cause impaired nitric oxide (NO) production and central nervous system sympathetic discharge, leading to endothelial dysfunction. PWV would therefore return to normal after the catecholamines have been cleared in the blood (Lemogoum et al. 2006).

Other studies have shown a longer period of recovery of up to 45 minutes after smoking 2 cigarettes (nicotine content 3mg) (Kim et al. 2005) and a recovery of 15 minutes after smoking 1 cigarette (nicotine content of 0.9mg) (Rhee et al. 2005). Both authors observed the recovery period of PWV in normotensive smokers. Their populations of Asians consisted of comparatively older males. In this study, nicotine content of 2.8 mg was used and it took 15 minutes for PWV in the elastic arteries (cfPWV) to return to baseline. The quick recovery noted

in this study could be explained by the faster metabolism of nicotine noted in blacks compared to the Asians (Benowitz et al. 2002).

It has been observed that Asian male smokers have higher plasma nicotine levels after overnight tobacco abstinence compared with other males (Muranaka et al. 1988). This could be the reason for the longer time taken for both Kim and Rhee's population to revert to baseline. Age is another factor that could have added to the difference noted in the recovery time. Clearance of nicotine decreases with age due to reduced liver blood metabolism (Messina et al. 1997).

The crPWV showed a significant difference between its lowest value 60 minutes post-smoking (10.3 ± 1.53 m/s) and that observed at 45 minutes post-smoking. In this study, the latter observation was noted as the true crPWV baseline. It can be deduced from this that it takes more than 45 minutes for muscular arteries (crPWV) to revert to normal PWV. This is similar to what Rhee et al. (2005) observed in his hypertensive, Asian male smokers. He reported that PWV did not return to baseline in the 15 minutes of observation after smoking one cigarette with 0.9 mg of nicotine. This increased time with elevated PWV places this population at a higher risk of hypertension.

6.2.2 Acute effects of smoking on the Arterial Stiffness Index (ASI)

The pulse wave is influenced by several factors, such as age, sex and body height. The ASI corrects for a subjects height and can therefore better determine levels of arterial stiffness (Woodman et al. 2003). In this study, cfASI and crASI increased insignificantly after smoking from the baseline values of 26.1 ± 6.0 m/s and 22.9 ± 3.52 to 28.6 ± 6.20 m/s and 24.3 ± 3.53 m/s respectively. The study shows a significantly decreased cfASI at the 45th minute (26.1 ± 3.90 m/s) when compared to the peak value noted immediately after smoking (28.6 ± 6.19 m/s). This implies an exaggerated initial baseline value obtained. Using the values obtained 45 minutes post-smoking as the true baseline value, it can be inferred that there was indeed a significant increase in cfASI due to smoking.

The ASI in the elastic arteries (cfASI) returned to baseline 15 minutes after cessation of smoking. A similar observation was made in the values obtained for crASI (muscular arteries), whose true baseline value was noted at the 60th minute of recovery time. This also suggests that there was a significant increase in crASI in the participants due to smoking. The values

observed during recovery were significantly higher than the true baseline value (21.7 ± 3.01 m/s - crASI at 60 minutes).

This implies that ASI of muscular arteries may take longer than 45 minutes to revert to baseline. Prabha (2013) detected a significant increase in ASI in smokers of more than double that noted in non-smokers. The increase in ASI values noted after smoking are indicative of large artery stiffness (Van Schooten et al. 1998; Woodman et al. 2003).

6.3 Acute effects of smoking on the haemodynamic indices (blood pressure and heart rate)

6.3.1 *Acute effects of smoking on the systolic blood pressure*

The SBP increased during smoking to 127.9 ± 13.80 mmHg from the baseline mean of 113.5 ± 13.15 mmHg (corresponding to a 13% increment, $p < 0.05$). The increase noted in this study was higher than SBP increase reported in white smokers by Rhee et al. (2005). He observed an increase in SBP of 4.1% from 121.2 ± 1.8 mmHg to 126.2 ± 1.6 mmHg ($p < 0.05$) after 5 minutes of smoking. This difference in increment between the two studies could be due to the difference in race in the two populations. Nicotine metabolism via N-glucuronidation and cotinine pathway, which is known to be mediated primarily by cytochrome P-450 2A6 (Nakajima et al. 1996), is slower in blacks compared to whites (Benowitz et al. 1999). As a result, black smokers have a higher plasma cotinine concentration at all levels of cigarette smoking (Caraballo et al. 1998) and have lower cotinine clearance (Benowitz et al. 1999). The black smokers are therefore prone to a higher increase in blood pressure compared to their white counterparts. Smokers have been observed to have higher SBP compared to non-smokers after smoking the same amount of nicotine (Vandanah et al. 2012). He observed that SBP in smokers increased to near hypertensive values (140 mmHg) after smoking and that of non-smokers only increased to normotensive values (120 mmHg) after smoking.

The acute increase in SBP in smokers is said to be due to greater contractility of the heart caused by increased sympathetic discharge during smoking. Enhanced contractility causes an increase in the volume of blood pumped by the heart, causing greater cardiac output. This increased cardiac output causes an increase in blood being pushed into the arterial tree, thus elevating the systolic pressure. The increase in heart rate also increases the cardiac output, elevating the SBP. The increase in SBP as observed in this study, according to Lewington (2002), can be associated with more than 40% greater risk of stroke and more than 30% greater

risk of death from coronary heart disease. The author further postulated that the observed increase in SBP could be associated with more than 10% and 7% greater risk of stroke mortality and ischaemic heart disease, respectively.

SBP values in this study were noted to return to baseline 30 minutes after cessation of smoking. The average time for SBP to revert to baseline, as noted by Lemogoum et al. (2006), was 30 minutes from the start of smoking. This was noted after 5 minutes of smoking cigarettes containing a total of 1.2 mg of nicotine. The slightly longer period of recovery in this study could be due to a higher amount of nicotine taken (2.8 mg in 15 minutes). According to Hukkanen et al. 2005, it takes 5 to 8 minutes for the inhaled smoke to be absorbed and reach blood maximum concentration. Extending the time of smoking would give increased time for absorption of the 2.8 mg of nicotine and hence increased recovery time. According to Benowitz et al. (1982), smoking represents a multiple dosing situation with considerable accumulation while smoking. The longer period of recovery of SBP in this population places these adolescents at a risk of cerebro-vascular injury, considering increased SBP is an index for stroke (Lewington et al. 2002).

6.3.2 Acute effects of smoking on the diastolic blood pressure

The mean DBP increased significantly during smoking to 85.6 ± 10.92 mmHg from a baseline mean of 79.5 ± 8.79 mmHg (corresponding to an 8% increment, $p < 0.05$). Lemogoum et al. (2006) reported a 5% increase in DBP in adolescent smokers from a baseline of 79 ± 5 mmHg to 83 ± 9 mmHg ($p < 0.05$) 5 minutes after smoking. The higher values observed in this study could be due to Lemogoum's population comprising both males and females. The latter generally have a lower mean DBP compared to males, hence lowering the average DBP values observed. Kim et al. (2005) observed a 5.9% increase in DBP in white smokers from a baseline of 68.2 mmHg to 72.7 mmHg ($p < 0.05$) after 5 minutes of smoking. It has been noted that whites have lower DBP values compared to blacks of African descent (Harding et al. 2009). This places the black smokers at a higher risk of the effects of smoking.

The increase in DBP in smokers is due to greater peripheral resistance being caused by an increase in sympathetic stimulation. Nicotine and other products in cigarettes cause blood vessels to constrict. This, in turn, increases the total peripheral resistance, which traps more blood as stressed volume in the arteries, increasing the pressure. Injury to blood vessel walls and speeding up of hardening of arteries lead to an increase in the peripheral resistance and this

elevates the diastolic pressure. The increase in DBP observed in this study is speculated to increase the chance of death from coronary heart disease by 30% (Lewington et al. 2002).

DBP in this study returned to baseline immediately after smoking from a peak value of 95.3 ± 16.72 bpm during smoking. This was a shorter period when compared to Rhee (2005), who noted more than 15 minutes as the time required for DBP to return to baseline after smoking. The population in Rhee's study had smokers with an average age of 39 years. Rhee's population was characterised by individuals who has been smoking for a longer time compared to the current study population. This could explain why the current study had a shorter time to revert to baseline even though they had a higher DBP than Rhee's population which had a DBP of 80.8 ± 1.2 mmHg 5 minutes after smoking. This entails that with increased duration of smoking, time to revert to normal blood pressure also increases. This could signify an increased arterial stiffness due to chronic smoking.

6.3.3 *Acute effects of smoking on the heart rate*

There was a significant increase in heart rate noted in this study from 74.3 ± 13.75 bpm at baseline to 95.2 ± 16.72 bpm during smoking (28% increment, $p < 0.05$). In this study, a comparatively higher increase in heart rate was noted when compared to Rhee et al. (2005), who observed a 22% increase in heart rate from 60.6 ± 1.6 bpm to 73.4 ± 2.0 bpm ($p < 0.05$). Farha et al. (2011) also noted a rise in heart rate in smokers from a baseline of 69.7 bpm to 76 bpm after smoking (15% increment, $p < 0.05$). Both Rhee and Farha had observed these changes in whites, Farha's population had both males and females. These differences could have contributed to the lower heart rate values in these studies compared to the current study and the comparatively lower increment due to effect of smoking.

Increased heart rate is due to nicotine that stimulates release of endogenous adrenergic neurotransmitters (Richard A, 1985). Within minutes of cigarette smoking, nicotine receptors in adrenal medulla are stimulated, triggering the release of epinephrine and norepinephrine i.e. smoking appears to induce increased sympathetic discharge and leads to an increase in plasma levels of the adreno-medullary hormones (McKenna et al. 1980; Levine P, 1973). Grassi in 1994 showed that the sympathetic activation induced by smoking depends on an increased release or a reduced clearance of catecholamine at the neuro-effector junctions. The heart rate in this study reverted to baseline in 15 minutes. This is in line with Rhee et al. (2005) and Kim et al. (2005), who also noted a 15 minute recovery period in their populations.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrates that smoking causes acute increases in arterial stiffness, SBP, DBP and heart rate in adolescent smokers. This group of individuals, highly targeted by tobacco companies, is therefore at an increased CVD risk, including myocardial infarction, heart failure, and mortality, as well as stroke, dementia, and renal disease in later life (Chae et al. 1999). This study has demonstrated that smoking has a significant increased effect on arterial stiffness and haemodynamic parameters.

PWV and ASI increased significantly in the adolescents due to smoking. The increased effect of PWV and ASI lasted for 15 minutes in elastic arteries (cfPWV and cfASI) and over 45 minutes in the muscular arteries (crPWV and crASI). Smoking is therefore a risk factor for high blood pressure and cerebro-vascular pathology.

Smoking caused a significant increase in both SBP and DBP which are indices for stroke and coronary heart disease respectively. The effect of increased SBP was noted to last for 30 minutes while DBP returned to baseline immediately after smoking. A significant increase in heart rate was also noted in the study. This is a factor for coronary artery disease. It stayed elevated for 15 minutes.

In order to reduce the risk of CVD related to increase in these parameters, awareness among this age group should be considered as a primary goal for both health practitioners and policy makers. As the methods used in this study are simple and reproducible, parameters such as cfPWV, crPWV may be a useful tool to assess the effect of smoking and also for screening the risk of CVD. This method can also be used to determine the extent of arterial stiffness in other vulnerable groups, long term smokers and those that have quit smoking. Considering the effects of certain confounders that might alter the blood nicotine concentration, like relation to puff volume and depth of inhalation, rate of puffing and type, a large sample-sized study would increase the power of analysis giving more insight into the acute non-physiologic hemodynamic changes of smoking.

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APPENDICES

Appendix A: Measurable parameters in vascular physiology

Arterial stiffness

Arterial stiffness describes the reduced capability of an artery to expand and contract in response to pressure changes. Parameters that describe vessel stiffness include compliance and distensibility. Compliance (C) is a measure of volume change (ΔV) in response to a change in blood pressure (ΔP ; $C = \Delta V / \Delta P$). In a stiff vessel the volume change, and therefore compliance, is reduced for any given pressure change. Distensibility is the relative change in diameter with pressure; compliance is the absolute change in diameter (or volume) with pressure.

Pulse wave velocity

Pulse Wave Velocity (PWV), the speed at which the pulse wave travels on an arterial segment, (Complior Analyse operator manual, 2013), is considered the “gold standard” in the measurement of arterial stiffness. This is due to its simple, non-invasive and reproducible nature.

The consequence of reduced compliance/distensibility is increased propagation velocity of the pressure pulse along the arterial tree, called Pulse Wave Velocity (PWV). PWV is calculated by measuring time taken for a pressure pulse to travel between two set points. Commonly used points are the carotid and femoral artery because they are superficial and easy to access.

Arterial Stiffness Index

The Arterial Stiffness Index (ASI) is a measure of the loss of elasticity in the arteries that occurs with onset of vascular disease and advancing age. It is a number that correlates with conditions in which fatty material collects along the walls of arteries. This fatty material thickens, hardens (forms calcium deposits) and may eventually block the arteries (Healthfair 2014). There is pronounced increase in ASI in cases of damaged vascular endothelium due to arterial stiffening (Prabha et al. 2013).

In order to determine the ASI, a patients Height / transit time [Transit time \rightarrow Time delay between systolic peak & Diastolic peak] is used in the analysis. The time delay between systolic peak & diastolic peak is called Pulse transit time (PTT or ΔT), is inversely proportional to arterial stiffness (Prabha et al. 2013).

Appendix B: Information sheet

You are invited to join a research study to investigate the effect of smoking on adolescents. Our aim is to find out if smoking has an effect on the state of your arteries by measuring your blood flow. The decision to join, or not to join, is up to you. If you decide to participate, you will be asked to have parameters that will determine the state of your arteries and how your blood is flowing taken. We think this will take about 3 hours.

The procedure will include getting general information about you, the participant. Blood pressure will be measured whilst in standing and lying down position and blood flow measurements will be obtained by placing probes on your neck, arm and leg. The probes may be slightly uncomfortable but non-invasive. A Dyasis machine will be worn throughout the observation period to obtain 15-minute measurements of blood flow variables. The machine will be attached to areas of your chest, non-invasively, and secured around your waist. After an hour of taking the readings, you will be asked to smoke two cigarettes after which the above readings shall be repeated for another hour at 15-minute intervals.

Discomfort may be felt once the probes are placed on the neck, arm and leg during the measurement. Participants will be required to abstain from alcohol and coffee beverages for 12 hours before the study period.

It is reasonable to expect the following benefits from this research: professionally analysed data of hemodynamic functions. Aside this, we cannot guarantee that you will personally experience benefits from participating in this study. Others may benefit in the future from the information we find in this study.

All measurements will be undertaken in a clinical research unit at the University of Zambia Ridgeway campus (UTH). Any abnormal results found during the study will be highlighted and feedback to the participant. The information collected in this study will be stored on trusted computers which will be password protected. No names will be stored. Only unique research laboratory numbers will identify participants.

Participation in this study is voluntary. You have the right not to participate at all or to leave the study at any time. Deciding not to participate or choosing to leave the study will not result in any penalty or loss of benefits to which you are entitled, and it will not harm your relationship with the researcher.

Appendix C: Consent form

The purpose of the study has adequately been explained to me and I understand the aim, benefits, risks and confidentiality of the study. I further understand that if I agree to take part in this study, I can withdraw at any time without having to give an explanation and that taking part in this study is purely voluntary.

I _____ (Full Names)

Consent to participate in this study

Signed; _____ date; _____ (Participant)

Participant's signature or thumbprint

Signed; _____ date; _____ (Witness)

Name of the interviewer; _____

Signed; _____ date; _____

PERSON TO CONTACT FOR ANYTHING

Chikopela Theresa, University of Zambia, School of Medicine, Department of Physiological Sciences, P.O. Box 50110, Lusaka, Zambia. Mobile Phone; +260977261540.

Or Chairperson, UNZABREC;

Telephone: 260-1-256067

Ridgeway Campus

P.O. Box 50110

Telex: UNZALU ZA 44370

Lusaka, Zambia

Fax: + 260-1-250753

E-mail: unzarec@zamtel.zm

Appendix D: Questionnaire

GENERAL INFORMATION

1. ID:
2. AGE (AT LAST BIRTHDAY) IN YEARS:
3. MARITAL STATUS: SINGLE MARRIED DIVORCED WIDOWED
4. OCCUPATION:
5. NATIONALITY:
 - a. ZAMBIAN: NON-ZAMBIAN:
 - b. IF NOT ZAMBIAN, NATIONALITY:

ALCOHOLIC CONSUMPTION

6. DO YOU DRINK ALCOHOLIC BEVERAGES? YES NO SKIP TO Q9
7. HOW OFTEN DO YOU CONSUME ALCOHOL?
 - a. EVERYDAY
 - b. 3-5 TIMES A WEEK
 - c. ONCE A WEEK
 - d. WEEKENDS ONLY
 - e. SPECIAL OCCASIONS
8. WHEN LAST DID YOU CONSUME ALCOHOL?

TOBACCO USE

1. DO YOU CURRENTLY SMOKE ANY TOBACCO PRODUCTS, SUCH AS CIGARETTES, CIGARS OR PIPES?
 - a. YES
 - b. NO (EXCLUDE FROM STUDY)
2. DO YOU CURRENTLY SMOKE TOBACCO PRODUCTS DAILY?
 - a. YES
 - b. NO
3. HOW OLD WERE YOU WHEN YOU FIRST STARTED SMOKING?
4. ON AVERAGE, HOW MANY CIGARETTES DO YOU SMOKE EACH DAY/WEEK?

PER DAY				PER WEEK
LESS THAN 5	5-10	10-20	MORE THAN 20	

5. WHAT TYPE OF CIGARETTES DO YOU SMOKE?
 - a. FILTERED
 - b. NON FILTERED

c. OTHER TYPE

6. WHAT BRAND OF CIGARETTES DO YOU SMOKE?

FAMILY MEDICAL HISTORY

7. IS THERE A HISTORY OF DIABETES MELLITUS IN YOUR FAMILY?

YES NO DON'T KNOW

8. IS THERE A HISTORY OF HYPERTENTION IN YOUR FAMILY?

YES NO DON'T KNOW

9. HAS A DOCTOR EVER TOLD YOU THAT YOU HAVE DIABETES?

YES NO SKIP TO Q11

10. ARE YOU CURRENTLY TAKING ANY MEDICATION TO CONTROL DIABETES?

YES NO IF YES, SPECIFY THE TYPE OF MEDICATION

11. ARE YOU CURRENTLY TAKING ANY MEDICATION TO CONTROL YOUR BLOOD PRESSURE?

YES NO IF YES, SPECIFY THE TYPE OF MEDICATION

12. ARE YOU CURRENTLY TAKING ANY MEDICATION TO CONTROL WEIGHT?

YES NO IF YES, SPECIFY THE TYPE OF MEDICATION

13. DO YOU HAVE ANY RESPIRATORY ILLNESSES SUCH AS BRONCHITIS OR ASTHMA?

YES NO IF YES, SPECIFY

PHYSICAL ACTIVITY

Next, I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment.

In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

14. IN A TYPICAL WEEK, ON HOW MANY DAYS DO YOU DO VIGOROUS-INTENSITY ACTIVITIES?

15. HOW OFTEN DO YOU EXERCISE? OFTEN MODERATELY RARELY NEVER

16. DO YOU WALK OR CYCLE TO SCHOOL FOR 10 MINUTES OR MORE? YES NO

17. IN A TYPICAL WEEK, ON HOW MANY DAYS DO YOU WALK OR BICYCLE FOR AT LEAST 10 MINUTES CONTINUOUSLY TO GET TO AND FROM PLACES?

LABORATORY ENTRY TOOL

1. ID:
2. DATE OF BIRTH:
3. VISIT DATE:
4. HEIGHT:
5. WEIGHT:

BEFORE SMOKING

1. **MEASURED BP:**

BP IN mmHg	STANDING		LYING DOWN	
	SBP	DBP	SBP	DBP
1 ST READING				
2 ND READING				
AVERAGE mmHg				

2. RIGHT CAROTID TO RADIAL DISTANCE:
3. RIGHT CAROTID TO FEMORAL DISTANCE:

4. **COMPLIOR (CAROTID-FEMORAL ARTERY) READINGS:**

TIME (MIN)	0	5	10	15	AVERAGE
VARIABLE					
PWV (m/sec)					
HEART RATE (Beats/min)					
TRANSIT TIME					

5. **COMPLIOR (CAROTID-RADIAL ARTERY) READINGS:**

TIME (MIN)	0	5	10	15	AVERAGE
VARIABLE					
PWV (m/sec)					
HEART RATE (Beats/min)					
TRANSIT TIME					

6. DYASIS READINGS:

TIME (MIN) \ VARIABLE	0	5	10	15	AVERAGE
SBP (mmHg)					
DBP (mmHg)					
HEART RATE (Beats/min)					

DURING SMOKING

1. DYASIS READINGS:

TIME (MIN) \ VARIABLE	0	5	10	15
SBP (mmHg)				
DBP (mmHg)				
HEART RATE (Beats/min)				

AFTER EXPOSURE

1. MEASURED BP:

BP IN mmHg \ VARIABLES	LYING DOWN	
	SBP	DBP
1 ST READING		
2 ND READING		
AVERAGE mmHg		

2. COMPLIOR (CAROTID-FEMORAL ARTERY) READINGS:

TIME (MIN) \ VARIABLE	0	15	30	45	60
PWV (m/sec)					
HEART RATE (Beats/min)					
TRANSIT TIME					

3. COMPLIOR (CAROTID-RADIAL ARTERY) READINGS:

TIME (MIN) \ VARIABLE	0	15	30	45	60
PWV (m/sec)					
HEART RATE (Beats/min)					
TRANSIT TIME					

4. DYASIS READINGS:

TIME (MIN) \ VARIABLE	0	15	30	45	60
SBP (mmHg)					
DBP (mmHg)					
HEART RATE (Beats/min)					

IF NOT COMPLETED, SPECIFY REASONS:

.....

.....

.....

.....

.....

.....

PARTICIPANT:

NAME:.....SIGNATURE:

RESEARCHER:

NAME:.....SIGNATURE:

DATE:/...../.....

Appendix E: Abstract of article published (Cardiology and Angiology; an International Journal) on the 24th July, 2015.



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Effects of Smoking on Arterial Stiffness in Male Adolescents in Lusaka, Zambia

Chikopela Theresa^{1*} and M. Goma Fastone²

¹Lusaka Apex Medical University, Basic Sciences, Zambia.

²The University of Zambia, School of Medicine, Zambia.

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ABSTRACT

Background: Tobacco smoke is harmful to health. In the acute phase it causes changes in the cardiovascular system that result in increase in blood pressure (BP). An increase in arterial stiffness due to arteriolar endothelial dysfunction has been cited among the causes. Pulse Wave Velocity (PWV) and Arterial Stiffness Index (ASI) are used as measures of arterial stiffness in the adult population.

Aim: To determine the acute effects of tobacco smoke on arterial stiffness in black male adolescents in Lusaka, Zambia.

Study Design: This was an observational study done at the University of Zambia School of Medicine Cardiovascular Research Laboratory in the month of December 2014.

Methodology: Twenty-two (22) black, male-adolescent (age range 19-25 years), active-smokers, consented to participate in the study. The Complior Analyse Unit (V1.9 Beta Version 2013; ALAM-Medical, France) protocol was used to obtain the carotid-femoral PWV (cfPWV) and carotid-femoral

*Corresponding author: E-mail: theresachikopela@yahoo.com, gomafm@unza.zm;