CIMT and cardiovascular risk in five-year-old children in a low socioeconomic population exposed to alcohol and nicotine during pregnancy: a case-control study

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ABSTRACT

CIMT AND CARDIOVASCULAR RISK IN FIVE-YEAR-OLD CHILDREN IN A LOW SOCIOECONOMIC POPULATION EXPOSED TO ALCOHOL AND NICOTINE DURING PREGNANCY: A CASE-CONTROL STUDY

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Cardiovascular diseases (CVD) are among the top 10 causes of death in all ages in South Africa. The prevalence of maternal smoking and alcohol consumption during pregnancy is alarmingly high in South Africa. *In utero* exposure to nicotine and alcohol may cause CVD later in life. There is a global need for early detection of CVD especially those vulnerable during early childhood, to prevent the development of CVD risk factors in adulthood. The aim of this study was to compare CVD risk in five-year-old children from a low socio-economic population with *in utero* dual exposure to nicotine and alcohol and *in utero* nicotine exposure by measuring carotid intima-media thickness (cIMT), anthropometric measurements and clinical measurements including blood pressure. A case-control study was conducted on 468 children at five years old through interviews to collect data on demographic characteristics and health statistics. The cIMT was measured using B-mode ultrasonography. Anthropometric measurements were taken such as skinfold thickness, waist circumference, height and weight to calculate Body Mass Index (BMI). Blood pressure measurements such as systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) were taken. The data was analysed using SPSS version 26. Descriptive and inferential statistics (Spearman's correlations, non-parametric partial correlations), Kruskal-Wallis H, Chi-square tests and logistic regression were used for statistical analysis. Results showed a significantly higher right cIMT (RcIMT) (0.36 ± 0.05 mm; $P < 0.01$) in children with *in utero* exposure to nicotine and alcohol during pregnancy and a higher RcIMT in males (0.37 ± 0.06 mm; $P < 0.01$) with *in utero* dual exposure to nicotine and alcohol when compared to females. A significant association was found between *in utero* dual exposure to nicotine and alcohol and a high RcIMT, specifically in females at five years old after the adjustment for confounders (B= -1.618, $P = 0.002$). Consequently, females in the dual exposed group were 7.6 times more likely to exhibit higher RcIMT with a relative risk of 2.6 times greater to children with no exposure. Females also had significantly higher SBP (U= 3829.50, $p < 0.01$), DBP (U= 3527.50, $p < 0.05$), MAP (U= 3561.00, $p < 0.05$) and HR (U= 3887.50, $p < 0.01$) in the dual exposed group. Cardiovascular risk factors were modestly prevalent at five years old in children with *in utero* teratogen exposures. However, increased adiposity indices were not observed in this population at five years old and were not associated with teratogen exposures. This may indicate that dual exposure to nicotine and alcohol has a significant effect on the intima-media thickness of the carotid arteries in children, but not necessarily on central and peripheral adiposity at five years old. Therefore, CVD risk factors need to be identified early in children in low socioeconomic regions with *in utero* exposure to nicotine and alcohol to prevent CVD later in life.

Key words: alcohol, cardiovascular risk, children, carotid intima-media thickness, *in utero* exposure, low-income population, nicotine.

DECLARATION

I declare that *"CIMT and cardiovascular risk in five-year-old children in a low socioeconomic population exposed to alcohol and nicotine during pregnancy: a case-control study"* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name.....**Tammy Charlene Hartel**..... Date.....**10 December 2020**.....

Signed.....

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LIST OF SCIENTIFIC ABBREVIATIONS

ABCA1	Cassette binding transporter A1
ABCG1	Cassette binding transporter G1
APC	Alcohol per capita consumption
ATP	Adenosine triphosphate
BMI	Body Mass Index
BMREC	Biomedical Research ethics committee
BP	Blood pressure
CCA	Common carotid artery
CD36+	Cluster of differentiation 36 macrophages
cIMT	Carotid intima-media thickness
CNS	Central nervous system
CpG site	cytosine-phosphate-guanine site
CVD	Cardiovascular disease
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic acid
DOHaD	Developmental Origins of Health and Disease
ET_B	Endothelin B
FTO gene	Fat mass and obesity gene
GA	Gestational age

HDL2-cholesterol	High-Density Lipoprotein 2-cholesterol
HDL3-cholesterol	High-Density Lipoprotein 3-cholesterol
HDL-cholesterol	High-Density Lipoprotein-cholesterol
HREC	Health and Research ethics committee
LDL-cholesterol	Low-Density Lipoprotein-cholesterol
MAP	Mean arterial pressure
nAChRs	Nicotinic acetylcholine receptors
NO	Nitric oxide
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SANHANES-1	South African National Health and Nutrition Examination Survey
SBP	Systolic Blood Pressure
SDG	Sustainable development goal
SFT	Skinfold thickness
SPS	Safe passage study
SPSS	Statistical package for Social Sciences
sSFT	Subscapular skinfold thickness
tSFT	Triceps skinfold thickness
UWC	University of the Western Cape
WC	Waist circumference

WHO		World Health Organization
WHR		Waist-to-hip ratio

UNITS OF MEASUREMENTS

bpm	=	beats per minute
cm	=	centimetres
kg	=	kilogram
kg/m^{-2}	=	kilograms per square metre
m	=	metre
mm	=	millimetres
mm Hg	=	millimetres of mercury

STATISTICAL UNITS

%	=	percentage
CI	=	Confidence Interval
OR	=	Odds ratio
RR	=	Relative risk
p	=	Significance level
SD	=	standard deviation
X	=	mean
H	=	Kruskal-Wallis H

U	=	Mann-Whitney U
χ^2	=	Chi-Square
IDV	=	Independent variable
B	=	Beta
df	=	degree of freedom
R^2	=	Nagelkerke R square value

CHAPTER 1: INTRODUCTION

1.1 Introduction

Many factors such as calorie-rich diets, genetic disorders, poverty, lack of physical activity and maternal lifestyle factors may contribute to abnormal lipid profiles which in turn affects the development of atherosclerosis, leading to the onset of CVD (Benjamin *et al.*, 2018; Peer *et al.*, 2018). It is evident that *in utero* exposure to alcohol and nicotine causes adverse effects to almost all organ systems particularly the cardiovascular system (Parkington *et al.*, 2010; Cupul-Uicab *et al.*, 2012; Benedict *et al.*, 2018). *In utero* teratogen exposure such as nicotine and alcohol has shown to affect atherosclerosis in coronary and carotid arteries, Body Mass Index (BMI), body fat, blood pressure, as well as birth weight which increases cardiovascular risk in offspring (Parkington *et al.*, 2010; Cupul-Uicab *et al.*, 2012; Benedict *et al.*, 2018; Kataria, Gaewsky and Ellervik, 2018).

Total cholesterol levels, triglycerides, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol levels play an important role in the development of atherosclerosis. The development of atherosclerosis begins in early childhood and therefore should be detected early (Rodrigues *et al.*, 2013). Atherosclerosis is the thickening and hardening of arterial walls and consists of a cascade of events which is characterized by an accumulation of lipid molecules such as inflammatory molecules, oxidised LDL-cholesterol and fibrin (Manco *et al.*, 2017). High density lipoprotein cholesterol has several important potentially protective functions against atherosclerosis, including promoting cholesterol efflux from macrophages and nitric oxide production from the endothelium, inhibiting lipid oxidation, platelet activation, impairing leucocyte adhesion and monocyte activation and preventing endothelial cell death and damage (Ayer *et al.*, 2011). The attachment and recruitment of circulating monocytes to the vascular endothelium and migration into the sub endothelium are

the main processes of atherosclerosis development in children (Manco *et al.*, 2017). The monocytes infiltrate the intima with the assistance of cellular adhesion surfaces on the vascular endothelial cells. When these monocytes differentiate into dendritic cells or cluster of differentiation 36 (CD36+) macrophages, they interact with atherogenic lipoproteins. These CD36+ macrophages are important for endocytosis of oxidised LDL-cholesterol and foam cell formation, inducing an immune response which results in the production and secretion of pro-inflammatory chemokines and cytokines, thus activating a vicious cycle (Manco *et al.*, 2017). This cycle will subsequently lead to the thickening of the tunica intima wall of the artery. It is suggested that all these molecules released in this cycle can be used as markers for atherosclerosis (Manco *et al.*, 2017).

Although the assessment of determinants of atherosclerosis onset and progression in paediatric cohorts is challenging, many markers have been identified for atherosclerosis. Atherosclerosis seems to be equally present in the carotid, cerebral and coronary arteries (Sharma *et al.*, 2014; Manco *et al.*, 2017). These arteries also show a close relationship between carotid, coronary and cerebral atherosclerotic disease with regard to histology and cIMT, and can therefore be a reliable marker for an overall reading on the presence of atherosclerosis (Sharma *et al.*, 2014; Manco *et al.*, 2017). Ultrasonography is used to distinguish between the three layers, namely intima, media, adventitia, as well as measure the intima-media thickness of the carotid artery which is associated with future risk for arterial disease (Myers and Clough, 2004).

1.2 Statement of the problem

Previous literature has shown that *in utero* exposure to nicotine and alcohol may result in CVD later in life which may contribute to the high rate of cardiovascular morbidity and mortality, now a considerable burden in South Africa. Women who are heavy smokers and consume excessive amounts of alcohol during pregnancy are negatively affecting the child *in utero*, which later in life affects their health and well-being, predisposing the child to CVD. In South

Africa, the concurrent use of nicotine and alcohol is considerably high, particularly during pregnancy. *In utero* dual exposure to nicotine and alcohol may have a double negative effect on the cardiovascular system of the fetus and may increase the risk of developing CVD risk factors in adulthood. Therefore, CVD risk factors need to be identified early in childhood, specifically in at risk populations in low socioeconomic regions to prevent the development of CVD in adulthood. The effects of dual exposure to alcohol and nicotine *in utero* on cIMT and cardiovascular risk compared to nicotine exposure alone has yet to be established. Few studies have reported on the monitoring of these children and their cardiovascular risk during their childhood. Therefore, this study will be conducted.

1.3 Aim of the study

The aim of this study was to compare CVD risk in five-year-old children from a low socio-economic population with dual *in utero* exposure to nicotine and alcohol and *in utero* nicotine exposure by measuring CIMT, anthropometric measurements (BMI, WC, SFT) and clinical measurements including blood pressure.

1. **4 The objectives of the study**

The objectives of the study are to:

1.4.1 Anthropometric measurements

a. Determine body mass index to classify level of childhood obesity
b. Measure waist circumference to classify central obesity
c. Measure subscapular and triceps skinfold thickness

1.4.2 Clinical measurements

a. Measure Carotid intima-media thickness using B-mode ultrasonography
b. Measure blood pressure to classify childhood hypertension

1.4.3 Statistical analyses

a. Determine the significant differences between CVD measurements between *in utero* nicotine exposed group, *in utero* dual exposure group (nicotine and alcohol), and the control group.
b. Compare cardiovascular risk between *in utero* nicotine exposed group, *in utero* dual exposed group and the control group.
c. Compare cardiovascular risk across gender.
d. Determine the associations, odds ratios and relative risk between CVD risk factors in the *in-utero* nicotine exposed group, *in utero* dual exposure group, and the control group.

1.5 Hypotheses of the study

- There will be a higher cardiovascular risk in five-year-old children with dual exposure to nicotine and alcohol compared to the control group.
- There will be a higher cardiovascular risk in five-year-old children with dual exposure to nicotine and alcohol compared to nicotine exposed group.
- There will be a significant difference in cardiovascular risk between the dual exposure group and nicotine exposure group in five-year-old children.
- Dual *in utero* exposure to nicotine and alcohol will be significantly associated with cardiovascular risk factors in five-year-old children after adjustment for confounders.

1.6 Definition of terms

Central obesity was defined as a waist circumference $\geq 90^{th}$ percentile (Rodrigues *et al.*, 2013). Central obesity was defined according to percentiles from the control group, which was used as the normal population in this study.

Overweight was defined as a BMI > 16.6 kg/m² in boys and a BMI >16.9 kg/m² in girls. BMI cut-off values for children were age and gender specific (WHO, 2007a, 2007b).

Obesity was defined as a BMI >18.3 kg/m² in boys and a BMI > 18.9 kg/m² in girls. BMI cut-off values for children were age and gender specific (WHO, 2007a, 2007b).

Prehypertension was defined as a systolic blood pressure and/or diastolic blood pressure reading between the 90th and 95th percentile which were adjusted for age and gender (Rao, 2016). Prehypertension was defined according to normotensive percentiles of an average of three blood pressure measurements taken in the control group.

Hypertension was defined as a systolic blood pressure reading and/or diastolic blood pressure reading < 95th percentile (Rao, 2016). Hypertension was defined according to normotensive percentiles of an average of three blood pressure measurements taken in the control group.

Systolic hypertension was defined as a systolic blood pressure reading ≥ 95th percentile (Rao, 2016). Systolic hypertension was defined according to normotensive percentiles of an average of three blood pressure measurements taken in the control group.

Diastolic hypertension was defined as a diastolic blood pressure reading ≥ 95th percentile (Rao, 2016). Diastolic hypertension was defined according to normotensive percentiles of an average of three blood pressure measurements taken in the control group.

Carotid intima-media thickness was defined as a double-line pattern formed by two anatomical boundaries (lumen-intima and media-adventitia interfaces) which can be visualized by echography on both walls in a longitudinal image (Touboul *et al.*, 2012).

High carotid intima-media thickness was defined as a thickness ≥ 75th percentile (Naqvi *et al.*, 2010). High Carotid intima-media thickness was defined according to percentiles from normal data such as the control group, which was used as the normal population in this study.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature review is based on the Developmental Origins of Health and Disease (DOHaD) (Bakker and Jaddoe, 2011; Rodríguez-Rodríguez *et al.*, 2018). The DOHaD hypothesises that fetal exposures will result in permanent adaptations in the structure, physiology and metabolism in the fetus (Bakker and Jaddoe, 2011). These adaptations are beneficial to the fetus for short term survival, but may result in the development of cardiovascular diseases (CVD) later in adulthood (Bakker and Jaddoe, 2011). Scientific literature has suggested that *in utero* exposure to nicotine and alcohol plays an important role in the DOHaD, specifically CVD (Rodrigues *et al.*, 2013; Akison *et al.*, 2019) This literature review will discuss the prevalence of CVD as well as the risk factors thereof. It will further discuss the prevalence and classification of these risk factors in both adulthood and childhood. The review then focusses on the prevalence of *in utero* exposures to nicotine and alcohol, and how this is associated with CVD risk factors in childhood and the development of CVD later in life.

2.2 Cardiovascular disease prevalence

Globally, CVD is the leading cause of death and is predicted to account for more than 23.6 million deaths by 2030 (Benjamin *et al.*, 2018). In America, an estimated 47% of the country have at least 1 of 3 risk factors for CVD such as tobacco smoking, hypertension and high cholesterol (Benjamin *et al.*, 2018). In 2012, global targets were adopted to reduce the premature mortality (30-70 years) by non-communicable diseases by 25% by 2025, of which six risk factors were agreed to be addressed by 2025 (Benjamin *et al.*, 2018). These risk factors included salt intake, alcohol use, obesity, raised blood pressure, and tobacco use (Benjamin *et al.*, 2018). If these risk factors are addressed, the CVD mortality rate will be reduced by 34% worldwide, preventing 11.4 million premature deaths and 15.9 million deaths over 70 years of age (Benjamin *et al.*, 2018).

In South Africa, the mortality prevalence of cerebrovascular diseases such as stroke was ranked as the fourth (5.1%) leading underlying natural cause of death in 2016, with a higher mortality rate (5.6%) in the Western Cape province (Statistics SA, 2018). Despite the decrease in mortality since 2014, stroke remains among the top 5 causes of death in South Africa and in the Western Cape (Statistics SA, 2018). In addition, other forms of heart disease including cardiac arrest and ischaemic heart diseases (chronic ischaemic heart disease, acute myocardial infarction) caused deaths in 5.1% and 2.8% of the South African population, being ranked as the third and ninth underlying natural cause of death in 2016, respectively (Statistics SA, 2018). In the Western Cape, other forms of heart disease caused a mortality rate of 3% in children aged 1-4, being ranked fourth in 2016 (Statistics SA, 2018). Therefore, cardiovascular and cerebrovascular diseases fall among the top 10 causes of death in all ages in South Africa (Statistics SA, 2018).

The high mortality rate of stroke, ischaemic heart disease and other forms of heart disease in South Africa could be due to the unhealthy lifestyle habits which many South Africans have. Many factors such as dietary intake, genetic disorders, poverty, maternal factors and toxins can contribute to the development of atherosclerosis which results in CVD or stroke (Benedict *et al.*, 2018; Rodríguez-Rodríguez *et al.*, 2018). However, these risk factors are modifiable and can be reduced through lifestyle changes. Risk factors for CVD include dyslipidaemia, hypertension, high blood cholesterol levels, dietary risks, high BMI, high fasting blood glucose, alcohol consumption, tobacco smoking and drug use (Benedict *et al.*, 2018; Peer *et al.*, 2018). In addition, risk factors for CVD mortality are obesity, high LDL-cholesterol, physical inactivity, high dietary salt and trans-fatty acids and low omega-3 fatty acids (Benedict *et al.*, 2018).

2.3 Cardiovascular disease risk factors

2.3.1 Dyslipidaemia

Dyslipidaemia is an abnormality in levels of circulating serum cholesterol, triglycerides, high-density lipoproteins (HDL-cholesterol) and low-density lipoproteins (LDL-cholesterol) (Oldewage, Egal and Grobler, 2017). Lipoprotein particles differ in size, density and composition and can be categorised into classes based on their chemical and physical limitations (Ivanova *et al.*, 2017). Low-density lipoprotein is highly atherogenic, specifically small density LDL, as it is common in the serum of patients with atherosclerosis and are susceptible to chemical modifications that increase their atherogenicity (Ivanova *et al.*, 2017). In addition, there is a strong association between CVD and elevated LDL-cholesterol and total serum cholesterol, as well as low HDL levels, obesity, hypertension and diabetes mellitus in children (Oldewage, Egal and Grobler, 2017).

Furthermore, a systematic review reported that carotid intima media thickness (cIMT) increased significantly in children and adolescents with diagnosed dyslipidaemia, obesity and hypertension (Lamotte *et al.*, 2011). According to the Heart disease and Stroke Statistics (2018), twenty-one percent of children and youth aged between 6 and 19 years old have one or more abnormal cholesterol measurement's (Benjamin *et al.*, 2018). A prospective longitudinal study conducted in Pretoria, South Africa reported that children whom were exposed to smoking during pregnancy had lower HDL-cholesterol and higher triglyceride levels (Ayer *et al.*, 2011). After the adjustment for potential confounders, such as maternal passive smoking, post-natal smoke exposure, gender, breast feeding duration, physical inactivity and adiposity, tobacco exposure during pregnancy remained significantly associated with lower HDL-cholesterol in children (Ayer *et al.*, 2011). Therefore, detection of dyslipidaemia and atherosclerosis early in children should be prioritised, as atherosclerotic plaque formation can begin as early as the fetal and neonatal period (Benedict *et al.*, 2018).

2.3.2 Dietary intake

Dietary quality, such as the intake of fruits, vegetables, fish and wholegrains have been shown to be beneficial in preventing CVD. In contrast, the intake of added sugars, sodium, refined carbohydrates and fats have been identified as potential risk factors for CVD in both children and adults (Oldewage, Egal and Grobler, 2017). The relationship between dyslipidaemia, dietary sodium and fat intake is well established as well as the association between high carbohydrate intake and hypertriglyceridemia in children (Oldewage, Egal and Grobler, 2017). A study conducted in the country Jordan showed that high caloric intake was significantly associated with increased serum total cholesterol in healthy adults (Tayyem, Hijjawi and Allehdan, 2018). Therefore, caloric intake should be monitored in children as additional calorie intake above basal metabolic rate, are associated with increased risk of dyslipidaemia and hypertriglyceridemia, whether the additional calories are from fat, protein, or carbohydrates (Tayyem, Hijjawi and Allehdan, 2018).

A study conducted by Oldewage, Egal and Grobler (2017) investigated the association between dietary intake and CVD risk factors in the Eastern Cape, South Africa and reported that high carbohydrate intakes were superfluous and two to three times more than the recommended intake (Oldewage, Egal and Grobler, 2017). Additionally, results showed that approximately 2.3% of children had hypercholesterolaemia, 2.1% had elevated LDL-cholesterol and 12.4% had triglycerides equal to or above 1.12 mmol/l for children between 0-9 years old. However, hypercholesteremia was only present in the 6-8-year-old age group. Low HDL-cholesterol levels (equal to or less than 1.04 mmol/l) were observed in 42.5% and hyperglycaemia was observed in 10.3% of children and none of the children were obese. The highest prevalence of abnormal lipid levels was in girls aged 6-8 years (Oldewage, Egal and Grobler, 2017). The prevalence of hypercholesteremia can be reduced by dietary change such as changing to a low-

fat diet, increasing the ratio of polyunsaturated or monounsaturated fats to saturated fatty acids and also increasing physical activity (Htet *et al.*, 2017).

Furthermore, previous literature reported that specific dietary patterns have also been revealed to affect cIMT (Mcclintock *et al.*, 2015; Maugeri *et al.*, 2019). Cardioprotective diets have been described as diets high in nuts, vegetables, monounsaturated fats, whereas harmful diets included trans-fatty acids, processed meats, refined grains, high fatty foods and foods with high glycaemic index (Mcclintock *et al.*, 2015). Study findings showed a positive correlation between a balanced diet such as low intake of saturated fat and total fat and a 4.95 μm decrease in cIMT, whereas a root vegetable diet was associated with an increased intima media thickness of 7.7 μm (Mcclintock *et al.*, 2015). Another study focussing on antioxidant intake and cIMT found an association between dietary zinc intake and decreased cIMT in women, but not in men (Maugeri *et al.*, 2019). For every 1 mg increase in dietary zinc intake, the cIMT decreased by 2.90 μm, which remained significant after the adjustment for confounding factors (Maugeri *et al.*, 2019). Thus, moderate caloric diets that are high in healthy fats and low-glycaemic carbohydrates should be emphasized during pregnancy and early childhood, to reduce the possibility of atherosclerotic plaque formation in children with *in utero* exposure to nicotine and alcohol.

2.3.3 Hypertension

2.3.3.1 Hypertension in adulthood

Hypertension in adults is defined as having a resting systolic blood pressure (SBP) of ≥ 140 mmHg and/or having a diastolic blood pressure (DBP) ≥ 90 mmHg or currently on antihypertensive medication (Riebe *et al.*, 2018). Hypertension is a major risk factor of CVD, of which obesity plays a major role in the development of hypertension (WHO, 2020). Risk factors of hypertension include overweight and obesity, male sex, ethnicity, older age, high salt diet, a high fat diet and a sedentary lifestyle (Rao, 2016; Riebe *et al.*, 2018). Hypertension is

significantly associated with obesity, prediabetes and diabetes (Owolabi *et al.*, 2017; Dong *et al.*, 2019). In West Africa, a study reported an overall hypertension prevalence of 41.2%, of which 27.3% and 13.9% of women and men had hypertension (Paolo and Michelle, 2016). In South Africa, hypertension was highly prevalent in 56.2% and 43.8% in obese and non-obese adults, respectively (Owolabi *et al.*, 2017). Hypertensive diseases were ranked as the sixth leading underlying natural cause of death in South Africa in 2016, accounting for 4.4% of deaths (Statistics SA, 2018).

2.3.3.2 Hypertension in childhood

Hypertension in children which was often considered rare is now a common health problem in childhood and adolescence as it is a predictor of hypertension in adulthood, however, it cannot be classified according to the adult hypertensive classification system (Rao, 2016; Dong *et al.*, 2019). Rao (2016) explains that blood pressure values are normally distributed and, therefore, a cut-off value of SBP and DBP can be classified as two standard deviations above the mean or approximately the 95th percentile for children and based on an average of three measurements (Rao, 2016). Normal blood pressure is classified as a SBP and/or DBP < 90th percentile, prehypertension in children aged 3-11 years old is classified as a SBP and/or DBP percentile between the 90th- <95th percentile, stage 1 hypertension between the 95th-99th percentile plus 5 mm Hg, and stage 2 hypertension as a percentile > 99th plus 5 mm Hg (Rodrigues *et al.*, 2013; Rao, 2016).

A systematic review observed a global trend of increasing prevalence of childhood hypertension during the past 20 years from 75% to 79% from 2000 to 2015 (Song *et al.*, 2019). In 2015, the global prevalence of childhood hypertension was 4.32% in children aged 6 years and older and 7.89% in children aged 14 years and older (Song *et al.*, 2019).

In the Western Cape, hypertension was prevalent among 2.6% of children between 7 and 18 years of age, with a prevalence of 2.9% in boys and 2.4% in girls (Negash *et al.*, 2017). A study in the Eastern Cape, reported an overall prevalence of hypertension in 29.5% of adolescents (13-18 years), with a prevalence of 32.8% in boys and 32.6% in girls (Sekokotla *et al.*, 2017).

2.3.4 Poverty

A study conducted in Myanmar (Yangon region) showed that urban citizens had a significantly higher total cholesterol, triglycerides and LDL-cholesterol, as well as lower HDL levels compared to rural citizens (Htet *et al.*, 2017). This association remained significant after adjustment for confounders (Htet *et al.*, 2017). The latter study suggested that an abnormal and unhealthy lipid profile in urban citizens could be due to the use of unhealthy oils containing saturated fats, such as refined oils, a high intake of foods rich in oil and low intake of fruits and vegetables (Htet *et al.*, 2017). Similarly, a study reported that urban residents in China had a higher prevalence of obesity and hypertension than their rural counterparts (Zhang, 2019). In West Africa, hypertension was more prevalent (25.7%) in semi-urban areas compared to rural areas (16.4%) (Paolo and Michelle, 2016). The trend of an increased CVD risk in urban areas could be due to urbanisation and urban living, such as physical inactivity, more access to high fat and energy dense diets (Paolo and Michelle, 2016; Zhang, 2019).

In South Africa, a cross-sectional study on socioeconomic status reported that all CVD risk factors including alcohol and tobacco use were more prevalent in urban areas compared to rural areas, diabetes, obesity and alcohol use were significantly different (Egbujie, Igumbor and Puoane, 2016). The increase in prevalence of CVD risk factors as well as lifestyle changes is associated with rapid urbanisation in low-middle income countries, particularly in South Africa (Egbujie, Igumbor and Puoane, 2016). In contrast, a longitudinal study (HAALSI study) reported a prevalence of dyslipidaemia in 67.3% among adults in rural villages, of which the majority (59.3%) had abnormal triglycerides levels (Reiger *et al.*, 2017). In a rural town in

South Africa, a study reported that 40% of adolescents were obese and female adolescents had significantly higher values for total cholesterol, LDL-cholesterol and HDL-cholesterol compared to male adolescents (Sekokotla *et al.,* 2017). Low socioeconomic status has also been consistently associated with cIMT in children aged 11-12 years old (Liu *et al.*, 2017). In contrast, in Indonesia, obesity and central obesity was observed in adults of high socioeconomic status compared to those with low socioeconomic status (Harbuwono *et al.*, 2018).

2.3.5 Air pollution exposure

Air pollutants such as oxides of nitrogen, particulate matter and carbon monoxide have been linked to CVD risk factors, subclinical CVD and cardiovascular mortality (Tibuakuu *et al.*, 2018). A recent study identified vulnerable populations that are highest at risk for poor CVD outcomes such as those with low socioeconomic status, women, the elderly, black ethnicity, those with comorbidities (such as Diabetes and a history of heart disease) as well as developing countries (Tibuakuu *et al.*, 2018). Smokers were also at risk for metabolic syndrome and cardiovascular mortality due to exposure to air pollution (Tibuakuu *et al.*, 2018). Therefore, smoking during pregnancy may put women at risk for CVD, specifically in developing countries where the concentrations of air pollution is higher (Tibuakuu *et al.*, 2018). In children, a study observed an association between long term exposure to air pollution and increased blood pressure and hypertension with stronger associations in obese children (Dong *et al.*, 2015). This means that overweight and obesity in children increased their susceptibility to the health effects of long term exposure to of ambient air pollution such as sulphur dioxide, nitrogen dioxide and ozone concentrations (Dong *et al.*, 2015).

2.3.6 Obesity

2.3.6.1 Obesity in adulthood

In South Africa, a study reported that approximately 24% of adults are overweight and 46% of adults are obese (Owolabi *et al.*, 2017). In other developing countries such as Indonesia, the prevalence of obesity and central obesity was 23.1% and 28%, respectively (Harbuwono *et al.*, 2018). Obesity is significantly associated with increased CVD risk factors such as hypertension, dyslipidaemia and diabetes (Owolabi *et al.*, 2017). Waist circumference (WC) or Waist-to-hip ratio (WHR) can also be used as a health risk assessment for obesity related diseases, as it is an indicator of abdominal obesity (Riebe *et al.*, 2018). Central obesity has been defined as a WC greater than 88cm in women and greater than 102cm in men and a WHR ratio greater than 0.9 in males and greater than 0.85 in females (Alpert *et al.*, 2014).

2.3.6.2 Childhood obesity

Globally, the prevalence of obesity in children and adolescents aged 5-19 years have doubled from 2.9% since 2000 to 6.8% in 2016 (WHO, 2020). In addition, overweight prevalence in children under 5 years old has shown an increasing trend with a prevalence of 38 million (5.6%) in 2019 compared to 30 million in 2000 (WHO, 2020). There is a significant association between excessive weight gain before 5 years old and the on-set of overweight and obesity in adulthood (Munthali *et al.*, 2017). According to the WHO, cut-offs values for children and adolescents, which are age and gender specific, overweight is classified as a BMI value of 16.6 kg/m² for boys and 16.9 kg/m² for girls (WHO, 2007a)(WHO, 2007b). Obesity is classified as a BMI value of 18.3 kg/m² for boys and 18.9 kg/m² for girls (Johnson *et al.*, 2007; WHO, 2007a, 2007b). Body mass index classification according to WHO cut-off values in children has been used in recent paediatric studies (Munthali *et al.*, 2017; Spinelli *et al.*, 2019). Paediatric studies have also used reference values from the International Obesity Task Force (IOTF) and z scores from the Centres for Disease and Control Growth Charts to classify BMI in children (Dong *et al.*, 2019).

According to the WHO world report, 13.3% and 11.3% of children in South Africa are overweight and obese, respectively (WHO, 2020). A longitudinal paediatric study reported a prevalence of excess weight of 11.06% and 13.58% in boys and girls, respectively (Munthali *et al.*, 2017). Similarly, a study in the Western Cape reported an overweight and obesity prevalence of 15.6% and 7.3% in school learners aged 7-18 years in 14 Western Cape schools, respectively (Negash *et al.*, 2017). Girls had a higher overweight and obesity prevalence of 19.7% and 9.1% compared to boys with a 9.4% and 4.5% prevalence, respectively (Negash *et al.*, 2017).

2.3.7 Tobacco smoking

2.3.7.1 Nicotine uptake

Nicotine is a neurotoxin that was first isolated from tobacco in 1828 by German chemist, Posselt and Rheimann (Alkam and Nabeshima, 2019). Nicotine is also one of 4700 ingredients in tobacco smoke and obtained from the tobacco plant *Nicotiana tabacum*, native to North and South America (Talhout *et al.*, 2011; Alkam and Nabeshima, 2019). Normally, approximately 70-80% of nicotine is metabolised to cotinine in the liver by enzymes cytochrome P450 2A6 (CYP2A6) (Alkam and Nabeshima, 2019). Cotinine is further broken down by CYP2A6 to trans-3′-hydroxycotinine (Alkam and Nabeshima, 2019). Nicotine is also broken down to other metabolites, namely nornicotine and norcotinine (Alkam and Nabeshima, 2019). Nicotine functions similarly to acetylcholine at the neuromuscular junction when binding to nicotinic acetylcholine receptors, whereby acetylcholine is broken down immediately by the enzyme acetylcholinesterase after an action potential has been propagated (Alkam and Nabeshima, 2019). The Nicotinic acetylcholine receptors (nAChRs) are located on plasma membranes of the central nervous system (CNS) neurons, of postganglionic cells in all autonomic ganglia. However, when nicotine binds to nicotinic acetylcholine receptors and exerts its effect by stimulating the receptor and opening the selective ion channels to cause de-polarization,

nicotine cannot be rapidly broken down to be ineffective similar acetylcholine. It thus has a prolonged stimulatory function, causing intracellular calcium concentrations to increase to pathological levels resulting in cellular dysfunction (Alkam and Nabeshima, 2019). The blood also contains high amounts of acetylcholinesterase, thus hydrolysing acetylcholine if it leaks into the venous circulation and, therefore, unlikely to reach the arteries (Alkam and Nabeshima, 2019). This means that when nicotine enters the blood circulatory system, it can travel to various organs as it cannot be rapidly broken down by acetylcholinesterase (Alkam and Nabeshima, 2019).

2.3.7.2 Tobacco smoking prevalence

According to WHO, tobacco causes 9 million deaths each year, of which 7 million deaths result directly from tobacco use and 1.2 million from non-smokers exposed to second-hand smoke (Health and Tobacco, 2019). Furthermore, 80% of the world's smokers reside in low-to middle income countries (Alkam and Nabeshima, 2019; Health and Tobacco, 2019). The global report on trends in prevalence of tobacco smoking reported a decrease in tobacco smoking among females from 10.9% in 2000 to 6.4% in 2015 (Geneva: World Health Organization, 2018). Therefore, females are expected to have a significant decrease in the prevalence in tobacco smoking to 5.3% by 2025 (Geneva: World Health Organization, 2018).

In South Africa, the prevalence of tobacco smoking among females was 7.9% in 2016 (Geneva: World Health Organization, 2018). However, global trends estimated a prevalence of 7.4% by 2020 and 6.8% by 2025 of tobacco smoking among females in South Africa (Geneva: World Health Organization, 2018). Current trends in South Africa show a decrease in tobacco smoking prevalence from 22% in 2000 to a prevalence of 20.1% in 2015 and an estimated 19.3% prevalence by 2025. However, the prevalence target is 15.79% by 2025 (a 30% reduction)(Geneva: World Health Organization, 2018). Therefore, it is evident that South Africa is not on track to achieving the target of a 30% reduction of tobacco use by 2025 set by

the WHO Global Action Plan for the Prevention and Control of Non-communicable Diseases 2013–2020 (Geneva: World Health Organization, 2018). Nevertheless, there remains a national annual decrease in tobacco smoking prevalence in South Africa.

2.3.7.3 Environmental tobacco smoke exposure

A study in Australia reported a significant association between environmental tobacco smoke exposure in childhood and increased cIMT (Viikari *et al.*, 2014). This meant that adults exposed to tobacco smoke during their childhood through maternal and paternal smoking had a greater cIMT than those with no exposure (Viikari *et al.*, 2014). In contrast, a prospective longitudinal study conducted in Australia reported that neither smoke exposure during pregnancy nor post-natal smoke exposure was associated with changes in cIMT in eight-year-old children (Ayer *et al.*, 2011). However, Ayer (2011) suggests that atherosclerosis could exist with tobacco smoke exposure later in life.

2.3.8 Alcohol consumption

Globally, alcohol contributes to a mortality rate of 5.3% annually, accounting for approximately 3 million deaths every year (World Health Organization, 2018). In 2016, alcohol was accountable for 1.7 million deaths, of which 0.6 million deaths were due to CVD (World Health Organization, 2018). In adults aged 24 to 39 years, alcohol consumption has been associated with the accumulation of fatty deposits in the carotid artery (Parkington *et al.*, 2010). A study reported that alcohol consumption in women increased the risk of cardiomyopathy later in life (Dejong, Olyaei and Lo, 2019). In contrast, studies reported that in Japanese men, heavy alcohol consumption may increase HDL-cholesterol rapidly, whereas little to moderate alcohol consumption was shown to increase HDL and decrease LDL-cholesterol which decreases cardiovascular risk (Kawamoto *et al.*, 2009; Parkington *et al.*, 2010; Tabara *et al.*, 2017). However, a new human genetic study argued that HDL-cholesterol itself is unlikely to be protective against CVD (Vitali, Khetarpal and Rader, 2017). Interestingly, new HDL sub-

classes are now coming to the fore in recent studies as a new approach to predict cardiovascular risk (Yang *et al.*, 2020). HDL- cholesterol comprises of subclass particles which vary in size, density and components, namely: High-density lipoprotein 2 cholesterol (HDL2-cholesterol) and High-density lipoprotein 3 cholesterol (HDL3-cholesterol) (Yang *et al.*, 2020). HDL2-cholesterol is less dense and more cholesterol-rich, whereas HDL3-cholesterol is more dense and cholesterol-poor (Superko *et al.*, 2012). Studies have suggested that HDL-cholesterol subclasses, specifically the subclass HDL3-cholesterol can refine CVD risk and Metabolic syndrome risk better than HDL-cholesterol (Yang *et al.*, 2020).

Conversely, the WHO global status report on alcohol and health discussed the complex relationship between the volume and patterns of alcohol consumption and the prevalence of CVD in many studies (World Health Organization, 2018). WHO reported that people who consume alcohol in low-to-moderate amounts and that do not drink heavily on an irregular basis have a lower risk of developing CVD, whereas, individuals who engage in heavy drinking irregularly and in high volumes have a high disease risk (World Health Organization, 2018). In addition, a meta-analysis was conducted on 37,494 individuals in North America and Europe found no evidence of a protective effect of alcohol consumption on cIMT (Britton *et al.*, 2016). In contrast to other studies, it was observed that heavy drinking during midlife had greater cIMT values than moderate drinkers and that moderate drinkers had similar cIMT values compared to non-drinkers (Britton *et al.*, 2016). The strength of this study was that it was a longitudinal study and was able to evaluate the long-term effects of alcohol over a period of 20 years, as opposed to a cross sectional study (Britton *et al.*, 2016).

For this reason, the United Nations have set goals to ultimately improve health worldwide and have included alcohol abuse as one of the sustainable development goal (SDG) targets, specifically SDG 3 “Strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol” which many countries including South Africa

have agreed to achieve by 2030 (World Health Organization, 2018). A recent study has reported that little seems to have been done in the majority of sub-Saharan countries in the African region to address the abuse of alcohol (Ferreira-Borges, Parry and Babor, 2017). In 2010, South Africa was classified as one of the countries with the highest alcohol per capita consumption (APC) in the WHO African Region, which was equivalent to 11 Litres of pure alcohol and of this consumption, 3% (2.9 Litres) per person (Ferreira-Borges, Parry and Babor, 2017). Determinants of the consumption and abuse of alcohol are often social and cultural characteristics within a community (Dias, Oliveira and Lopes, 2011). For example, drinking was perceived as notions of masculinity, and therefore men were more likely to consume alcohol if conformed to the norms of society (Dias, Oliveira and Lopes, 2011). Cultural and social determinants may also be the reason why alcohol consumption is relatively high in South Africa.

Therefore, evidence suggests that alcohol has an effect on atherosclerotic development (Ferreira-Borges, Parry and Babor, 2017). However, the relationship between the volume and pattern of alcohol consumption and the prevalence of CVD are not consistent among many epidemiological studies and thus needs to be further investigated.

2.3.9 Gender

According to previous literature, women are generally more protected from CVD than men, but the roles are reversed as age increases (Pucci *et al.*, 2017). According to a recent systematic review, women have an equal and higher risk of CVD compared to men due to the higher prevalence of metabolic syndrome, especially in older women (Pucci *et al.*, 2017). It is to be noted that an increase in cardiovascular risk in women could be due to associated biological disturbances caused by hormonal changes during menopause, metabolic syndrome or polycystic ovarian syndrome, such as insulin sensitivity, HDL-cholesterol and body fat distribution and percentage (Pucci *et al.*, 2017). A study conducted in the Slovak Republic,

reported a significant difference in BMI, WHR and body fat percentage between male and female medical students, of which males had a higher cardiovascular risk factor prevalence (Rimárová *et al.*, 2018).

In South Africa, a study conducted among adolescents aged between 13-18 years in Mthatha, reported that female adolescents had a higher prevalence (47.5%) of overweight/obesity compared to male adolescents (Sekokotla *et al.*, 2017). Sekokotla, et al (2017) also reported a significantly higher BMI and WC in female adolescents compared to their male counterparts. However, male adolescents had a higher prevalence of having three to five cardiovascular risk factors (Sekokotla *et al.*, 2017). In contrast, Oldewage-Theron, Egal and Grobler (2017), reported that no CVD associated trends were observed across gender in children in the Eastern Cape, South Africa.

2.3.10 Genetics and maternal factors

2.3.10.1 Dyslipidaemia

Maternal lipid levels have been shown to have an effect on cholesterol and triglyceride levels of neonates and it has been suggested that cholesterol is transported to the fetus through the placenta via ATP (adenosine triphosphate) cassette binding transporter A1 (ABCA1) and ATP cassette binding transporter G1 (ABCG1) (Benedict *et al.*, 2018). These transporters have been further studied and seem to play a role in a disorder of fetal synthesis of cholesterol (Benedict *et al.*, 2018). Maternal hypercholesteremia has been reported to increase fatty streak formation in spontaneously aborted foetuses (Benedict *et al.*, 2018). In new-borns, some fatty streak formations would revert, while others would progressively accelerate during childhood (Benedict *et al.*, 2018).

2.3.10.2 Malnutrition

During the Dutch famine, a 5-month period of life-threatening food shortages from 1944 to 1945 resulted in maternal undernutrition and fetuses being exposed to restricted nutrition

during early gestation (Lunde *et al.*, 2016). Poor nutrition during pregnancy resulted in the offspring having a predisposition to chronic diseases in adulthood with increased morbidity of CVD, coronary artery disease, adult obesity and coronary heart disease as well as a higher risk of developing hypertension, stoke and hyperlipidaemia (Lunde *et al.*, 2016). In addition, the offspring had an increased atherogenic lipid profile and higher levels of plasma fibrinogen (Lunde *et al.*, 2016). Other maternal factors may also influence or trigger events during fetal development such as endocrine disruptors, obesity, maternal stress, maternal diabetes, substance abuse and poor maternal nutrition (Lunde *et al.*, 2016).

2.3.10.3 Psychosocial stress

Psychosocial stressors include anxiety, pregnancy related anxiety, job strain, depressive symptoms and parenting stress (van Dijk *et al.*, 2012). Psychosocial stress is a potential CVD risk factor that seem to contribute to all the underlying mechanisms of cardiac events, such as endothelial dysfunction and atherosclerosis, plaque rupture, thrombosis, cardiac ischaemia, malignant arrhythmias as well as the clustering of traditional CVD risk factors (Merz *et al.*, 2002). Studies have shown that people who are socioeconomically disadvantaged may have higher levels of psychosocial stress, partially due to the experience of poorer living conditions, anxiety and daily challenges (Eick *et al.*, 2019).

Eick et al (2019) observed that women at a socioeconomic disadvantage had higher psychosocial stress during pregnancy and higher levels of oxidative stress. However, elevated oxidative stress which is normally associated with psychosocial stress in normal populations, was not associated with psychosocial stress during pregnancy (Eick *et al.*, 2019). Lower socioeconomic status (SES) and elevated levels of psychosocial stress have been associated with adverse pregnancy outcomes (Loomans *et al.*, 2013; Eick *et al.*, 2019). For example, a study reported that infants born to mothers who were characterised as ‘high depression, high anxiety and moderate job strain’ had a significantly lower birth weight when compared to

infants from women with low depression, low anxiety and moderate job strain (Loomans *et al.*, 2013). In addition, a prospective paediatric study reported an association between multiple psychosocial stressors during pregnancy with an increase of 1.5 mmHg systolic and diastolic BP as well as a 1.5 mmHg increase in mean arterial pressure (MAP) children aged 5-7 years old (van Dijk *et al.*, 2012). Therefore, it is evident that increasing psychosocial stress during pregnancy, specifically in low socioeconomic populations may result in the development of CVD risk factors in the offspring, predisposing the child to CVD later in life.

2.4 Maternal smoking

Tobacco smoking during pregnancy ranged from 1.2% to 29% in other countries, such as the Netherlands and Israel (Geerts *et al.*, 2008a; Jaddoe *et al.*, 2008; Leybovitz-Haleluya *et al.*, 2018). Results from the South African National Health and Nutrition Examination Survey (SANHANES-1) conducted in 2011-12 reported a 5% prevalence of maternal smoking and a 3.7% prevalence of alcohol consumption during pregnancy in South Africa (Davoudi-Kiakalayeh *et al.*, 2017). A study conducted in South Africa reported a prevalence of 24% of prenatal smoking, of which 94% of mothers reported a daily smoking habit of 1.2 pack-years which is equivalent to 20 cigarettes per day per year (Vanker *et al.*, 2016). However, measurements of cotinine levels of infants at birth showed that 56% of infants had urine cotinine indicating nicotine exposure during pregnancy (Vanker *et al.*, 2016). Another study conducted in South Africa reported a prevalence of 18% and 30% of infants born with urine cotinine levels, through active smoking and passive smoking exposure, respectively (Vanker, Gie and Zar, 2018). Leybovitz-Haleluya et al. (2018) reported CVD morbidity in 1.3% and heart disease in 0.9% of participants 18 years after fetal exposure to nicotine, which was higher than those with no exposure (Leybovitz-Haleluya et al., 2018).

Therefore, it is evident that *in utero* exposure to nicotine could have severe long-term effects on cardiovascular health in offspring. However, the underlying mechanism of nicotine on the cardiovascular system *in utero* is not well understood.

2.4.1 *In utero* nicotine exposure and cardiovascular outcomes

In utero exposure to nicotine causes adverse pregnancy outcomes such as changes in birth weight, increased perinatal mortality, oxidative stress and atherosclerotic lesions in the coronary vessels of neonates (Benedict *et al.*, 2018). In Israel, a study reported a prevalence of 2861 (1.2%) new-borns exposed to maternal smoking during pregnancy (Leybovitz-Haleluya *et al.*, 2018). In the cardiovascular system, nicotine increases atherosclerotic plaque formation which leads to atherogenic and ischemic changes, increasing the likelihood of hypertension and cardiovascular morbidity later in life.

2.4.2 *In utero* nicotine exposure and adiposity

Obesity is caused by a chronic energy imbalance whereby the energy intake exceeds the energy expenditure (Haghighi *et al.*, 2013). *In utero* exposure to nicotine was associated with an increase in BMI and WC in adulthood compared to those with no exposure, which remained significant after the adjustment for risk factors in all life stages (Power, Atherton and Thomas, 2010; Bakker and Jaddoe, 2011; Kataria, Gaewsky and Ellervik, 2018). Power, Atherton and Thomas (2010) observed a dose-response relationship between BMI and WC and the number of cigarettes smoked per day (Power, Atherton and Thomas, 2010). A general population study and meta-analysis reported that adults with *in utero* nicotine exposure had an increased adjusted odds ratio of 1.34 and 1.46 for overweight and obesity, respectively (Kataria, Gaewsky and Ellervik, 2018). Similarly, a study reported an odds ratio of 1.53 for obesity in women exposed to nicotine *in utero* after adjusting for age, education and personal smoking (Cupul-Uicab *et al.*, 2012). The regulation of dietary preferences for fat is controlled by the brain stem and hypothalamus which functions to regulate energy balance, as well as the amygdala, nucleus

accumbens and the orbitofrontal cortex which forms part of the reward mechanisms (Haghighi *et al.*, 2013). Adolescents with *in utero* exposure to nicotine showed a significantly smaller amygdala volume (P<0.001) compared to adolescents with no exposure, and remained significant after adjustment for socioeconomic status, perinatal factors and maternal BMI (Haghighi *et al.*, 2013). Smaller amygdala volume also correlated significantly (P< 0.006) with fat intake in adolescents with *in utero* nicotine exposure (Haghighi *et al.*, 2013).

2.4.3 *In utero* nicotine exposure and atherosclerosis

In utero nicotine exposure has also been associated with increased total cholesterol and is suggested to result in adverse lipoprotein profiles in children (Jaddoe *et al.*, 2008; Bakker and Jaddoe, 2011). In contrast, other studies reported that total cholesterol was not associated with *in utero* exposure to nicotine (Power, Atherton and Thomas, 2010). It has been suggested that nicotine affects the vascular wall, eventually leading to atherosclerotic plaque formation (Bakker and Jaddoe, 2011; Chaturvedi *et al.*, 2015; Benedict *et al.*, 2018). In the Netherlands, a retrospective study on offspring approximately 30 years after exposure to maternal and paternal smoking during pregnancy, reported that offspring with nicotine exposure had a significantly greater cIMT (13.4 µm) than those with no exposure which remained significant after adjustment for known CVD risk factors including age, gender, BMI, pulse pressure, and LDL-cholesterol (Geerts *et al.*, 2008a). Furthermore, higher cIMT values were associated with maternal smoking during pregnancy and a dose-response relationship was found between the average cigarettes smoked per day and cIMT values in offspring (Geerts *et al.*, 2008a). However, it is important to note that the majority (38.1%) of the offspring with *in utero* exposure to nicotine were current smokers, which may have been a contributing factor to the higher cIMT values (Geerts *et al.*, 2008a).

In animal studies, rats exposed to 1 mg/kg/day nicotine during pregnancy and lactation showed irregular and abnormal alignment of the abdominal aorta wall with an irregular arrangement of

the vascular smooth muscle cells at the tunica media layers (Alfourti, Azzwali and Azab, 2019). This irregularity of the vascular smooth muscle cells predisposed the rats to atherosclerotic plaque deposition (Alfourti, Azzwali and Azab, 2019).

2.4.4 *In utero* nicotine exposure and hypertension

Studies by Bruin, Gerstein and Holloway, (2010); Cupul-Uicab *et al.* (2012) reported an association between *in utero* exposure to nicotine and the development of hypertension in adulthood. A Norwegian mother and child cohort study also reported an association between *in utero* exposure to nicotine and hypertension in adulthood (Cupul-Uicab *et al.*, 2012). Adults with *in utero* nicotine exposure had an adjusted odds ratio of 1.17 for hypertension compared to adults with no exposure (Kataria, Gaewsky and Ellervik, 2018). Whereas another study reported a slightly higher odds ratio of 1.68 for hypertension in women with *in utero* nicotine exposure (Cupul-Uicab *et al.*, 2012). Bruin, Gerstein and Holloway (2010) explains that animal studies have demonstrated that nicotine increases the risk of the development of hypertension even after adjustment of confounding factors, which suggest that nicotine alone may be accountable for adverse health outcomes in adulthood. This was evident in another study that demonstrated that *in utero* nicotine exposure during gestation and lactation increased blood pressure in male offspring (Alfourti, Azzwali and Azab, 2019).

2.4.5 Proposed mechanisms of *in utero* nicotine exposure

2.4.5.1 *In utero* nicotine uptake and atherogenic changes

During pregnancy, nicotine crosses the placental barrier in pregnant women and accumulates in the amniotic fluid, fetal serum and is also detectable in breastmilk during lactation (Alfourti, Azzwali and Azab, 2019; Alkam and Nabeshima, 2019). Chaturvedi et al (2015) suggests that nicotine affects the structural and functional characteristics of blood vessels and endothelial cells by increasing the release of fibroblast growth factors and thus preventing the production of growth factor β1 (Chaturvedi et al., 2015). These changes will essentially lead to an

increased mitogenic activity, increased deoxyribonucleic acid (DNA) synthesis, endothelial proliferation and eventually atherosclerotic plaque formation (Chaturvedi et al., 2015).

2.4.5.2 Chronic hypoxia

A suggested mechanism is *in utero* exposure to nicotine through chronic hypoxia by an increased placental resistance, increase carboxyhaemoglobin and decreased uterine blood flow (Leybovitz-Haleluya et al., 2018). Carbon monoxide, a hazardous tobacco component binds to hemoglobin, the molecule in blood that carries oxygen (Soothill and Ayida, 1996; Talhout *et al.*, 2011). When carbon monoxide is bound to hemoglobin, oxygen cannot bind, thereby decreasing the amount of oxygen delivered to all cells (Soothill and Ayida, 1996). Furthermore, the presence of carboxyhaemoglobin reduces the oxygen carrying capacity of the erythrocytes, leading to reduced blood flow and oxygen to the fetus (Soothill and Ayida, 1996).

2.4.5.3 Disturbance in endocrine control

It has been suggested that *in utero* nicotine exposure can result in increased adiposity by altering endocrine homeostatic control of body weight and regulation of appetite and satiety, which is controlled by the hypothalamus (Bruin, Gerstein and Holloway, 2010). For instance, the biological mechanism accountable for the association between *in utero* nicotine exposure and childhood adiposity could be due to disturbances in hypothalamic regulation, resulting in increased appetite (Bruin, Gerstein and Holloway, 2010).

2.5. Maternal alcohol consumption

A systematic review across 50 countries and six WHO regions, reported a global prevalence of 9.8% of alcohol consumption during pregnancy (Popova *et al.*, 2017). The European region had the highest prevalence of alcohol consumption during pregnancy which included five countries: Russia (36.5%), United Kingdom (41.3%), Denmark (45.8%), Belarus (46.6%) and Ireland (60.4%), whereas the African region had the third highest prevalence of 10% alcohol use during pregnancy (Popova *et al.*, 2017). Popova et al (2017) also observed that the African

region had a low prevalence of heavy episodic drinking (16.4%), but an extremely high alcohol consumption per capita (19.5 litres) and can be assumed that women consuming alcohol during pregnancy are more likely to practise heavy drinking (Popova *et al.*, 2017). A study conducted on alcohol consumption among pregnant women in Canada and the United states reported a prevalence of 11.5% of current drinking (at least 1 drink) and 3.9% binge drinking (4 or more drinks on at least one occasion) among pregnant women (Denny *et al.*, 2019).

In South Africa, approximately 3.7% of women reported alcohol use during pregnancy, with a prevalence of 7.2% in the Northern Cape, 7.3% in the Free State and 6.1% prevalence in the Western Cape, these being the highest prevalence's of maternal alcohol consumption among the nine provinces in South Africa (Peltzer and Pengpid, 2019). Another study reported a prevalence of maternal smoking in more than 50% of mothers, of which 18% and 30% of infants were born with urine cotinine levels indicating active and passive smoking exposure, respectively (Vanker, Gie and Zar, 2018). In a South African national population-based study, a prevalence of 60% of women reported alcohol consumption once or twice per week during their pregnancy, 23.7% reported alcohol consumption 3-4 times a week, 7.8% consumed alcohol 5-6 times per week, 5.4% consumed alcohol once or twice a day and 4.1% of women consumed alcohol at least 3 times a day during their pregnancy (Peltzer and Pengpid, 2019).

A study in Cape Town reported a 28% prevalence of self-reported antenatal alcohol use among mixed race, and 8% in black Africans (Vanker *et al.*, 2016). Another study conducted in Cape Town reported that majority of pregnant women (92%) self-reported severe alcohol abuse, ranging from 4 to 84 drinks per week (Steven *et al*., 2015). It was believed that it was a normal habit to consume alcohol during pregnancy and that the fetus will not be affected (Steven *et al*., 2015). Another study in the Western Cape reported a prevalence of 13% of hazardous alcohol use during pregnancy and a prevalence of 28% of hazardous tobacco use (Myers *et al.*, 2018). The majority of mothers who consumed alcohol during pregnancy did not complete

school, were unemployed, had a low household income and were of mixed ancestry (Vanker *et al.*, 2016; Myers *et al.*, 2018; Peltzer and Pengpid, 2019). Concurrent alcohol consumption and tobacco use seem to be prevalent among pregnant women in the Western Cape (Davoudi-Kiakalayeh *et al.*, 2017; Myers *et al.*, 2018; Peltzer and Pengpid, 2019). This is of concern as alcohol and nicotine have harmful effects on most organ systems in the fetus, including the cardiovascular system (Parkington *et al.*, 2010; Cupul-Uicab *et al.*, 2012; Benedict *et al.*, 2018).

2.5.1 *In utero* alcohol exposure and cardiovascular outcomes

In utero exposure to alcohol results in several developmental defects such as brain abnormalities (fetal alcohol syndrome), malfunctions in the CNS, reproductive systems, immune system and growth deficiencies in fetal organs and organ systems (Parkington *et al.*, 2010; Benedict *et al.*, 2018; Akison *et al.*, 2019; Weeks *et al.*, 2020). These adverse effects are collectively known as fetal alcohol syndrome (Caputo, Wood and Jabbour, 2016). Despite the harmful effects of *in utero* alcohol exposure on the CNS, alcohol also affects the cardiovascular system, specifically cholesterol levels and intima medial thickness (Parkington *et al.*, 2010; Britton *et al.*, 2016; Caputo, Wood and Jabbour, 2016; Benedict *et al.*, 2018; Dejong, Olyaei and Lo, 2019; Ehrhart *et al.*, 2019; Weeks *et al.*, 2020). Studies have reported the association between prenatal alcohol exposure and structural anomalies including cardiac malformations and vascular endothelial dysfunction in the fetus (Parkington *et al.*, 2010; Rodríguez-Rodríguez *et al.*, 2018; Dejong, Olyaei and Lo, 2019).

2.5.2 *In utero* alcohol exposure and adiposity

A systematic review observed that the effect of *in utero* exposure to alcohol varied among studies, of which human studies showed no effect of alcohol on adiposity, whereas animal models with high ethanol exposure reported increased adiposity in their offspring (Akison *et*

al., 2019). In mice, gestational ethanol exposure was associated with increased adiposity in adult male offspring compared to controls at 12 weeks of age (Zhang *et al.*, 2019).

In contrast, an animal study observed no effect on adiposity in mice offspring and also did not increase adiposity risk in offspring on high fat diets (Amos-Kroohs *et al.*, 2018). Amos-Kroohs et al (2018) suggested that prior reports of obesity in adults with *in utero* exposure was due to additional gestational calories and not through the pharmacological action of alcohol and therefore dietary practices should be considered.

2.5.3 *In utero* alcohol exposure and atherosclerosis

Altered lipid metabolism as a result of ethanol exposure *in utero* has been reported in several preclinical studies, specifically hypercholesterolaemia and/or dyslipidaemia in offspring with *in utero* alcohol exposure, at least in animal studies (Akison *et al.*, 2019; Weeks *et al.*, 2020). Although results showed altered lipid metabolism despite dose or timing of alcohol exposure, Akison et al (2019) suggested that a dose-dependent relationship exists between *in utero* alcohol consumption and the development of dyslipidaemia in offspring (Akison *et al.*, 2019). A retrospective cross-sectional study reported that adult patients with prenatal alcohol exposure had increased metabolic abnormalities such as low HDL-cholesterol (31.9%), elevated triglycerides (34.5%), and overweight/obesity (64.9%) (Weeks *et al.*, 2020). However, males had a higher incidence of metabolic abnormalities (61.2%) despite their low BMI, compared to females (35.9%) (Weeks *et al.*, 2020). Weeks et al (2020) further used a zebrafish model for prenatal alcohol exposure which showed that prenatal alcohol exposure is a risk factor for diet-induced obesity in male zebrafish. This shows evidence that maternal alcohol consumption may play an important role in dyslipidaemia and therefore cIMT in the offspring.

2.5.4 *In utero* alcohol exposure and hypertension

In animal studies, *in utero* alcohol exposure significantly increased heart rate in male rats, but had no effect on blood pressure in male rat offspring at 19 weeks of age (Amos-Kroohs *et al.*,

2018). Amos-Kroohs e al (2018) suggested that the age of 17-22 weeks was too young to exhibit metabolic dysfunctions in rat models of prenatal alcohol exposure and concluded that prenatal alcohol exposure did not alter cardiac function. In contrast, Gray et al (2010) observed a significantly higher mean arterial blood pressure of 10 mmHg in both male and female offspring at 6 months of age compared to the control group. However, no changes in heart rate were observed (Gray *et al.*, 2010). Gray et al (2010) reported that alcohol exposure caused a permanent reduction of approximately 10 to 20% in nephron number, which has been associated with the onset of hypertension in adulthood.

2.5.5 Proposed mechanisms of *in utero* alcohol exposure

2.5.5.1 *In utero* alcohol uptake

The distribution of ethanol is also particularly relevant during pregnancy, as 1-2 hours after maternal alcohol ingestion, the fetal alcohol concentrations reach levels nearly equivalent to the maternal levels (Hernández, López-Sánchez and Rendón-Ramírez, 2016). The elimination of ethanol by the fetus is impaired due to its reduced metabolic capacity, and thus, *in utero* exposure is prolonged through the reuptake of amniotic-fluid containing ethanol (Hernández, López-Sánchez and Rendón-Ramírez, 2016). Ultimately, the elimination of alcohol from the fetus depends on the mother's metabolic capacity, which inevitably, is a process that occurs late, meaning that the fetus is exposed to the toxicological effects of alcohol before the toxin is eliminated (Hernández, López-Sánchez and Rendón-Ramírez, 2016).

Many animal studies have shown evidence to support the DOHaD and programming of CVD later in life which have recommended for longitudinal studies to be conducted to study the long-term effects of *in utero* exposure to alcohol (Hernández, López-Sánchez and Rendón-Ramírez, 2016).

2.5.5.2 DNA methylation

In utero alcohol exposure has consistently demonstrated alterations in blood vessels, such as remodelling, endothelial dysfunction and stiffening which may be mediated by epigenetic modulation of expression of genes that play a role in cardiovascular growth and control (Rodríguez-Rodríguez et al., 2018). Epigenetics refers to a series of biochemical processes causing heritable changes in gene transcription during the life cycle of an organism, with no change in the actual DNA sequence (Lunde *et al.*, 2016; Vaiserman, 2020). DNA methylation is the binding of a methyl group to the DNA sequence, specifically where cytosine is positioned next to guanine, called a cytosine-phosphate-guanine site (CpG site). DNA methylation at CpG sites inhibits genes expression by inhibiting transcription factors from binding to the promoter regions of the DNA sequence (Felix and Cecil, 2018). Teratogens such as alcohol and nicotine can also programme the fetus for the development of CVD later in life (Parkington *et al.*, 2010; Rodríguez-Rodríguez *et al.*, 2018). This is because maternal smoking exposes the fetus to toxins which can directly damage the genetic material of the fetus (Leybovitz-Haleluya *et al.*, 2018). Studies suggest that *in utero* exposure nicotine or alcohol can cause significant changes in DNA methylation in the fetus (Parkington *et al.*, 2010; Bakker and Jaddoe, 2011; Vaiserman, 2020).

2.5.5.3 Oxidative stress

Studies have proposed that alcohol causes oxidative stress which leads to redox alterations (Rodríguez-Rodríguez *et al.*, 2018). Oxidative stress is an imbalance in reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) which leads to oxidative damage in macromolecules (Hernández, López-Sánchez and Rendón-Ramírez, 2016; Rodríguez-Rodríguez *et al.*, 2018). When the redox balance is altered during pregnancy, it causes an excess production of ROS resulting in a pro-oxidative state which will compromise fetal growth as the embryo has a low antioxidant capacity (Rodríguez-Rodríguez et al., 2018).

Oxidative stress also decreases Nitric Oxide (NO) synthesis, a vasodilator which inhibits platelet aggregation and adhesion and therefore atherosclerosis (Bosco and Diaz, 2012). Toda and Ayajiki (2012) reported that low concentrations of alcohol increase the NO production in human endothelial cells, whereas high concentrations induce endothelial dysfunction and apoptosis. Conversely, Parkington, Coleman and Wintour (2010) concluded that alcohol in fact enhances NO production and has a direct effect on vascular smooth muscle by increasing the density of Endothelin B (ET_B) receptors on endothelial cells which in turn promotes NO release. However, when endothelial cells are persistently challenged with an inhospitable environment, it can lead to oxidative stress, endothelial dysfunction and a decrease in NO bioavailability (Parkington *et al.*, 2010).

2.6 Concurrent use of tobacco and alcohol during pregnancy

Hazardous tobacco use increases the odds of alcohol abuse among pregnant women (Myers *et al.*, 2018). Additionally, studies have reported concurrent use of tobacco and alcohol during pregnancy (Davoudi-Kiakalayeh *et al.*, 2017; Peltzer and Pengpid, 2019). Results from the SANHANES-1 conducted in 2011-12 reported a prevalence of 31.5% of South African mothers that had been consuming alcohol in combination with smoking during pregnancy (Davoudi-Kiakalayeh *et al.*, 2017). Dual exposure to nicotine and alcohol may have a double negative effect on the cardiovascular system of the fetus and may considerably increase the risk of developing CVD risk factors in adulthood (De Smidt *et al.*, 2019).

2.7 Conclusion

Cardiovascular disease risk factors such as alcohol consumption and tobacco smoking are highly prevalent in South Africa, specifically among women during pregnancy. Studies have also reported a prevalence of CVD risk factors in children which further increases their risk of the development of atherosclerosis. Studies have observed an association between *in utero* nicotine exposure and an increased adiposity and BP in children. *In utero* exposure to alcohol

has shown effects on cholesterol levels, cIMT and hypertension in animal studies. Furthermore, *in utero* exposure to nicotine and alcohol has been suggested to cause endothelial dysfunction through biological mechanisms such as DNA methylation, oxidative stress, chronic hypoxia and a disturbance in endocrine control.

Further studies are required to determine the effects of *in utero* dual exposure to nicotine and alcohol on the development of CVD risk factors in childhood which predisposes children to the onset of CVD later in life. Screening for CVD risk factors in children should be prioritized to prevent the development of CVD later in life. Further research is required to determine the odds/relative risk of children exposed to nicotine and alcohol during pregnancy compared to children with no exposure.

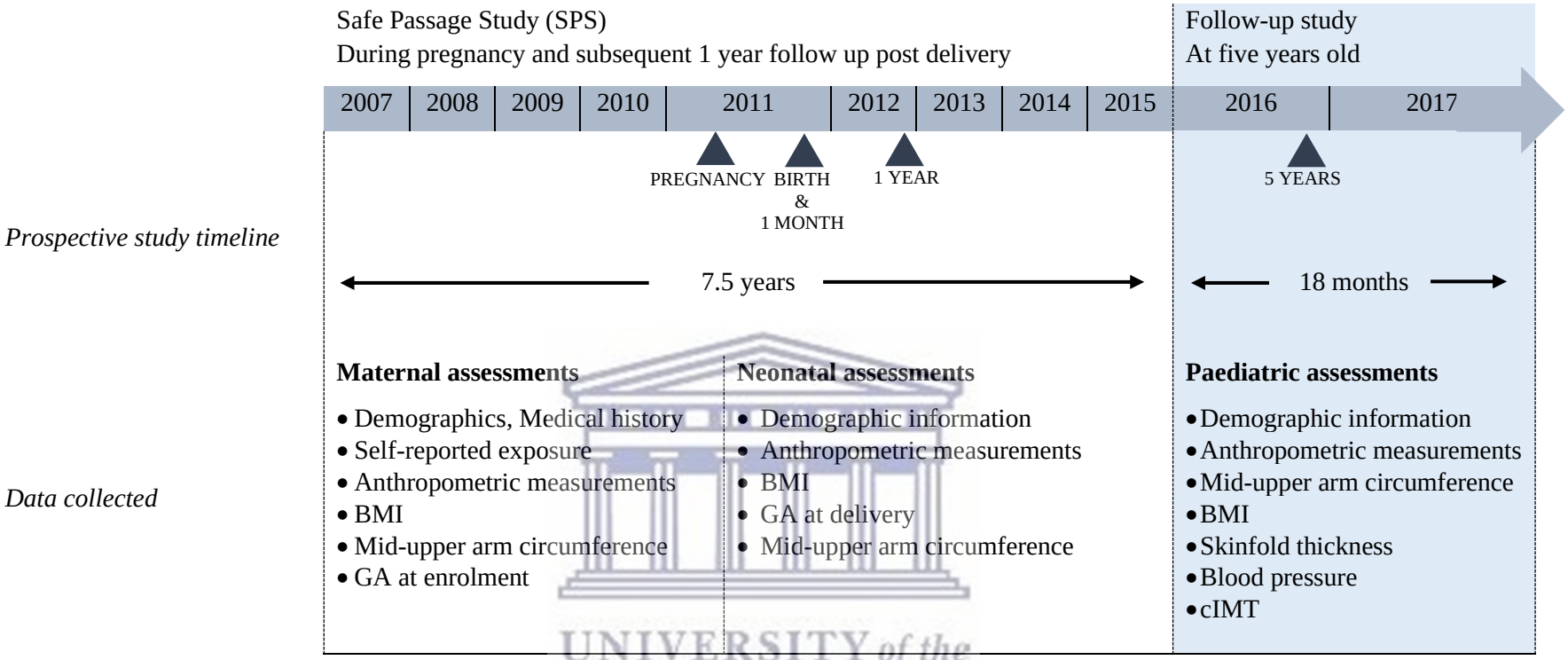
CHAPTER 3: METHODOLOGY

3.1 Introduction

This section describes the study design and participant recruitment, followed by the paediatric procedures. Paediatric assessments included a questionnaire, anthropometry, and clinical examination of cIMT and BP. All measurements were taken three times, of which the average was used as the final value. The data was then captured into Microsoft excel and transferred to SPSS (version 26) to be statistically analysed. The demographic characteristics, anthropometric measurements and cIMT values was presented using tables and graphs.

3.2 Research design

This was a case-control follow-up study of a larger longitudinal study called the Safe Passage Study (SPS) (Dukes *et al.*, 2014). In the SPS study, 500 children were assessed to determine the effect of antenatal exposure to alcohol in still births and sudden unexplained infant deaths. In this follow-up study, information on paediatric health was collected at the age of five years to explore the effects of *in utero* alcohol and nicotine exposure on cardiovascular risk factors. In Figure 3.1, the prospective study timeline shows the duration of the study and the data collected at each assessment.



Prospective study timeline

Data collected

Figure 3.1: Prospective study timeline and data collected in each study. GA at enrolment: Gestational age at enrolment; GA at delivery: Gestational age at delivery; cIMT: Carotid intima medial thickness. The study highlighted in blue forms part of the current analysis.

3.3 Participant recruitment

A total of 468 participants aged five years old from low socioeconomic communities were recruited to participate in this study. Consent was obtained from both parent or guardian and child. Participants were contacted telephonically, through SMS or by delivering letters to the addresses with no contactable numbers to invite them to participate in the study.

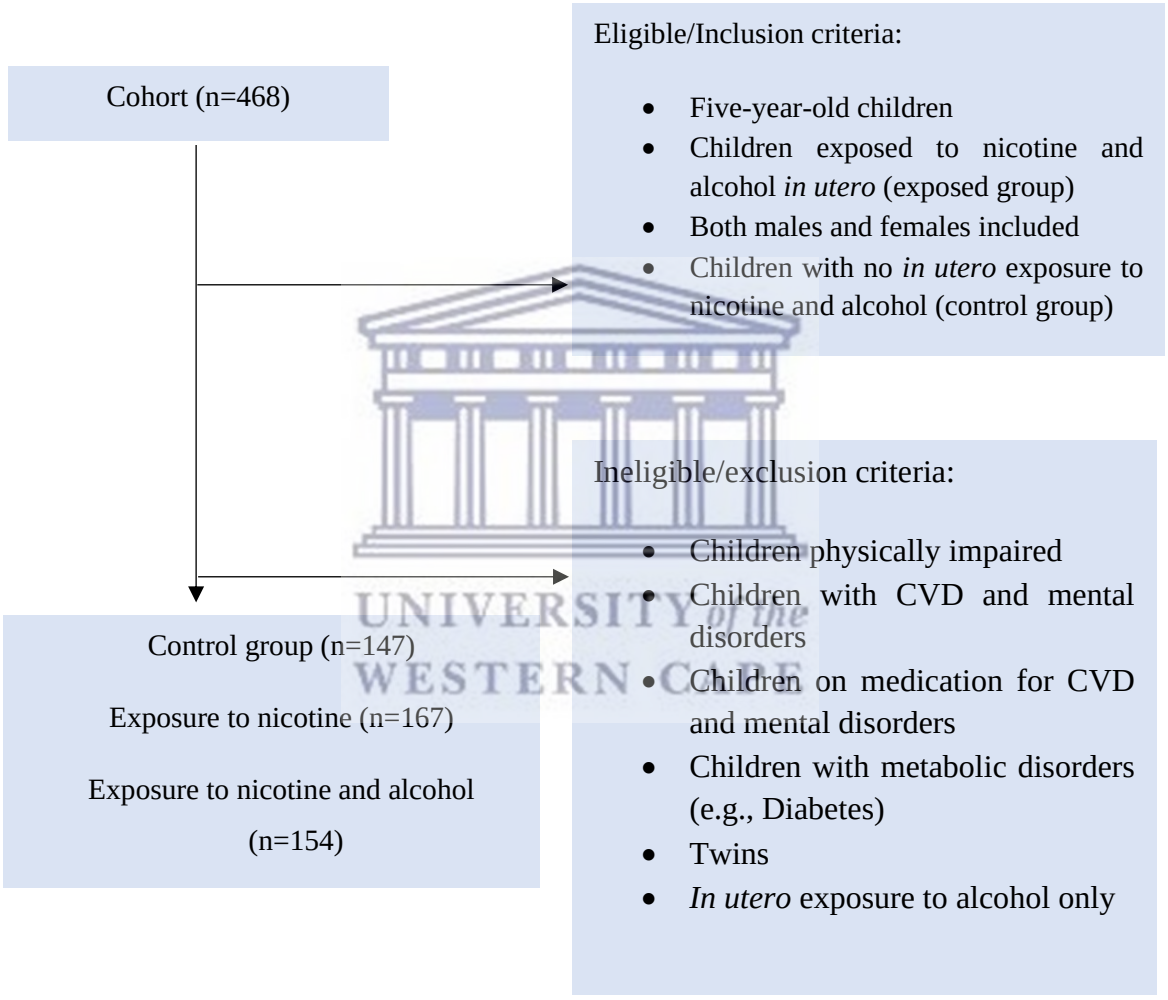


Figure 3.2: A flow diagram of participant selection criteria for the study.

3.4 Paediatric assessments

3.4.1. Participant selection and screening

In utero exposure, maternal characteristics and birth measurements were obtained from the SPS during visits at the Belhar antenatal clinic. Self-reported alcohol and tobacco use during

pregnancy by mothers determined *in utero* exposure as described by Dukes et al (2014). *In utero* exposure to nicotine was validated using meconium analysis after birth. These analyses correlated with the self-reported information provided by the mothers antenatally (Dukes *et al.*, 2014). In the present study, participants were interviewed in a private room with the parent and child and a study questionnaire was used to collect information on their demographic characteristics such as age, ethnicity, and education level. Information regarding their health status and lifestyle was asked such as their health status, self-reported conditions and diseases and prescribed medications.

The cohort was divided into three groups:

Nicotine exposed group: Children with *in utero* exposure to nicotine

Dual exposed group: Children with *in utero* exposure to nicotine and alcohol

Control group: Children with no *in utero* exposure to nicotine and alcohol

3.4.2 Body weight and stretch stature

Body weight and body length was measured at birth and at five years old. Weight was measured in kilograms (kg) using a Mellerware, Munich scale and rounded off to the nearest 0.01 kg. Stature was measured in centimetres (cm) using a mechanical Panamedic stadiometer height rod. The patient stood upright and barefoot with the buttocks, upper back and heels touching the stadiometer height rod. The stadiometer rod was lowered onto the top of the head as flat as possible and rounded off to the nearest 0.1 cm. Height was measured three times and the average was recorded as the final measurement.

3.4.3 Body Mass Index

Body Mass Index was measured at birth and at five years old, and was calculated by dividing the weight (kg) over the height (m^2). Overweight was classified as a BMI value of 16.6 kg/m^2

for boys and 16.9 kg/m² for girls (WHO, 2007a, 2007b). Obesity was classified as a BMI value of 18.3 kg/m² for boys and 18.9 kg/m² for girls (Johnson *et al.*, 2007; WHO, 2007a, 2007b).

3.4.4 Waist circumference

The waist circumference was measured between the tenth rib (the lowest rib margin) and the iliac crest or by the umbilicus level. The waist circumference procedure was followed according to the SANHANES clinical staff manual. The readings from a tape measure were taken three times and rounded to the nearest 0.1 cm. Waist circumference is an indicator of health risk assessment for obesity related diseases, as it is an indicator of central obesity.

3.4.5 Skinfold thickness

Triceps skinfold thickness (tSFT) and Subscapular skinfold thickness (sSFT) were measured using Holtain callipers. Each measurement was read approximately three seconds after the release of the calliper tension. The readings were recorded to the nearest 0.1 mm. The tSFT was measured at the midpoint of the posterior side of the right upper arm midway between the acromion process and the tip of the olecranon. The sSFT was measured approximately 20 mm below the inferior angle and a 45° angle, laterally, with the patients' shoulders and arms relaxed. These procedures were followed according to the SANHANES clinical staff manual.

3.4.6 Blood pressure

Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and MAP were measured at five years old using an automated digital sphygmomanometer (CAS Medical Systems, Inc. (Branford, CT), 740 MAXNIBP) and paediatric sized cuff. Blood pressure was measured on the right upper arm while the patient was in a sitting position. All measurements were taken three times with 1-2 minutes between measurements. The mean values were recorded as the final measurement.

3.4.7 CIMT B-mode Ultrasonography

All the cIMT ultrasonography procedure was performed and analysed by a single trained ultrasonographer, using a Voluson E8 ultrasound system (GE Healthcare, Kretz ultrasound, Zipf, Austria), using a 9L-D (3.1–10 MHz) transducer specialized for vascular application on paediatrics. The patient was placed in supine position on a bed with their head resting comfortably. The head of the patient was hyperextended and rotated in the opposite direction of the probe. The left and right Common Carotid artery (CCA) intima-media thickness were measured at five years old using the Mannheim Consensus, an international protocol (Touboul *et al.*, 2012). Touboul (2012) defined cIMT as a double-line pattern formed by two anatomical boundaries (lumen-intima and media-adventitia interfaces) which can be visualized by echography on both walls in a longitudinal image.

3.5 Data analysis

All the data was captured into a Microsoft Office Excel spreadsheet and exported to the Statistical Package for the Social Sciences (SPSS)® version 26 for statistical analysis. The data was coded and cleaned for errors. Each participant was allocated with a unique identity code to protect their identity.

3.6 Statistical analysis

The data was analysed using SPSS® software version 26. Frequency distributions were conducted on the demographic characteristics of the participants in both exposed group and control group. Descriptive statistical analysis was expressed as means, standard deviations and confidence intervals (95%) was calculated for each measurement that was taken including demographic characteristics. A Shapiro-Wilks test was used to test for normality. Majority of the data were not normally distributed and therefore, non-parametric tests were used. Descriptive statistical analysis was expressed as means and standard deviations (95% CI). Inferential statistics were generated using Spearman's r correlation coefficient, Pearson's chi

squared (X^2) test, Kruskal-Wallis H test and Mann Whitney U test. A p value of <0.05 was used to indicate statistical significance.

Spearman's r correlation coefficient was used to determine if there was a statistically significant correlation between the continuous variables (BMI, WC, sSFT, tSFT, SBP, DBP, MAP, HR). A Spearman's partial correlation was used to perform correlations while controlling for potential confounders. Potential confounders were selected based on previous knowledge in published literature and the possible association with the outcome variable. These included BMI at five years, WC at five years, length and weight at five years which were identified as confounding variables where applicable.

Pearson's chi-squared test was used to determine if there was a statistically significant association between categorical variables (nicotine exposure, dual exposure, gender, maternal anaemia, maternal hypertension, overweight, obesity, central obesity, prehypertension, systolic hypertension, diastolic hypertension, hypertension, high LcIMT, high RcIMT). Kruskal-Wallis H test was used to test if there was a significance difference between continuous variables across the control and exposure groups. Mann-Whitney U test was applied with a post hoc Bonferroni correction. After a Bonferroni correction, a p value of <0.0167 (0.05 ÷ 3 comparative groups) was used to indicate statistical significance. Correlations were reported as described by Akoglu (2018). A binomial logistic regression was performed to ascertain the independent association between dual exposure to nicotine and alcohol and high cIMT by adjustment for covariates (length at 5 years old, weight at 5 years old, BMI at 5 years old, and WC). All assumptions were met before a logistic regression model was constructed. A Bonferroni correction was applied by using all 9 items in the model resulting in a significance of p< 0.005. Based on this assessment, all continuous independent variables (length at 5 years old, weight at 5 years old, BMI at 5 years old, and WC) were found to be linearly related to the logit of the dependent variable (RcIMT).

3.7. Ethical clearance

Ethical clearance was obtained by the Health and Research ethics Committee (HREC) of Stellenbosch University as well as the Biomedical Research Ethics Committee (BMREC) of the University of the Western Cape (UWC). Written informed consent was obtained from the mother or guardian of the child.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter presents the results of the data analysis. In this study, a total of 468 -mother-child pairs from a low socioeconomic population, participated in this study. The average age of the children was 5.4 years. The cohort was separated into three groups: 31.4% in the control group (n=147), 35.7% in the nicotine exposed group (n=167) and 32.9% in the group with dual exposure to nicotine and alcohol (n=154). Overall, 50.9% of children were male and 49.1% were female. Cardiovascular risk factors were categorised according to percentile cut off values generated from a normal population (control group) which were gender and age specific for this population.

4.2 Maternal and neonatal characteristics

Maternal weight and BMI were significantly lower in the dual exposed group with maternal weight: 63.66 ± 14.49 kg at $p < 0.01$ and BMI: 25.058 ± 5.61 Kg/m^2 at $p < 0.01$. And, in the nicotine exposed group with maternal weight: 63.51 ± 14.83 kg at $p < 0.01$ and BMI: 25.14 ± 5.62 Kg/m^2 at $p < 0.01$, compared to the control group where maternal weight was 68.91 ± 18.29 at $p < 0.01$ and BMI Kg/m^2: 27.43 ± 7.07 at $p < 0.01$ as shown in Table 4.1. Gestational age at enrolment was significantly higher in the dual exposed group with GA at enrolment: 142.66 ± 49.37 days at $p < 0.01$ compared to the control group with GA at enrolment: 126.24 ± 44.69 days at $p < 0.01$ and the nicotine exposed group with GA at enrolment: 134.56 ± 46.20 days at $p < 0.01$ as shown in Table 4.1. Birth length was significantly lower in the dual exposed group with birth length: 48.31 ± 2.41 cm at $p < 0.05$ and in the nicotine group with birth length: 48.62 ± 2.12 cm at $p < 0.05$) compared to the control group with birth length: 49.06 ± 2.09 cm, $p < 0.05$) as shown in Table 4.1. Gestational age at delivery was significantly earlier in the dual exposed group with GA at delivery: 271.73 ± 14.16 days at $p < 0.05$ and nicotine exposed

group with GA at delivery: 269.87 ± 15.47 days at $p < 0.05$ compared to the control group with GA at delivery: 274.14 ± 13.78 days at $p < 0.05$ as shown in Table 4.1. However, there were no significant differences in birth weight as well BMI at birth when comparing the control and exposure groups as shown in Table 4.1.

Table 4. 1: Maternal and neonatal characteristics at enrolment according to exposure groups.

	Overall **N= 468**	**Control** **N= 147**	**Dual exposed** **N= 154**	**Nicotine exposed** **N= 167**	**p value**
Maternal characteristics	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**	
Length (cm)	158.86 ± 6.53	158.22 ± 6.93	159.62 ± 6.50	158.74 ± 6.15	0.173
Weight (kg)	65.24 ± 16.04	68.91 ± 18.29	63.66 ± 14.49	63.51 ± 14.83	0.004**
BMI (Kg/m^2)	25.83 ± 6.19	27.43 ± 7.07	25.058 ± 5.61	25.14 ± 5.62	0.001**
GA at enrolment (days)	134.62 ± 47.17	126.24 ± 44.69	142.66 ± 49.37	134.56 ± 46.20	0.010*
Neonatal characteristics at birth					
Birth length (cm)	48.66 ± 2.22	49.06 ± 2.09	48.31 ± 2.41	48.62 ± 2.12	0.026*
Birth weight (kg)	3.00 ± 0.56	3.06 ± 0.52	2.98 ± 0.57	2.98 ± 0.59	0.350
BMI at birth (Kg/m^2)	12.61 ± 2.04	12.60 ± 1.94	12.62 ± 1.81	12.61 ± 2.32	0.976
GA at delivery (days)	271.83 ± 14.99	274.14 ± 13.78	271.73 ± 14.16	269.87 ± 15.47	0,035*

Note: * indicates statistically significant differences < 0.05; ** indicates statistically significant differences < 0.01. BMI: body mass index, GA at enrolment: Gestational age at enrolment, GA at delivery: Gestational age at delivery.

Overall, 40% of mothers had anaemia, of which 13.1% of anaemic mothers were from the control group, followed by 14.1% of mothers from the nicotine exposed group and 12.8% from the dual exposed group. Overall, 9.8% of mothers were hypertensive, of which 4.0% of mothers

were from the control group, 3.2% were from the nicotine exposed group and 2.6% were from the dual exposed group as seen in Table 4.2.

Table 4.2: Maternal medical conditions at enrolment according to exposure groups.

	Overall	Control	Dual exposed	Nicotine exposed
Maternal medical conditions	**N= 468**	**N= 147**	**N= 154**	**N= 167**
Maternal Anaemia	187 (40%)	61 (13.1%)	60 (12.8%)	66 (14.1%)
Maternal Hypertension	46 (9.8%)	19 (4.0%)	12 (2.6%)	15 (3.2%)

4.3 Paediatric characteristics at five years old

Anthropometric measurements such as length, weight, tSFT and WC differed significantly between the control group and exposure groups (Table 4.3). Length and weight at five years old were significantly lower in the dual exposed group (109.01 ± 50.18 cm and 180.16 ± 20.71 kg) and in the nicotine exposed group (108.23 ± 40.49 cm and 170.69 ± 20.29 kg) compared to the control group (110.04 ± 50.19 cm and 180.69 ± 30.58 kg) with $p < 0.01$ as shown in Table 4.3. When comparing the nicotine exposed group and dual exposed group, length and weight at five years old were significantly higher in the dual exposed group (109.01 ± 50.18 cm and 180.16 ± 20.71 kg) compared to the nicotine exposed group (108.23 ± 40.49 cm and 170.69 ± 20.29 kg) with $p < 0.01$ as shown in Table 4.3. This was a 5.55% difference in weight and 0.72% difference in length between the dual exposed group and the nicotine exposed group. Weight was significantly the lowest in the nicotine group (170.69 ± 20.29 kg) compared to the control group (180.69 ± 30.58 kg) and dual exposed group (180.16 ± 20.71 kg) with $p < 0.01$ as shown in Table 4.3. Weight in the nicotine exposed group was 5.88% lower than the control group and 5.55% lower than the dual exposed group. However, there were no significant difference in BMI between the control and exposure groups (Table 4.3).

With regard to clinical measurements, SBP was significantly higher in the dual exposed group (107.15 ± 10.02 mmHg) and significantly lower in the nicotine exposed group (104.27 ± 9.33

mmHg) compared to the control group (106.49 ± 1.66 mmHg) with $p < 0.05$ in Table 4.3. Systolic blood pressure was 7.15% higher in the dual exposed group compared to the control group and SBP was 4.27% lower in the nicotine exposed group compared to the control group. Diastolic blood pressure, MAP and HR did not differ significantly between the control group and exposure groups.

Furthermore, the ultrasound measurements, specifically RcIMT was significantly higher in the dual exposed group (0.36 ± 0.05 mm) compared to the nicotine exposed group (0.34 ± 0.05 mm) and control group (0.34 ± 0.04 mm) with $p < 0.01$ as shown in Table 4.3 and Figure 4.1. This was a 5.88% higher RcIMT in the dual exposed group compared to the nicotine exposed group and the control group. The LcIMT did not differ significantly between the control group and exposure groups $p = 0.331$ (Table 4.3).

Table 4.3: Paediatric cardiovascular risk measurements at five years old according to exposure groups.

	Overall	Control	Dual exposed	Nicotine exposed	p value
	N= 468	N= 147	N= 154	N= 167	
Anthropometric measurements	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**	
Age (days)	1984.87 ± 50.86	1984.27 ± 51.39	1988.88 ± 52.68	1981.71 ± 48.71	0.446
Length (cm)	109.05 ± 40.99	110.04 ± 50.19	109.01 ± 50.18	108.23 ± 40.49	0.006**
Weight (kg)	180.16 ± 20.91	180.69 ± 30.58	180.16 ± 20.71	170.69 ± 20.29	0.010*
BMI (Kg/m^2)	150.20 ± 10.65	150.35 ± 20.07	150.21 ± 10.47	150.07 ± 10.37	0.311
sSFT (cm)	90.60 ± 20.97	70.66 ± 40.24	70.03 ± 10.89	60.92 ± 20.33	0.063
tSFT (cm)	70.18 ± 30.08	100.14 ± 30.77	90.38 ± 20.65	90.34 ± 20.71	0.040*
WC (cm)	510.30 ± 40.25	510.93 ± 50.20	510.43 ± 30.92	500.66 ± 30.53	0.036*
Clinical measurements					

SBP (mmHg)	105.91 ± 10.04	106.49 ± 1.66	107.15 ± 10.02	104.27 ± 9.33	0.026*
DBP (mmHg)	64.93 ± 9.06	65.25 ± 9.07	65.75 ±9.05	63.88 ± 9.01	0.160
MAP	78.49 ± 9.37	78.84 ± 9.48	79.45 ± 9.28	77.29 ± 9.30	0.104
HR (b/min)	91.84 ± 13.39	91.72 ± 12.43	92.47 ± 14.42	91.35 ± 13.27	0.749
Ultrasound measurements					
Left cIMT (mm)	00.35 ± 00.05	00.35 ± 00.05	00.36 ± 00.05	00.35 ± 00.05	0.331
Right cIMT (mm)	00.34 ± 00.04	00.34 ± 00.04	00.36 ± 00.05	00.34 ± 00.04	0.001**

Note: * indicates statistically significant differences < 0.05; ** indicates statistically significant differences < 0.01. BMI: body mass index, WC: waist circumference, tSFT: triceps skinfold thickness, sSFT: subscapular skinfold thickness, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, Right cIMT: right carotid intima-media thickness, left cIMT: left carotid intima-media thickness.

After a Bonferroni correction was applied for multiple comparisons to mitigate type 1 error, length, weight, tSFT, WC and SBP were not significantly different between the control group, nicotine exposed group and dual exposed group as shown in Table 4.4. However, RcIMT remained significantly higher in the dual exposed group when compared to the control group and nicotine exposed group with $p < 0.0167$ as shown in Table 4.4 and Figure 4.1.

Table 4.4: Results of Mann Whitney U test illustrating the differences between cardiovascular measurements in five-year-old children after a Bonferroni correction with a P value < 0.0167 according to exposure groups.

Exposure groups	BMI	WC	sSFT	tSFT	SBP	DBP	MAP	HR	LcIMT	RcIMT
Control vs Nicotine	0.577	0.13	0.376	0.078	0.096	0.224	0.237	0.697	0.864	0.427
Control vs Dual exposure	0.799	0.828	0.559	0.23	0.615	0.435	0.443	0.712	0.216	0.001*
Nicotine vs Dual exposure	0.333	0.037	0.116	0.608	0.021	0.052	0.032	0.905	0.292	0.01*

Note: * indicates statistically significant differences < 0.0167; BMI: body mass index, WC: waist circumference, tSFT: triceps skinfold thickness, sSFT: subscapular skinfold thickness, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, RcIMT: right carotid intima-media thickness, LcIMT: left carotid intima-media thickness.

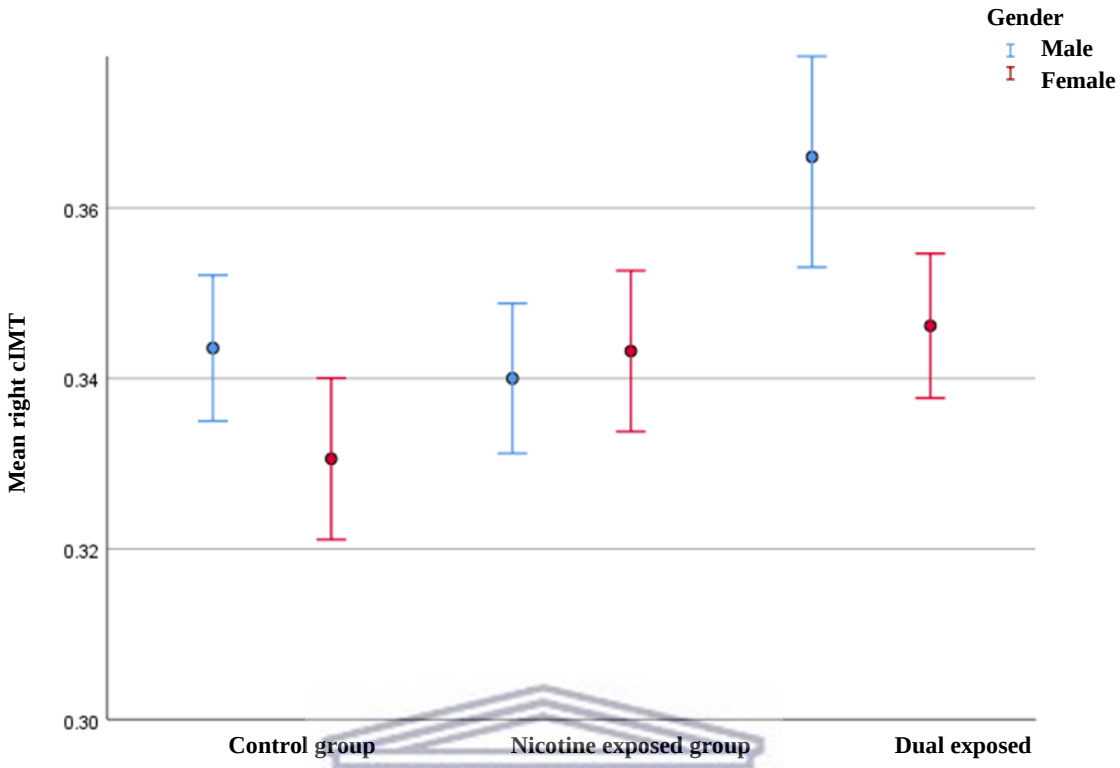


Figure 4.1: Mean RcIMT and 95% confidence intervals according to gender across the exposure groups.

In Table 4.5, there was a borderline significant difference in length in females at five years old across the exposure groups with $p < 0.05$. Regarding clinical measurements, there was a significant difference in SBP and MAP in females across the exposure groups with $p < 0.01$. In addition, there was a borderline significant difference in DBP in females across the exposure groups with $p = 0.05$, where the average DBP was highest in females in the control group (65.32 ± 8.72 mmHg), followed by the nicotine exposed group (65.31 ± 8.70 mmHg) and the dual exposed group (65.19 ± 9.13 mmHg). Regarding ultrasound measurements, the average RcIMT was significantly different in males across the exposure groups with $p < 0.01$, where the average RcIMT was highest in the dual exposed group (0.37 ± 0.06 mm), followed by the control group (0.35 ± 0.05 mm) and the nicotine exposed group (0.34 ± 0.04 mm).

Table 4. 5: Paediatric cardiovascular measurements at five years old across gender according to exposure groups.

		Overall	Control	Dual exposed	Nicotine exposed	P value
		N= 468	N= 147	N= 154	N= 167	
Anthropometric measurements	**Gender**	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**	
Age (days)	Male	1984.35 ±49.39	1984.67±49.49	1983.68±49.78	1985.47±51.06	0.562
	Female	1985.41±52.44	1984.78±52.45	1984.40±50.86	1986.28±51.64	0.624
Length(cm)	Male	109.17± 4.94	109.31±5.02	108.92±4.90	109.11±4.97	0.062
	Female	108.93±5.06	109.26±5.09	108.76±4.86	108.97±5.11	0.048*
Weight (kg)	Male	18.20±2.80	18.32±3.09	18.07±2.70	18.16±2.75	0.207
	Female	18.12±3.02	18.26±3.08	18.01±2.83	18.16±2.97	0.285
BMI (Kg/m^2)	Male	15.20±1.52	15.26±1.77	15.17±1.46	15.19±1.52	0.503
	Female	15.21±1.77	15.22±1.75	15.17±1.69	15.23±1.69	0.883
sSFT (cm)	Male	6.76±2.47	7.12±3.31	6.87±2.44	6.94±2.37	0.961
	Female	7.62±3.35	7.48±3.32	7.36±3.12	7.37±3.07	0.208
tSFT (cm)	Male	8.89± 2.85	9.37±3.25	9.15±2.83	9.21±2.87	0.378
	Female	10.35±3.15	10.10±3.24	9.91±3.10	9.91±3.06	0.356
WC (cm)	Male	51.21±3.98	51.45±4.49	51.05±3.88	51.28±3.96	0.458
	Female	51.40±4.51	51.45±4.49	51.22±4.27	51.39±4.38	0.221
Clinical measurements						
SBP (mmHg)	Male	104.91±9.88	105.57±10.37	104.58±9.49	106.06±10.01	0.816

	Female	106.95±10.13	106.52±10.00	106.37±10.20	106.39±10.09	0.001**
DBP (mmHg)	Male	64.25±9.43	64.69±9.38	64.11±8.95	64.96±9.39	0.956
	Female	65.6245±8.63	65.32±8.72	65.19±9.13	65.31±8.70	0.046*
MAP	Male	77.85±9.43	78.35±9.73	77.53±9.25	78.61±9.63	0.956
	Female	79.15±8.99	78.79±9.05	78.81±9.44	78.85±9.07	0.008**
HR (b/min)	Male	90.08±12.87	90.87±12.72	90.32±12.80	91.64±13.88	0.275
	Female	93.67±13.69	92.75±13.53	93.09±13.75	92.37±13.41	0.282
Ultrasound measurements						
Left cIMT (mm)	Male	0.36±0.05	0.36±0.05	0.37±0.06	0.36±0.05	0.105
	Female	0.35±0.05	0.34±0.05	0.35±0.05	0.35±0.05	0.497
Right cIMT (mm)	Male	0.34±0.04	0.35±0.05	0.37±0.06	0.34±0.04	0.005**
	Female	0.34±0.04	0.33±0.04	0.35±0.04	0.34±0.04	0.053

Note: * indicates statistically significant differences < 0.05; ** indicates statistically significant differences < 0.01; BMI: body mass index, WC: waist circumference, tSFT: triceps skinfold thickness, sSFT: subscapular skinfold thickness, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, Right cIMT: right carotid intima-media thickness, Left cIMT: left carotid intima-media thickness.

In Table 4.6, when comparing gender, females had a significantly higher sSFT in the control group (U = 3384,00, p < 0.01), in the nicotine exposed group (U = 4138.00, p < 0.05) and dual exposed group (U = 4020.50, p < 0.001) when compared to males. Percentage differences in sSFT between males and females was 5.06% in the control group, 6.20% in the nicotine exposed group and 7.13% in the dual exposed group. Females had a significantly higher tSFT in the control group (U = 3575.00, p < 0.01), in the nicotine exposed group (U = 4564.50, p < 0.001) and dual exposed group (U = 4048.00, p < 0.001) when compared to males. Percentage differences in tSFT between males and females was 7.79% in the control group, 7.60% in the nicotine exposed group and 8.31% in the dual exposed group. In the dual exposed group, females had a significantly higher SBP (U = 3829.50, p < 0.01), DBP (U = 3527.50, p < 0.05), MAP (U = 3561.00, p < 0.05) and HR (U = 3887.50, p < 0.01) when compared to males. Systolic blood pressure was 1.71% higher, DBP was 1.69% higher, MAP was 3.07% higher and HR was 3.07% higher in females when compared to males in the dual exposed group. In addition, males had a significantly higher LcIMT with a 5.71% difference when compared to females in the dual exposed group (U = 2234.50, p < 0.05). In addition, in the dual exposed group, there was a significant difference in RcIMT when compared to females (U = 2293.50, p < 0.05). In other words, males in the dual exposed group had a 5.71% higher LcIMT as well as RcIMT compared to their female counterparts. In the control group, males also had a significantly higher LcIMT with a 5.88% difference when compared to females (U = 2087.50, p < 0.05) (Figure 4.2). Also, RcIMT was also significantly higher in males with a 6.10% difference when compared to females in the control group (U = 2153.50, p = 0.05).

Table 4. 6: Results of Mann Whitney U test illustrating the differences in cardiovascular risk measurements in five-year-old children according to gender.

	Control group			Nicotine exposed group			Dual exposed group		
	Males vs Females		p value	Males vs Females		p value	Males vs Females		p value
	N= 77	N=70		N=89	N=78		N=72	N=82	
	mean (SD)	mean (SD)		mean (SD)	mean (SD)		mean (SD)	mean (SD)	
BMI	15.26±1.77	15.22±1.75	0.780	15.19±1.52	15.23±1.69	0.633	15.17±1.46	15.17±1.69	0.548
WC	51.45±4.49	51.45±4.49	0.759	51.28±3.96	51.39±4.38	0.718	51.05±3.88	51.22±4.27	0.821
sSFT	7.12±3.31	7.48±3.32	0.004**	6.94±2.37	7.37±3.07	0.032*	6.87±2.44	7.36±3.12	0.000**
tSFT	9.37±3.25	10.10±3.24	0.001**	9.21±2.87	9.91±3.06	0.000**	9.15±2.83	9.91±3.10	0.000**
SBP	105.57±10.37	106.52±10.00	0.137	106.06±10.01	106.39±10.09	0.380	104.58±9.49	106.37±10.20	0.001**
DBP	64.69±9.38	65.32±8.72	0.072	64.96±9.39	65.31±8.70	0.789	64.11±8.95	65.19±9.13	0.037*
MAP	78.35±9.73	78.79±9.05	0.052	78.61±9.63	78.85±9.07	0.718	77.53±9.25	78.81±9.44	0.027*
HR	90.87±12.72	92.75±13.53	0.144	91.64±13.88	92.37±13.41	0.841	90.32±12.80	93.09±13.75	0.001**
LcIMT	0.36±0.05	0.34±0.05	0.024*	0.36±0.05	0.35±0.05	0.833	0.37±0.06	0.35±0.05	0.013*
RcIMT	0.35±0.05	0.33±0.04	0.046*	0.34±0.04	0.34±0.05	0.863	0.37±0.05	0.35±0.04	0.022*

Note: * indicates statistically significant differences < 0.05; ** indicates statistically significant differences < 0.01; BMI: body mass index, WC: waist circumference, tSFT: triceps skinfold thickness, sSFT: subscapular skinfold thickness, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, Right cIMT: right carotid intima-media thickness, Left cIMT: left carotid intima-media thickness.

4.4 Cardiovascular risk factor prevalence

4.4.1 Cardiovascular risk factor prevalence across exposure groups

Table 4.7 illustrates the prevalence of obesity, central obesity, high cIMT and hypertension in five-year-old children across the exposure groups. In the nicotine exposed group, 8.4% of children were overweight, followed by 7.8% in the dual exposed group and the lowest prevalence of 6.8% in the control group (Table 4.7). Children with no exposure had the highest obesity prevalence of 6.1%, followed by the nicotine exposed group and dual exposed group with a prevalence of 1.8% and 1.3%, respectively. In the control group, 13.6% of children had central obesity, with a lower prevalence of 5.8% in the dual exposed group and 4.2 % in the nicotine exposed group. In the dual exposed group, 5.2% of children were prehypertensive, followed by the control group and nicotine exposed group with a prevalence of 4.8% and 3.0%, respectively. In the control, 1.4% of children were hypertensive, followed by 0.6% in both the nicotine group and exposure group. The left cIMT was high in 24% of children with dual exposure, with similar prevalence's of 19.7% and 19.2% in the control group and nicotine exposed group, respectively. High right cIMT was highly prevalent in the dual exposed group with 33%, followed by 25.1% in the nicotine exposed group, and 17.7% in the control group.

Table 4.7: Prevalence of obesity, central obesity and hypertension in five-year-old children according to age and gender across the exposure groups.

CVD risk factors	Overall	Control	Dual exposed	Nicotine exposed
	N= 468	N= 147	N= 154	N= 167
	n (%)	n (%)	n (%)	n (%)
Overweight (Males BMI 16.6 kg/m², Females BMI >16.9 kg/m²) *	36 (7,7%)	10 (6,8%)	12 (7.8%)	14 (8.4%)
Obesity (Male BMI >18.3 kg/m², Females BMI > 18.9 kg/m²)	14 (3.0%)	9 (6,1%)	2 (1.3%)	3 (1.8%)
Central obesity (High WC ≥90th percentile)	36 (7.7%)	20 (13.6%)	9 (5.8%)	7 (4.2%)
Prehypertension (SBP/DBP 90th- <95th percentile) *	20 (4,3%)	7 (4.8%)	8 (5.2%)	5 (3.0%)
Systolic hypertension (≥95th percentile)	2 (0.4%)	1 (0.7%)	0 (0%)	1 (0.6%)
Diastolic hypertension (≥95th percentile)	3 (0.6%)	1 (0.7%)	1 (0.6%)	1 (0.6%)
Hypertension (SBP/DBP <95th percentile)	4 (0.9%)	2 (1.4%)	1 (0.6%)	1 (0.6%)
High LcIMT (≥75th percentile) *	98 (20.9%)	29 (19.7%)	37 (24%)	32 (19.2%)

High RcIMT (≥75th percentile) *	119 (25.4%)	26 (17.7%)	51 (33%)	42 (25.1%)

Note: Italics indicates supporting measurements. * indicates pre-clinical measurements. BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, High RcIMT: high right carotid intima-media thickness, High LcIMT: high left carotid intima-media thickness.

4.4.2 Cardiovascular risk factor prevalence in males

Figure 4.2 illustrates the prevalence of CVD risk factors in males at five years old according to exposure groups. The prevalence of overweight males using BMI was highest in the dual exposed group, with 8.33%, followed by the nicotine group with 6.74% and the control group with 3.90%. The prevalence of obesity in males was highest in the control group, with 6.49%, followed by the nicotine group, with 3,37% and the dual exposed group, with 1.39%. The prevalence of central obesity was highest in the control group, with 11.69%, followed by the dual exposed group, with 5.56% and the nicotine group with 3.37%.

In Figure 4.2, the prevalence of prehypertension in males was similar in the nicotine exposed group, with 3.37% and in the dual exposed group, with 2.78%, followed by the control group, with 1.30%. Systolic hypertension in males was only present the nicotine exposed group, with 1.12%. Diastolic hypertension was most prevalent in the dual exposed group, with 2.78%, followed by the control group, with 1.30% and in the nicotine exposed group, with 1.12%. Hypertension in males was most prevalent in the dual exposed group, with 1.39%, followed by the control group with 1.30%, and the nicotine exposed group with 1.12%.

In males, high RcIMT was most prevalent in the dual exposed group, with 38.89%, follow by the nicotine exposed group, with 25.84%, and the control group, with 22.08%. High LcIMT in males was most prevalent in the dual exposed group, with 30.56%, followed by the control group, with 23.38%, and the nicotine exposed group, with 19.10% (Figure 4.2).

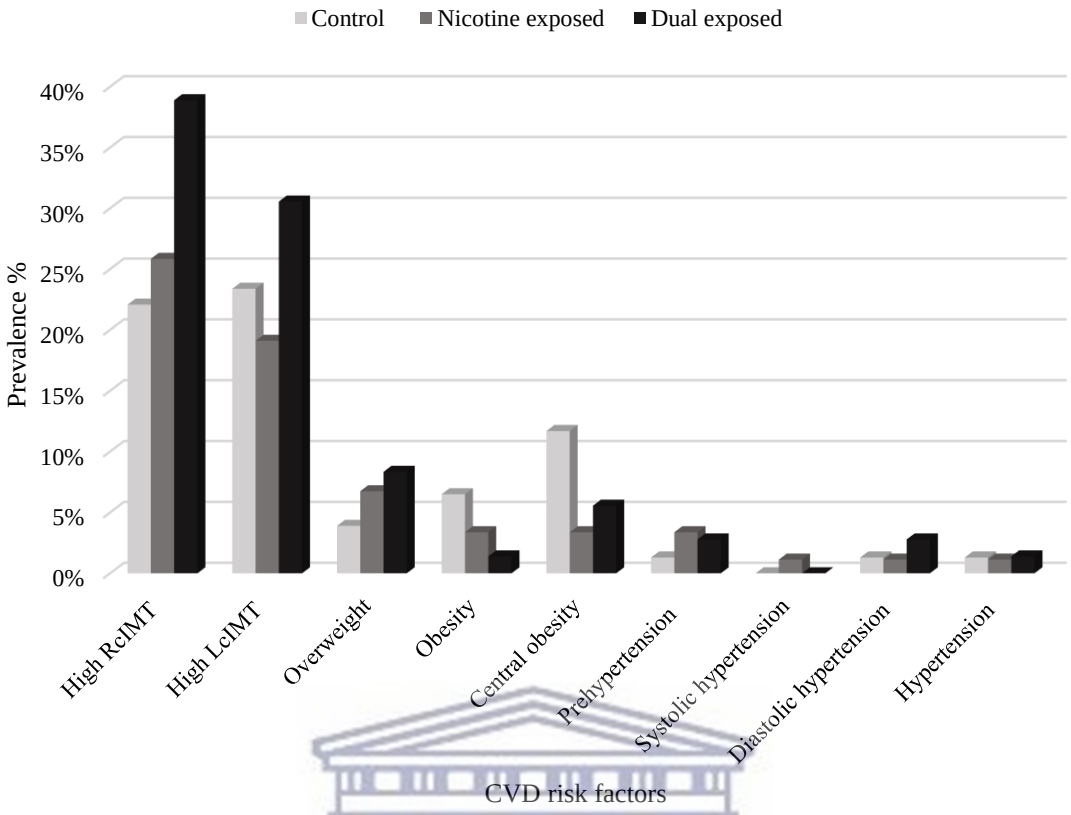


Figure 4.2: Prevalence of CVD risk factors in males at five years old according to exposure groups.

4.4.3 Cardiovascular risk factor prevalence in females

Figure 4.3 illustrates the prevalence of CVD risk factors in females at five-year-old according to exposure groups. Furthermore, the prevalence of overweight females using BMI was highest in the nicotine group, with 10.26%, followed by the control group with 10.00% and the dual exposed group with 7.32%. The prevalence of obesity in females was highest in the control group, with 5.71%, followed by the dual exposed group, with 1.22%. The prevalence of central obesity was highest in the control group, with 15.71%, followed by the dual exposed group, with 6.10% and in the nicotine group with 5.13%.

In Figure 4.3, the prevalence of prehypertension in females was highest in the control group, with 8.57%, followed by the dual exposed group, with 7.32%, followed by the nicotine exposed group, with 2.56%. Systolic hypertension was only present in the control group, with 1.43%.

Diastolic hypertension was not prevalent in females across the exposure groups. Hypertension in females was only prevalent in the control group, with 1.43%.

In females, high RcIMT was highest in the dual exposed group, with 28.05%, followed by the nicotine exposed group, with 24.36%, and the control group, with 12.86%. High LcIMT in females was most prevalent in the nicotine exposed group, with 19.23%, followed by the dual exposed group, with 18.29%, and the control group, with 15.71% (Figure 4.3).

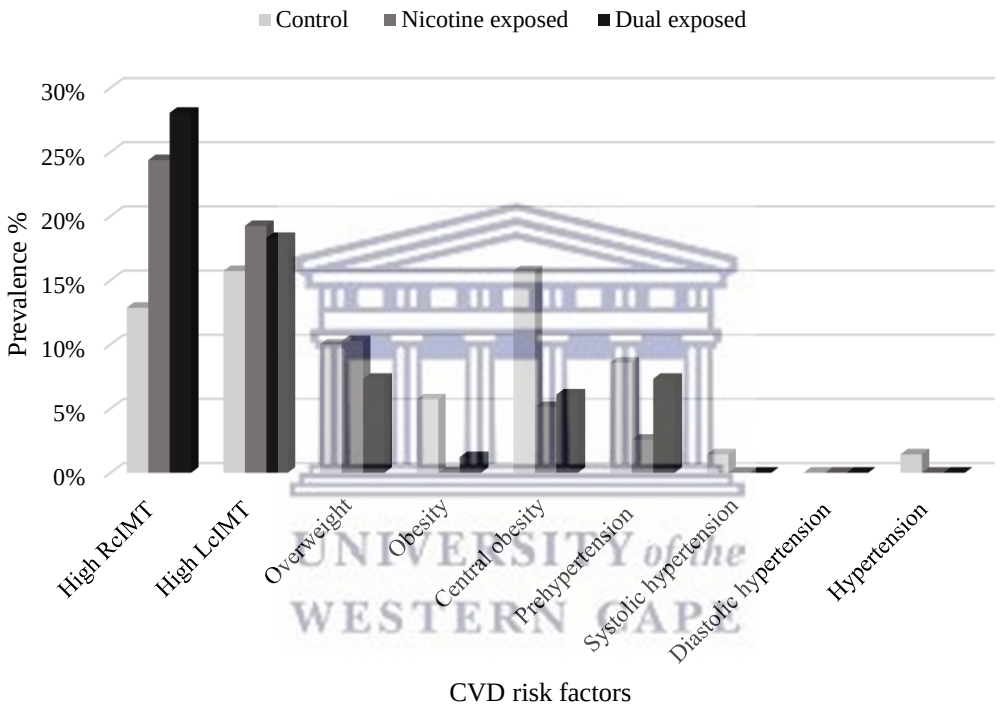


Figure 4.3: Prevalence of CVD risk factors in females at five years old according to exposure groups.

4.5 Relationship between cardiovascular risk measurements

4.5.1 Relationship between cardiovascular risk measurements across exposure groups

4.5.1.1 Relationship between adiposity indices

In Table 4.8, there was a positive and significant strong correlation between BMI and WC ($r = 0.840$; $p < 0.01$) in the control group, between BMI and WC ($r = 0.820$; $p < 0.01$) in the nicotine exposed group, and between BMI and WC ($r = 0.821$; $p < 0.01$) in the dual exposed group. There

was a positive and significant moderate correlation between BMI and sSFT ($r = 0.656$; $p < 0.01$) in the control group, between BMI and sSFT ($r = 0.627$; $p < 0.01$) in the nicotine exposed group, and between BMI and sSFT ($r = 0.543$; $p < 0.01$) in the dual exposed group. There was a positive and significant moderate correlation between BMI and tSFT ($r = 0.604$; $p < 0.01$) in the control group, a positive and significant fair correlation between BMI and tSFT ($r = 0.468$; $p < 0.01$) in the dual exposed group, and a positive and significant fair correlation between BMI and tSFT ($r = 0.517$; $p < 0.01$) in the nicotine exposed group.

4.5.1.2 Relationship between adiposity indices and BP measurements

As shown in Table 4.8, there was a positive and significant correlation between BMI and SBP ($r = 0.200$; $p < 0.01$), a positive and significant correlation between BMI and DBP ($r = 0.182$; $p < 0.05$), and a positive and significant correlation between BMI and MAP ($r = 0.156$; $p < 0.05$) in the nicotine exposed group. There was also a positive and significant correlation between sSFT and SBP ($r = 0.164$; $p < 0.05$) as well as sSFT and MAP ($r = 0.172$; $p < 0.05$) in the control group. A positive and significant correlation was found between tSFT and DBP ($r = 0.222$; $p < 0.01$) as well between tSFT and MAP ($r = 0.197$; $p < 0.05$) in the control group. Furthermore, a positive and significant correlation was found between sSFT and SBP ($r = 0.191$; $p < 0.05$), sSFT and DBP ($r = 0.197$; $p < 0.05$), sSFT and MAP ($r = 0.181$; $p < 0.05$), and sSFT and HR ($r = 0.197$; $p < 0.05$) in the dual exposed group.

4.4.1.3 Relationship between BP measurements

In Table 4.8, as expected, a positive and significant moderate correlation was found between SBP and DBP ($r = 0.748$; $p < 0.01$) in the control group, between SBP and DBP ($r = 0.727$; $p < 0.01$) in the nicotine exposed group, and between SBP and DBP ($r = 0.741$; $p < 0.01$) in the dual exposed group. There was a positive, significant and strong correlation found between SBP and MAP ($r = 0.873$; $p < 0.01$) in the control group, between SBP and MAP ($r = 0.845$; $p < 0.01$) in the nicotine exposed group, and between SBP and MAP ($r = 0.856$; $p < 0.01$) in the dual exposed group. In

addition, there was a positive and significant fair correlation between SBP and HR ($r = 0.303$; $p < 0.01$) in the control group, a positive and significant correlation between SBP and HR ($r = 0.258$; $p < 0.05$) in the nicotine exposed group, and a positive and significant moderate correlation between SBP and HR ($r = 0.447$; $p < 0.01$) in the dual exposed group.

Furthermore, in Table 4.8, a positive, significant and strong correlation was found between DBP and MAP ($r = 0.892$; $p < 0.01$) in the control group, between DBP and MAP ($r = 0.904$; $p < 0.01$) in the nicotine exposed group between DBP and MAP ($r = 0.904$; $p < 0.01$) in the dual exposed group. There was a positive and significant fair correlation between DBP and HR ($r = 0.361$; $p < 0.01$) in the control group, DBP and HR ($r = 0.451$; $p < 0.01$) in the dual exposed group, and a positive and significant correlation between DBP and HR ($r = 0.275$; $p < 0.05$) in the nicotine group. Furthermore, a positive and significant fair correlation was found between MAP and HR ($r = 0.320$; $p < 0.01$) in the control group, MAP and HR ($r = 0.426$; $p < 0.01$) in the dual exposed group, and a positive and significant correlation between MAP and HR ($r = 0.263$; $p < 0.01$) in the nicotine group.

4.4.1.4 Relationship between cIMT and adiposity indices

There was a positive and significant correlation between RcIMT and BMI ($r = 0.223$; $p < 0.05$) and a positive and significant correlation between LcIMT and BMI ($r = 0.169$; $p < 0.05$) in the nicotine exposed group. However, the correlation between LcIMT and BMI in the nicotine exposed group was not significant after the adjustment for WC at five years old ($r = 0.023$); $p = 0.773$). Similarly, RcIMT was not significantly correlated with BMI in the nicotine exposed group after the adjustment for WC at five years old ($r = 0.138$; $p = 0.086$). There was a positive and significant correlation between sSFT and LcIMT ($r = 0.180$; $p < 0.05$) as well as sSFT and RcIMT ($r = 0.203$; $p < 0.05$) in the nicotine exposed group. There was a negative and significant correlation between sSFT and LcIMT ($r = -0.194$; $p < 0.05$) in the dual exposed group. In addition, a positive and significant correlation was observed between RcIMT and WC ($r = 0.165$; $p < 0.05$)

in the dual exposed group, which remained significant after the adjustment for BMI at five years old (r = 0.176; P = 0.033). Furthermore, a positive and significant correlation was observed between WC and RcIMT (r = 0.200; p < 0.05) and between WC and LcIMT (r = 0.175; p < 0.05) in the nicotine exposed group. However, the significant correlation between RcIMT and WC in the nicotine exposed group was also attenuated after the adjustment for BMI at five years old (r = 0.005; P = 0.955). The significant correlation between LcIMT and WC in the nicotine exposed group was also attenuated after the adjustment for BMI at five years old (r = 0.084; P = 0.301).

4.4.1.5 Relationship between cIMT and BP measurements

As shown in Table 4.8, there was a negative and significant correlation between RcIMT and HR (r = -0.186; p < 0.05) in the nicotine exposed group. In the dual exposed group, a negative and significant correlation was found between RcIMT and HR (r = -0.160; p < 0.01) as well as LcIMT and HR (r = -0.210; p < 0.01).

Table 4.8: Relationship between cardiovascular risk measurements across exposure groups.

	Group	BMI	WC	sSFT	tSFT	SBP	DBP	MAP	HR
WC	**Control group**	0.840**							
	Nicotine group	0.820**							
	Dual exposure group	0.821**							
sSFT	**Control group**	0.656**	0.588**						
	Nicotine group	0.627**	0.540**						
	Dual exposure group	0.543**	0.539**						
tSFT	**Control group**	0.604**	0.560**	0.683**					
	Nicotine group	0.517**	0.477**	0.611**					
	Dual exposure group	0.468**	0.408**	0.632**					
SBP	**Control group**	0.094	0.117	0.164*	0.150				
	Nicotine group	0.200**	0.095	0.089	0.088				
	Dual exposure group	0.080	0.132	0.191*	0.158				
DBP	**Control group**	0.076	0.077	0.107	0.222**	0.748**			
	Nicotine group	0.182*	0.058	0.129	0.159*	0.727**			
	Dual exposure group	0.099	0.131	0.197*	0.100	0.741**			
MAP	**Control group**	0.076	0.077	0.172*	0.197*	0.873**	0.892**		
	Nicotine group	0.156*	0.034	0.075	0.081	0.845**	0.904**		
	Dual exposure group	0.093	0.115	0.181*	0.142	0.856**	0.904**		
HR	**Control group**	-0.019	0.000	0.039	0.144	0.303**	0.361**	0.320**	
	Nicotine group	0.149	0.008	0.034	0.127	0.258*	0.275*	0.263**	

	Dual exposure group	-0.016	0.034	0.197*	0.080	0.447**	0.451**	0.426**	
RcIMT	**Control group**	0.149	0.160	-0.038	0.041	-0.006	0.003	-0.004	-0.074
	Nicotine group	0.223*	0.200*	0.203*	0.058	0.120	0.023	0.088	-0.186*
	Dual exposure group	0.058	0.165*	-0.058	-0.053	0.001	-0.015	0.064	-0.160*
LcIMT	**Control group**	0.096	0.098	0.040	0.043	0.028	0.073	0.069	0.020
	Nicotine group	0.169*	0.175*	0.180*	0.069	0.092	0.038	0.098	-0.123
	Dual exposure group	0.008	0.046	-0.194*	-0.028	-0.044	-0.134	-0.035	-0.210*

Note: * indicates statistical significance P<0.05. ** indicates statistical significance P<0.01; BMI: body mass index, WC: waist circumference, tSFT: triceps skinfold thickness, sSFT: subscapular skinfold thickness, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, RcIMT: right carotid intima-media thickness, LcIMT: left carotid intima-media thickness.

4.5.2 Relationship between cardiovascular risk measurements across gender

4.5.2.1 Relationship between adiposity indices

In Table 4.9, in males, there was a positive and significant strong correlation between BMI and WC ($r = 0.833$; $p < 0.01$) in the control group, between BMI and WC ($r = 0.801$; $p < 0.01$) in the nicotine exposed group, and between BMI and WC ($r = 0.832$; $p < 0.01$) in the dual exposed group. In females, there was a positive and significant strong correlation between BMI and WC ($r = 0.822$; $p < 0.01$) in the control group, between BMI and WC ($r = 0.840$; $p < 0.01$) in the nicotine exposed group, and between BMI and WC ($r = 0.814$; $p < 0.01$) in the dual exposed group. In males, there was a positive and significant moderate correlation between BMI and sSFT ($r = 0.609$; $p < 0.01$) in the control group, between BMI and sSFT ($r = 0.620$; $p < 0.01$) in the nicotine exposed group, and between BMI and sSFT ($r = 0.632$; $p < 0.01$) in the dual exposed group. In females, there was a positive and significant moderate correlation between BMI and sSFT ($r = 0.697$; $p < 0.01$) in the control group, between BMI and sSFT ($r = 0.645$; $p < 0.01$) in the nicotine exposed group, and a positive and significant fair correlation between BMI and sSFT ($r = 0.577$; $p < 0.01$) in the dual exposed group. In males, there was a positive and significant fair correlation between BMI and tSFT ($r = 0.480$; $p < 0.01$) in the control group, between BMI and tSFT ($r = 0.479$; $p < 0.01$) in the dual exposed group, and a positive and significant moderate correlation between BMI and tSFT ($r = 0.538$; $p < 0.01$) in the nicotine exposed group. In females, there was a positive and significant moderate correlation between BMI and tSFT ($r = 0.719$; $p <$

0.01) in the control group, and a positive and significant fair correlation between BMI and tSFT ($r = 0.511$; $p < 0.01$) in the dual exposed group, and between BMI and tSFT ($r = 0.537$; $p < 0.01$) in the nicotine exposed group.

In Table 4.9, in males, there was a positive and significant correlation between WC and sSFT ($r = 0.538$; $p < 0.01$) in the control group, between WC and sSFT ($r = 0.549$; $p < 0.01$) in the nicotine exposed group, and a positive and significant moderate correlation between WC and sSFT ($r = 0.656$; $p < 0.01$) in the dual exposed group. In females, there was a positive and significant fair correlation between WC and sSFT ($r = 0.584$; $p < 0.01$) in the nicotine group, between WC and sSFT ($r = 0.502$; $p < 0.01$) in the dual nicotine exposed group, and a positive and significant moderate correlation between WC and sSFT ($r = 0.643$; $p < 0.01$) in the control group. In males, there was a positive and significant fair correlation between WC and tSFT ($r = 0.480$; $p < 0.01$) in the control group, between WC and tSFT ($r = 0.537$; $p < 0.01$) in the nicotine exposed group, and between WC and tSFT ($r = 0.527$; $p < 0.01$) in the dual exposed group. Among females, there was a positive and significant moderate correlation between WC and tSFT ($r = 0.705$; $p < 0.01$) in the control group, and a positive and significant fair correlation between WC and tSFT ($r = 0.359$; $p < 0.01$) in the dual exposed group.

Furthermore, in Table 4.9, males had a positive and significant fair correlation between sSFT and tSFT ($r = 0.538$; $p < 0.01$) in the control group, between sSFT and tSFT ($r = 0.540$; $p < 0.01$) in the dual exposed group, and a positive and significant moderate correlation between sSFT and tSFT ($r = 0.617$; $p < 0.01$) in the nicotine exposed group. Among females, there was a positive and significant moderate correlation between sSFT and tSFT ($r = 0.709$; $p < 0.01$) in the control group, between sSFT and tSFT ($r = 0.665$; $p < 0.01$) in the dual exposed group, and a positive and significant fair correlation between sSFT and tSFT ($r = 0.534$; $p < 0.01$) in the nicotine exposed group.

4.5.2.2 Relationship between adiposity indices and BP measurements

As shown in Table 4.9, there was a positive and significant correlation between BMI and SBP ($r = 0.231$; $p < 0.05$) in females in the nicotine exposed group, whereas a positive and significant correlation was found between BMI and DBP ($r = 0.0.236$; $p < 0.05$) in males in the nicotine exposed group. There was also a positive and significant correlation between sSFT and HR ($r = 0.239$; $p < 0.05$) in males in the dual exposed group. Females had a positive and significant correlation between tSFT and HR ($r = 0.228$; $p < 0.05$) in the dual exposed group.

4.4.2.3 Relationship between BP measurements

In Table 4.9, as expected, males had a positive and significant moderate correlation between SBP and DBP ($r = 0.725$; $p < 0.01$) in the control group, between SBP and DBP ($r = 0.743$; $p < 0.01$) in the nicotine exposed group, and between SBP and DBP ($r = 0.740$; $p < 0.01$) in the dual exposed group. Females had a positive and significant moderate correlation between SBP and DBP ($r = 0.767$; $p < 0.01$) in the control group, between SBP and DBP ($r = 0.721$; $p < 0.01$) in the nicotine exposed group, and between SBP and DBP ($r = 0.730$; $p < 0.01$) in the dual exposed group. Males had a positive, significant and very strong correlation between SBP and MAP ($r = 0.831$; $p < 0.01$) in the control group, between SBP and MAP ($r = 0.854$; $p < 0.01$) in the nicotine exposed group, and between SBP and MAP ($r = 0.849$; $p < 0.01$) in the dual exposed group. Similarly, females had a positive, significant and very strong correlation between SBP and MAP ($r = 0.897$; $p < 0.01$) in the control group, between SBP and MAP ($r = 0.843$; $p < 0.01$) in the nicotine exposed group, and between SBP and MAP ($r = 0.856$; $p < 0.01$) in the dual exposed group. In males, there was a positive and significant fair correlation between SBP and HR ($r = 0.300$; $p < 0.01$) in the control group, between SBP and HR ($r = 0.331$; $p < 0.01$) in the dual exposed group, and a positive and significant weak correlation between SBP and HR ($r = 0.256$; $p < 0.05$) in the nicotine exposed group. In females, there was a positive and significant poor correlation between SBP and HR ($r = 0.293$; $p < 0.05$) in the control group, between SBP and HR ($r = 0.245$; $p < 0.01$) in the nicotine

exposed group, and a positive and significant fair correlation between SBP and HR (r = 0.446; p < 0.01) in the dual exposed group. Furthermore, in Table 4.9, males had a positive, significant and very strong correlation between DBP and MAP (r = 0.898; p < 0.01) in the control group, between DBP and MAP (r = 0.908; p < 0.01) in the nicotine exposed group between DBP and MAP (r = 0.908; p < 0.01) in the dual exposed group. Females also had a positive, significant and very strong correlation between DBP and MAP (r = 0.888; p < 0.01) in the control group, between DBP and MAP (r = 0.900; p < 0.01) in the nicotine exposed group between DBP and MAP (r = 0.894; p < 0.01) in the dual exposed group. In males, there was a positive and significant fair correlation between DBP and HR (r = 0.332; p < 0.01) in the control group, DBP and HR (r = 0.427; p < 0.01) in the dual exposed group, and a positive and significant poor correlation between DBP and HR (r = 0.265; p < 0.05) in the nicotine group. Similarly, in females, there was a positive and significant fair correlation between DBP and HR (r = 0.372; p < 0.01) in the control group, DBP and HR (r = 0.406; p < 0.01) in the dual exposed group, and a positive and significant poor correlation between DBP and HR (r = 0.263; p < 0.05) in the nicotine group. Males had a positive and significant fair correlation between MAP and HR (r = 0.322; p < 0.01) in the control group, MAP and HR (r = 0.349; p < 0.01) in the dual exposed group, and a positive and significant poor correlation between MAP and HR (r = 0.249; p < 0.05) in the nicotine group. Similarly, females had a positive and significant fair correlation between MAP and HR (r = 0.312; p < 0.01) in the control group, MAP and HR (r = 0.424; p < 0.01) in the dual exposed group, and a positive and significant poor correlation between MAP and HR (r = 0.271; p < 0.05) in the nicotine group.

4.4.2.4 Relationship between cIMT and adiposity indices

In females, there was a positive and significant correlation between RcIMT and BMI (r = 0.352; p < 0.01) and a positive and significant correlation between LcIMT and BMI (r = 0.248; p < 0.05) in the nicotine group. However, the correlation between LcIMT and BMI in females in the nicotine exposed group was not significant after adjusting for WC at five years old (r = 0.149); p = 0.208).

However, RcIMT remained significantly correlated with BMI in females in the nicotine exposed group after the adjustment for WC at five years old (r = 0.367); p = 0.001). Females had a positive and significant correlation between tSFT and LcIMT (r = 0.245; p < 0.05) in the dual exposed group. Females also had a positive and significant correlation between RcIMT and WC (r = 0.234; p < 0.05) in the nicotine group, between LcIMT and tSFT (r = 0.245; p < 0.05) in the control group. However, the significant relationship between WC and RcIMT (r = 0.234; p < 0.05) in females in the nicotine exposed group was attenuated after the adjustment for BMI at five years old (r = -0.188; P = 0.112).

In males, there was a positive and significant correlation between RcIMT and BMI (r = 0.235; p < 0.05), between RcIMT and WC (r = 0.248; p < 0.05) in the dual exposed group, and a positive and significant correlation between RcIMT and MAP (r = 0.424; p < 0.05) in the control group. However, there was no significant correlation between RcIMT and BMI in males in the dual exposed group after the adjustment for WC at five years old (r =0.050; P = 0.679). Males had a positive and significant correlation between WC and RcIMT (r = 0.248; p < 0.05) in the dual exposed group. However, this significance was attenuated after the adjustment for BMI at five years old (r = 1.000; P = 0.411).

4.4.2.5 Relationship between cIMT and BP measurements

In Table 4.9, males had a negative and significant correlation between RcIMT and HR (r = -0.284; p < 0.05) in the dual exposed group, whereas females had a negative and significant correlation between RcIMT and HR (r = -0.413; p < 0.01) in the nicotine group.

Table 4.9: Relationship between cardiovascular risk measurements according to gender and exposure groups.

	Gender	Group	BMI	WC	sSFT	tSFT	SBP	DBP	MAP	HR
WC	Males	Control group	0.833**							
		Nicotine group	0.801**							
		Dual exposure group	0.832**							
	Females	Control group	0.822**							
		Nicotine group	0.840**							
		Dual exposure group	0.814**							
sSFT	Males	Control group	0.609**	0.538**						
		Nicotine group	0.620**	0.549**						
		Dual exposure group	0.632**	0.656**						
	Females	Control group	0.697**	0.643**						
		Nicotine group	0.645**	0.584**						
		Dual exposure group	0.577**	0.502**						
tSFT	Males	Control group	0.480**	0.408**	0.556**					
		Nicotine group	0.538**	0.537**	0.617**					
		Dual exposure group	0.479**	0.527**	0.540**					
	Females	Control group	0.719**	0.705**	0.709**					
		Nicotine group	0.511**	0.491	0.534**					
		Dual exposure group	0.537**	0.359**	0.665**					
SBP	Males	Control group	-0.004	0.051	0.047	-0.022				
		Nicotine group	0.183	0.094	0.095	0.109				
		Dual exposure group	0.124	0.092	0.106	0.042				
	Females	Control group	0.134	0.128	0.168	0.189				
		Nicotine group	0.231*	0.059	0.080	0.060				
		Dual exposure group	0.055	0.157	0.087	0.099				
DBP	Males	Control group	0.048	0.022	0.015	0.145	0.725**			
		Nicotine group	0.236*	0.166	0.114	0.192	0.743**			
		Dual exposure group	0.206	0.185	0.178	0.048	0.740**			
	Females	Control group	0.032	0.078	0.047	0.162	0.767**			
		Nicotine group	0.146	-0.064	0.112	0.107	0.721**			

		Dual exposure group	0.029	0.092	0.106	0.039	0.730**			
MAP	**Males**	**Control group**	-0.043	-0.009	0.094	0.092	0.831**	0.898**		
		Nicotine group	0.200	0.107	0.095	0.085	0.854**	0.908**		
		Dual exposure group	0.189	0.166	0.135	0.105	0.849**	0.917**		
	Females	**Control group**	0.118	0.101	0.124	0.167	0.897**	0.888**		
		Nicotine group	0.135	-0.059	0.037	0.075	0.843**	0.900**		
		Dual exposure group	0.032	0.084	0.125	0.094	0.856**	0.894**		
HR	**Males**	**Control group**	-0.079	-0.094	-0.047	0.082	0.300**	0.332**	0.322**	
		Nicotine group	0.104	0.021	0.024	0.059	0.256*	0.265*	0.249*	
		Dual exposure group	0.019	-0.013	0.239*	-0.037	0.331**	0.427**	0.349**	
	Females	**Control group**	0.036	0.097	0.005	0.147	0.293*	0.372**	0.312**	
		Nicotine group	-0.003	-0.007	0.020	0.228*	0.245*	0.263*	0.271*	
		Dual exposure group	-0.025	0.053	-0.004	0.007	0.446**	0.406**	0.424**	
RcIMT	**Males**	**Control group**	0.081	0.086	0.059	0.091	0.072	0.064	0.424**	0.039
		Nicotine group	0.096	0.165	0.201	0.090	0.077	0.001	0.084	0.003
		Dual exposure group	0.235*	0.248*	-0.014	0.179	0.193	0.112	0.217	-0.152
	Females	**Control group**	0.206	0.208	-0.043	0.092	0.000	-0.027	-0.046	-0.179
		Nicotine group	0.352**	0.234*	0.212	-0.012	0.177	0.066	0.097	-0.413**
		Dual exposure group	-0.116	0.087	0.010	-0.156	-0.079	-0.080	-0.037	-0.100
LcIMT	**Males**	**Control group**	0.086	0.040	0.117	-0.036	0.029	0.034	0.065	0.140
		Nicotine group	0.105	0.129	0.199	0.081	0.164	0.070	0.165	-0.142
		Dual exposure group	0.080	0.086	-0.140	0.065	-0.051	-0.182	-0.044	-0.284*
	Females	**Control group**	0.115	0.13	0.057	0.245*	0.028	0.154	0.118	-0.085
		Nicotine group	0.248*	0.208	0.207	0.081	0.013	0.002	0.021	-0.085
		Dual exposure group	-0.071	0.018	-0.123	0.030	0.040	-0.062	0.002	-0.081

Note: * indicates statistical significance P<0.05. ** indicates statistical significance P<0.01; BMI: body mass index, WC: waist circumference, tSFT: triceps skinfold thickness, sSFT: subscapular skinfold thickness, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, HR: heart rate, RcIMT: right carotid intima-media thickness, LcIMT: left carotid intima-media thickness.

In Table 4.10, there was a significant association between obesity and nicotine exposure [$\chi^2(1) = 4.097$; $p < 0.05$; OR = 0.3 (95% CI: 0.1-1.0); RR = 0.3 (95% CI: 0.1-1.1)]. The results showed that children with nicotine exposure *in utero* have a reduced risk of 0.3, indicating that they are 0.7 times less likely to develop obesity compared to children in the control group (Table 4.10). In addition, there was a significant association between nicotine exposure and central obesity [$\chi^2(1) = 8.816$; $p < 0.01$; OR = 0.3 (95% CI: 1,5-8,8); RR = 0.3 (95% CI: 0.1-1.2)]. Children with nicotine exposure *in utero* were 0.7 times less likely to develop central obesity compared to children in the control group (Table 4.10). Furthermore, exposure to nicotine and alcohol *in utero* was significantly associated with obesity [$\chi^2(1) = 5.031$; $p < 0.05$; OR = 0.2 (95% CI: 0-0.9); RR = 0.2 (95% CI: 0.1-0.9)]. However, children with exposure to nicotine and alcohol *in utero* were 0.8 times less likely to become obese compared to the control group (Table 4.10). Exposure to nicotine and alcohol *in utero* was also significantly associated with central obesity [$\chi^2(1) = 5.204$; $p < 0.05$; OR = 0.4 (95% CI: 0-0.9); RR = 0.4 (95% CI: 0.1-0.9)]. The results showed that children with exposure to nicotine and alcohol *in utero* were 0.6 times less likely to have central obesity when compared to the control group (Table 4.10).

Table 4.10: Relationship between overweight, obesity, central obesity, gender and exposures.

	Overweight (BMI)				Obesity (BMI)				Central obesity (High WC)			
	Yes	P value	OR (95% CI)	RR (95% CI)	Yes	P value	OR (95% CI)	RR (95% CI)	Yes	P value	OR (95% CI)	RR (95% CI)
	Count (%)				Count (%)				Count (%)			
Nicotine exposure	14 (8.6)	0.737	1.2 (0.5-2.7)	1.14 (0.5-2.5)	3 (2)	0.043*	0.3 (0.1-1)	0.3 (0.1-1.1)	7 (4.2)	0.003**	0.3 (0.1-0.7)	0.3 (0.1-0.7)
No exposure	10 (7.5)				9 (6.8)				20 (13.6)			
Dual exposure	12 (8.1)	0.867	1.1 (0.5-2.6)	1.1 (0.5-2.4)	2 (0.7)	0.025*	0.2 (0-0.9)	0.2 (0.1-0.9)	9 (14.8)	0.023*	0.4 (0.2-0.9)	0.4 (0.2-0.9)
No exposure	10 (7.5)				9 (6.8)				20 (13.6)			
Nicotine exposure	12 (8.1)	0.864	1.0 (0.4-2.1)	1.0 (0.5-2.0)	2 (1.4)	*1.000*	0.7 (0.1-4.4)	0.7 (0.1-4.2)	9 (5.8)	0.497	0.7 (0.3-1.9)	1.4 (0.5-3.7)
Dual exposure	14 (8.6)				3 (2.6)				7 (4.2)			
Male	15 (6.7)	0.278	0.7 (0.3-1.4)	0.7 (0.4-1.3)	9(4.1)	0.332	1.7 (0.6-5.2)	1.7 (0.6-5.0)	16 (6.7)	0.423	1.3 (0.7-2.6)	1.0 (0.4-1.5)
Female	21 (9.5)				5(2.4)				20 (8.7)			

Note: Italics represents P value of Fisher's exact test. * indicates statistical significance P<0.05. ** indicates statistical significance P<0.01. OR (95% CI) = odds ratio (95% confidence interval), RR (95% CI) = relative risk (95% confidence interval).

In Table 4.11, there was a significant association between exposure to nicotine and alcohol and a high RcIMT [$\chi^2(1) = 9.406$; $p < 0.01$; OR = 2.3 (95% CI: 1.3-4.0); RR = 1.9 (95% CI: 1.2-2.8)]. Children exposed to alcohol and nicotine *in utero* were 2.3 times more likely to have an increased RcIMT with a relative risk of 1.9 compared to children with no exposure. There were no significant association between gender and overweight, obesity and central obesity across the exposure groups (Table 4.11).

Table 4.11: Relationship between ultrasound measurements, gender and exposure groups.

	High right cIMT				High left cIMT			
	Yes	**P value**	**OR (95% CI)**	**RR (95% CI)**	**Yes**	**P value**	**OR (95% CI)**	**RR (95% CI)**
	Count (%)				**Count (%)**			
Nicotine exposure	42 (25.1)	0.109	1.7 (0.9-2.7)	1.4 (0.9-2.2)	32 (19.2)	0.899	0.9 (0.6-1.7)	1.0 (0.9-1.1)
No exposure	26 (17.7)				29 (19.7)			
Dual exposure	51 (33.1)	0.002**	2.3 (1.3-4.0)	1.9 (1.2-2.8)	37 (24)	0.368	1.3 (0.7-2.2)	1.2 (0.7-2.2)
No exposure	26 (17.7)				29 (19.7)			
Nicotine exposure	51 (33.1)	0.116	1.5 (0.9-1.9)	1.3 (0.9-1.9)	37 (24)	0.289	1.3 (0.8-2.3)	1.3 (0.8-1.9)
Dual exposure	42 (25.1)				32 (19.2)			
Male	68 (26.6)	0.112	1.4 (0.9-2.1)	1.3 (0.9-2.1)	57 (23.9)	0.104	1.5 (0.9-2.3)	1.3 (0.9-1.9)
Female	51 (22.2)				41 (17.8)			

Note: * indicates statistical significance P<0.05. ** indicates statistical significance P<0.01. OR (95% CI) = odds ratio (95% confidence interval), RR (95% CI) = relative risk (95% confidence interval).

Table 4.12 confirmed the association between dual exposure to nicotine and alcohol and high RcIMT after classifying the group according to males and females. There was a significant association between exposure to nicotine and alcohol and a high RcIMT in males [$\chi^2(1) = 0.976$; $p < 0.05$; OR = 2.3 (95% CI: 1.1-1.6); RR = 1.5 (95% CI: 1.1-2.0)] as well as in females [$\chi^2(1) = 5.244$; $p < 0.05$; OR = 2.6 (95% CI: 1.1-6.1); RR = 1.5 (95% CI: 1.1-1.9)].

Table 4.12: Relationship between ultrasound measurements and dual exposure classified according to males and females.

		High RcIMT				High LcIMT			
		Yes	P value	OR (95% CI)	RR (95% CI)	Yes	P value	OR (95% CI)	RR (95% CI)
		Count (%)				Count (%)			
Females	**Dual exposure**	23 (28.0)	0.022*	2.6 (1.1-6.1)	1.5 (1.1-1.9)	15 (18.3)	0.674	1.2 (0.5-2.2)	1.1 (0.8-1.6)
	No exposure	9 (12.9)				11 (15.7)			
Males	**Dual exposure**	28 (38.9)	0.026*	2.3 (1.1-4.6)	1.5 (1.1-2.0)	22 (30.6)	0.323	1.4 (0.7-3.0)	1.2 (0.9-1.7)
	No exposure	17 (22.1)				18 (23.4)			

Note: * indicates statistical significance P<0.05. OR (95% CI) = odds ratio (95% confidence interval), RR (95% CI) = relative risk (95% confidence interval).

In table 4.13, a binomial logistic regression was performed to ascertain the effects of dual exposure to nicotine and alcohol, on the likelihood that children have high RcIMT by adjusting for covariates (length, weight, BMI, and WC at five years old). The logistic regression model was statistically significant ($X^2(5) = 11.449$, p = 0.043). The model explained 6.5% of variance in high RcIMT. Children with dual exposure to nicotine and alcohol had 1.8 times higher odds to exhibit higher RcIMT compared to children with no exposure as seen in Table 4.13. Dual exposure was significantly associated (*B* = 0.629, P < 0.05) with higher RcIMT in children at five years after adjustment of covariates such as length, weight, BMI, and WC at 5 years old.

This association was further analysed by separating gender. For males in the dual exposure group, the logistic regression model was not significant ($X^2(5) = 7.968$, p = 0.158). The model explained 7.9% of variance in high RcIMT in males. Males with dual exposure to nicotine and alcohol had 2.2 times higher odds to exhibit higher RcIMT compared to males with no exposure (Table 4.13). Dual exposure was not significantly associated (*B* = -0.759, P = 0.053) with higher RcIMT in males at five years after adjustment of covariates (length, weight, BMI, and WC at 5 years old). However, the association was at borderline significance. In females in the dual exposure group, the logistic regression model was significant ($X^2(5) = 28.332$, p = < 0.001). The model explained 3.1% of variance in high RcIMT in females. Females with dual

exposure to nicotine and alcohol had 7.6 times higher odds to exhibit higher RcIMT compared to females with no exposure (Table 4.13). Dual exposure remained significantly associated (*B* = -1.618, P = 0.002) with higher RcIMT in females at five years after adjustment of covariates such as length, weight, BMI, and WC at 5 years old.

Table 4.13: Logistic regression models illustrating the effect of dual exposure to nicotine and alcohol on high RcIMT after the adjustment for confounders.

	B	**X^2**	***df***	**R^2**	**P value**	**OR (95% CI)**
Model: High RcIMT	0.629	11.449	8	0.065	0.043*	
IDV: Dual exposure	0.629		1		0.044*	1.87 (1.0-3.5)
Adjusted variables						
Length	0.007		1		0.984	1.00 (0.5-2.0)
Weight	0.154		1		0.884	1.17 (0.2-9.2)
BMI	-0.232		1		0.861	0.79 (0.1-10.7)
WC	0.078		1		0.414	1.08 (0.9-1.3)
Model: High RcIMT in males	-0.759	7.967	5	0.079	0.158	
IDV: Dual exposure	0.765		1		0.053	2.15 (0.9-4.7)
Adjusted variables						
Length	-0.012		1		0.971	1.0 (0.5-1.9)
Weight	0.018		1		0.986	1.0 (0.2-7.0)
BMI	0.091		1		0.944	1.1(0.8-1.3)
WC	0.051		1		0.674	1.1 (0.83-1.3)
Model: High RcIMT in females	-1.618	28.332	5	0.311	0.000*	
IDV: Dual exposure	2.03		1		0.002*	7.6 (2-27.8)
Adjusted variables						
Length	0.31		1		0.441	1.4 (0.6-3.0)
Weight	-0.348		1		0.766	0.7 (0.1-7.0)
BMI	-0.122		1		0.936	0.9 (0.1-17.8)
WC	0.246		1		0.098	1.3 (1.0-1.7)

Note: * indicates statistical significance < 0.05; *B*: Beta, X^2: Chi-square, *df*: degree of freedom, R^2: Nagelkerke R square value, OR (95% CI) = odds ratio (95% confidence interval, IDV: Independent variable; BMI: body mass index, WC: waist circumference.

In addition, there were no significant associations between the *in-utero* teratogen exposures and systolic hypertension, diastolic hypertension, prehypertension and hypertension at five

years old. Furthermore, there were no significant associations between maternal hypertension, maternal anaemia and systolic hypertension, diastolic hypertension, prehypertension and hypertension in the five-year-old children (Table 4.14).

Table 4.14: Relationship between clinical measurements, gender, maternal hypertension and maternal anaemia across exposure groups.

	Systolic hypertension				Diastolic hypertension				Prehypertension				Hypertension			
	Yes	P value	OR (95% CI)	RR (95% CI)	Yes	P value	OR (95% CI)	RR (95% CI)	Yes	P value	OR (95% CI)	RR (95% CI)	Yes	P value	OR (95% CI)	RR (95% CI)
	Count (%)				Count (%)	Count (%)			Count (%)				Count (%)			
Nicotine exposure	1 (0.6)	*1.000*	0.8 (0.1-13.3)	0.82 (0.1-13.0)	1 (0.6)	158 (99.4)	0.8 (0.1-13.5)	0.84 (0.1-13.3)	5 (3.1)	0.386	0.6 (0.2-1.9)	0.61 (0.2-1.9)	1 (0.6)	*1.000*	0.8 (0.1-13.5)	0.8 (0.1-13.3)
No exposure	1 (0.7)				1 (0.7)	132 (99.2)			7 (5.0)				1 (0.8)			
Dual exposure	0 (0)	*0.493*	№	1 (0.9-1)	1 (0.7)	140 (99.3)	0.9 (0.1-15.2)	0.9 (0.1-14.3)	8 (5.4)	0.888	1.1 (0.4-3.1)	1.1 (0.4-2.9)	1 (0.7)	0.617	0.5 (0.0-5.3)	0.5 (0.0-5.3)
No exposure	1 (0.7)				1 (0.8)	132 (99.2)			7 (5.0)				2 (1.5)			
Nicotine exposure	0 (0)	*1.000*	№	1.0 (0.9-1.0)	1 (0.7)	140 (99.3)	1.1 (0.1-18.2)	1.1 (1.0-17.9)	8 (5.4)	0.304	1.8 (0.6-5.6)	0.8 (0.6-5.3)	1 (0.7)	*1.000*	1.2 (0.1-18.9)	1.2 (0.1-1.0)
Dual exposure	1 (0.6)				1 (0.6)	158 (99.4)			5 (3.1)				1 (0.6)			
Maternal hypertension	0 (0)	*1.000*	№	1.0 (0.9-1.0)	0 (0)	41 (100)	№	1.0 (0.9-1.0)	4 (8.9)	*0.123*	2.4 (0.8-7.4)	2.2 (0.8-6.4)	0 (0)	*1.000*	№	1.0 (1.0-1.0)
Normal	2 (0.5)				3 (0.8)	385 (99.2)			16 (4.0)				4 (1.0)			
Maternal Anaemia	2 (1.1)	*0.166*	№	0.9 (1.0-1.0)	2 (1.2)	171 (98.8)	3.0 (0.3-33.7)	3.0 (0.3-32.9)	9 (5.0)	0.641	1.2 (0.5-3.1)	1.2 (0.5-2.9)	3 (1.7)	0.307	4.5 (0.5-43.7)	4.5 (0.5-42.4)
Normal	0 (0)				1 (0.4)	259 (99.6)			11 (4.1)				1 (0.4)			
Male	1 (0.4)	*1.000*	0.9 (0.1-15.0)	0.93 (0.0-15.0)	3 (1.3)	220 (98.7)	№	0.987 (1.0-1.0)	6 (2.7)	0.064	0.4 (0.2-1.1)	0.4 (0.2-1.1)	3 (1.4)	*0.624*	2.8 (0.3-27.3)	2.8 (0.3-26.7)
Female	1 (0.5)				0 (0)	210 (100)			14 (6.3)				1 (0.5)			

Note: Italics represents P value of Fisher's exact test. * indicates statistical significance P<0.05. ** indicates statistical significance P<0.01. № indicates no cases. OR (95% CI) = odds ratio (95% confidence interval), RR (95% CI) = relative risk (95% confidence interval).

CHAPTER 5: DISCUSSION

5.1 Introduction

Cardiovascular disease risk factors such as alcohol consumption and tobacco smoking are highly prevalent in South Africa, specifically among women during pregnancy (Davoudi-Kiakalayeh *et al.*, 2017). Studies have reported a prevalence of CVD risk factors in children which further increases their risk of the development of atherosclerosis (Jaddoe *et al.*, 2008). Studies have observed an association between *in utero* teratogen exposures such as nicotine or alcohol and increased adiposity, adverse lipoprotein profiles and BP in children (Jaddoe *et al.*, 2008; Bakker and Jaddoe, 2011). Therefore, *in utero* exposure to nicotine and alcohol may result in CVD later in life. The aim of this study was to compare cardiovascular risk measurements in five-year-old children from a low socio-economic population with dual *in utero* exposure to nicotine and alcohol and *in utero* exposure to nicotine by measuring cIMT, anthropometric measurements (BMI, WC, SFT) and clinical measurements including blood pressure. The main findings in this study was that there was a significant and independent association between *in utero* exposure to alcohol and nicotine during pregnancy and high RcIMT, specifically in females at five years old.

5.2 *In utero* teratogen exposure and infant birth weight and length

Birth length was significantly higher for infants in the control group and significantly lower in the dual exposed group and the lowest for infants in nicotine exposed group. Birth weight was slightly lower in the nicotine and dual exposed group when compared to infants in the control group. However, birthweight was not significantly different in infants when comparing the exposure groups and the control group. This may be due to this cohort being affected by poverty and therefore poor dietary intake, or maternal factors such as maternal BMI, which in turn can influence birth weight (Ellison, 2013). Although low birth weight is often used as an indication

of intrauterine growth restriction, Ellison (2013) emphasizes that it is possible for malnourished mothers to give birth to normal sized infants, despite having suffered from an energy restricted environment. Furthermore, it may be necessary to investigate the effects of dose and duration of alcohol consumption during pregnancy as higher doses of alcohol may lead to birth defects, whereas lower doses can result in subtle alterations in birth that may not show visible phenotypes at birth, for example: low birth weight (Lunde *et al.*, 2016).

Infants born to mothers who smoke during pregnancy normally have a lower birth weight when compared to infants of non-smokers (Yaghoubi *et al.*, 2020). It is well established that prenatal nicotine exposure is associated with lower birth weight and also intrauterine growth restriction (Molnar *et al.*, 2017). Birthweight is strongly related to maternal nutritional status and mothers with a lower BMI are more like to have smaller sized infants (Ellison, 2013). Infants exposed to intrauterine growth restriction and born with low birth weight and shorter length is associated with an increased risk of increased cIMT in young children, specifically those with a postnatal growth "catch up" period (Oren *et al.*, 2004). Infants born with low birth weight may also be more susceptible to increased BMI, dyslipidaemia and increased BP compared to infants with normal birth weight (Oren *et al.*, 2004).

Literature has correspondingly supported that prenatal nicotine exposure increases the risk of developing childhood overweight and obesity from infancy into adulthood (Molnar *et al.*, 2017; Kataria, Gaewsky and Ellervik, 2018). This is particularly important because childhood obesity has been increasing in developing countries and, thus, needs to be prevented as it may persist into adulthood (Rodrigues *et al.*, 2013).

5.3 *In utero* teratogen exposure and adiposity indices

5.3.1 Central obesity at five years old

In this study, central obesity (WC $\geq 90^{th}$ percentile) was observed in 13.6% of children with no exposure to alcohol and nicotine, followed by a prevalence of 5.8% in the dual exposed group

and 4.2% in the nicotine exposed group. In contrast, a study reported a higher prevalence of central obesity (WC > 90th percentile) in adolescents with *in utero* exposure to nicotine when compared to adolescents with no exposure (Stevens *et al.*, 2018). When comparing nicotine exposure and dual exposure in the present study, central obesity was most prevalent in 6.10% of females and in 5.56% of males with *in utero* exposure to nicotine and alcohol. Thereafter, central obesity was prevalent in 5.13% of females and 3.37% of males with *in utero* exposure to nicotine. Consequently, more females had central obesity than males in both the dual exposed group and the nicotine exposed group. A similar trend was observed in adolescents aged 12-15 years old in the United States, where the prevalence of central obesity was more prevalent in females than in males in the nicotine exposed group (Stevens *et al.*, 2018). A similar prospective study reported a significantly higher WC at eight-years-old in children with *in utero* exposure to nicotine (Ayer *et al.*, 2011).

5.3.2 Overweight and obesity at five years old

Overall, children with *in utero* exposure to teratogens were slightly more overweight at five years old than children with no exposure, with a similar prevalence of 8.4% in the nicotine exposed group and 7.8% in the dual exposed group compared to 6.8% in the control group. In a similar paediatric study conducted in the United Sates, children in the nicotine exposed group had a higher overweight (BMI > 85th percentile) prevalence in both males with 40.4% and females with 43.0% when compared to the control group (Stevens *et al.*, 2018). When comparing overweight across gender, overweight males using BMI cut off values were most prevalent in the dual exposed group with 8.33% when compared to the nicotine group with 6.74%. However, the opposite was observed in females, with females being more overweight than males in the nicotine exposed group with 10.26% in the present study. Similarly, a study observed a comparable trend of a higher overweight prevalence in females when compared to males in the nicotine exposed group (Stevens *et al.*, 2018). Nevertheless, excessive weight gain

before five years old is significantly associated with the on-set of overweight and obesity in adulthood and therefore, placing the child at risk for CVD (Munthali *et al.*, 2017).

Regarding obesity, the prevalence of obesity in males was higher in the nicotine group, with 3.37% when compared to the dual exposed group, with a 1.39% prevalence. In females, the prevalence of obesity was highest in the control group, with 5.71%, followed by the dual exposed group, with 1.22%. In contrast, a study in the United States reported that adolescents with *in utero* nicotine exposure had the highest obesity prevalence's in 23.2% of males and a slightly higher prevalence in females with 24.9% when compared to adolescents with no exposure (Stevens *et al.*, 2018).

Adolescents exposed to nicotine compared to those with no exposure during pregnancy had a marginally higher BMI ($p = 0.05$), body weight ($p = 0.10$) and significantly higher total body fat ($p < 0.009$) after adjustment for potential confounders including age, gender, height, body weight and total body fat when appropriate (Haghighi *et al.,* 2013). Maternal BMI and increased weight gain during pregnancy is also likely to increase BMI in children, which in turn may increase cardiovascular risk such as an increase in BP in adulthood (Arnold *et al.*, 2019). Another study reported a significant association between *in utero* nicotine exposure and increased odds ratio of being overweight and obese in adulthood, but not with WC and hypertension (Kataria, Gaewsky and Ellervik, 2018). However, in this study, increased adiposity indices were not observed at five years old across the exposure groups. Nevertheless, *in utero* nicotine exposure may affect adiposity indices in females more than males as observed in this study.

Conversely, a recent study's findings suggested that prenatal nicotine exposure is not a risk factor for childhood obesity with no significant association between prenatal nicotine exposure and childhood obesity (Yaghoubi *et al.*, 2020). Also, Molnar et al. (2017) reported that prenatal

nicotine exposure was not associated with conditional weight-for-length gain by the first two years of life, in fact, children with prenatal nicotine exposure had a reduced risk of developing obesity compared to children with no exposure (Molnar *et al.*, 2017; Yaghoubi *et al.*, 2020).

When looking at birth weight, children with a higher birth weight is associated with overweight and obesity in children aged 3 to 6 years old (Gaskins *et al.*, 2010). However, low birth weight may also be associated with increased risk of developing childhood overweight and obesity (Gaskins *et al.*, 2010). As seen with lower birth weight and length in this study, the low prevalence of overweight and obesity in the exposed groups may be due to the thrift phenotype hypothesis. The thrifty phenotype hypothesis explains that fetuses exposed to poor *in utero* environments such as teratogen exposure learn to compensate for their nutrient deprivation during pregnancy, resulting in a catch-up growth period during childhood when provided with sufficient nutrition (Molnar *et al.*, 2017). This could explain the results in the present study, with children still being in the catch-up phase (Molnar *et al.*, 2017). It is important to note that exposure to undernutrition during pregnancy is often seen in low socioeconomic populations and may also be accountable for lower birth weight in offspring (Mandy and Nyirenda, 2018). Mandy and Nyirenda (2018) suggests that the DOHaD may be driving the increasing prevalence of non-communicable diseases in developing countries as poverty, malnutrition and low birth weight are often prevalent in the same regions in developing countries.

Furthermore, studies suggest that *in utero* nicotine exposure can result in increased adiposity by altering endocrine homeostatic control of body weight, appetite and satiety (Bruin, Gerstein and Holloway, 2010). The association between *in utero* nicotine exposure and childhood adiposity could be explained by disturbances in hypothalamic regulation, resulting in an increased appetite through the upregulation of specific genes such as the fat mass and obesity associated (FTO) gene which has become of particular interest in paediatric populations (Bruin, Gerstein and Holloway, 2010; Rhee, Phelan and McCaffery, 2012). The FTO gene is located

on chromosome 16, expressed in the hypothalamic nuclei and is associated with increased weight gain (Rhee, Phelan and McCaffery, 2012).

Mandy and Nyirenda (2018) explains that undernutrition in early life causes permanent changes in the neuroendocrine system and homeostatic systems which can ultimately lead to an increased risk of cardiovascular and cardiometabolic diseases later in life (Mandy and Nyirenda, 2018). Equally important, a paediatric study observed a significantly smaller amygdala volume in adolescents with *in utero* exposure to nicotine when compared to adolescents with no exposure which remained significant after the adjustment for confounders such as socioeconomic status, perinatal factors and maternal BMI. A smaller amygdala volume correlated significantly with fat intake in adolescents with nicotine exposure during pregnancy (Haghighi *et al.*, 2013). Therefore, *in utero* nicotine exposure may enhance dietary preference for fat through structural changes in the amygdala (Haghighi *et al.*, 2013).

In animal studies, *in utero* exposure to alcohol subtly increased the volume of the hypothalamus in male offspring, which is associated with long-term increase in weight and obesity (Zhang *et al.*, 2019). This increase was suggested to be mediated by neurogenesis (Zhang *et al.*, 2019). The hypothalamus is an important regulator of metabolism and energy homeostasis by regulating appetite (Follin, 2019; Zhang *et al.*, 2019). Hypothalamic structural damage may lead to leptin resistance, resulting in the inability to determine satiety after high energy stores, consequently leading to obesity (Follin, 2019). It is evident that *in utero* exposure to nicotine and *in utero* exposure to alcohol influence the structure and function of the hypothalamus as seen in separate studies (Follin, 2019; Zhang *et al.*, 2019). Therefore, *in utero* exposure to both nicotine and alcohol may have a compounding effect on key brain structures that regulate appetite and satiety predisposing children to lifelong weight gain.

In this study, data was not collected at 5 years old to determine dietary fat preferences and therefore no conclusions can be made at this point. It is also important that dietary intake be collected to determine if it is influenced by the *in-utero* exposure to nicotine and alcohol, potentially mediated through structural changes in the brain.

5.3.3 Skinfold thickness at five years old

In this study, there were no significant differences in sSFT and tSFT when comparing exposure groups and the control group. However, sSFT and tSFT were significantly different when comparing gender in the control group, nicotine exposed group and dual exposed group, with females having a significantly higher sSFT and tSFT than males. Females with significantly higher body composition (sSFT, tSFT and BMI) than males were also observed in other paediatric studies (Silva, Lima and Tremblay, 2018). Furthermore, there was a significant correlation between sSFT and HR in males, as well as a significant correlation between tSFT and HR in females in the dual exposed group. This was also confirmed in a paediatric population in Southern Brazil, where the sum of skinfold thickness was associated with resting heart rate (Silva, Lima and Tremblay, 2018). An increase in one mm in the sum of skinfold thickness resulted in an increase in 0.5 beats per minute in resting heart rate (Silva, Lima and Tremblay, 2018). An increase in adipose tissue may increase the activity of the sympathetic nervous system due to adipose tissue secreting angiotensinogen which stimulates angiotensin II formation, further activating the sympathetic nervous system resulting in an increased heart rate (Silva, Lima and Tremblay, 2018).

5.4 *In utero* teratogen exposure and Carotid intima-media thickness

In the present study, there was a significant difference in cIMT in five-year-old children in a low socioeconomic population across the exposure groups as well as across gender. In addition, the intima-media thickness of the right carotid artery was significantly higher in males in the dual exposed group when compared to both the control group and nicotine exposed group. A

similar trend was observed in the Atherosclerosis Risk in Young Adults study in the Netherlands that reported a significant difference in cIMT in adolescents born to mothers who smoked during pregnancy compared to adolescents with no exposure, with a 13.4 μm thicker cIMT in the nicotine exposed group (Geerts *et al.*, 2008b). A longitudinal study reported that family socioeconomic status was found to be inversely associated with higher risk of RcIMT (>75th percentile) in early childhood (Liu *et al.*, 2017). Children aged 2-3 years old in the lowest quartile for socioeconomic position had the highest risk for high RcIMT, with a relative risk of 1.43 (95% CI, 1.10–1.86), but was not attenuated in adjusted models (Liu *et al.*, 2017).

When comparing increased cIMT across exposure groups, high RcIMT (≥ 75th percentile) as well as high LcIMT (≥ 75th percentile) were most prevalent in males in the dual exposure group with 38.89% and 30.56%, respectively, when compared to the nicotine exposed group and control group. Females with high RcIMT was most prevalent in the dual exposed group, with 25.84%, followed by females with high RcIMT in the nicotine exposed group with a prevalence of 24.36% when compared to the control group. Females with high LcIMT was also most prevalent in the nicotine group with 19.23% when compared to the control group.

When comparing gender, RcIMT and LcIMT were significantly higher in males in the dual exposed group when compared to females. This is consistent with previous literature, where cIMT values was reported to be significantly higher in males than in females which is normally more evident with an increase in age (Łoboz-Rudnicka *et al.*, 2016). There are also gender-related differences in the effects of CVD risk factors on cIMT, where age independently determined cIMT in males and age, increased WC as well as pulse pressure independently determined cIMT in females (Łoboz-Rudnicka *et al.*, 2016). However, both age and BMI determined cIMT in both males and females (Łoboz-Rudnicka *et al.*, 2016). In this study, both LcIMT and RcIMT was not associated with gender. However, other studies found a significant correlation between gender and both LcIMT and RcIMT (Luo *et al.*, 2011).

Furthermore, there was a significant association between *in utero* exposure to nicotine and alcohol and high RcIMT at five years old. This relationship remained significant after controlling for confounding variables including weight, length, BMI and WC of the child at five years old. This association was further analysed by separating gender. *In utero* dual exposure to nicotine and alcohol was significantly associated with RcIMT in males, with males having 2.2 times higher odds to exhibit higher RcIMT compared to males with no exposure. However, *in utero* dual exposure to nicotine and alcohol was not significantly associated with higher RcIMT in males after the adjustment for covariates such as length, weight, BMI, and WC at five years old. However, it is to be noted that this association was at borderline significance. In addition, *in utero* dual exposure to nicotine and alcohol was significantly associated with higher RcIMT in females at five years old which remained significant after the adjustment for covariates such as length, weight, BMI, and WC at five years old. Females with *in utero* exposure to nicotine and alcohol were 7.6 times more likely to exhibit high RcIMT compared to females with no exposure. These results were confirmed by a similar study that indicated a significant association between teratogen exposure such as smoking during pregnancy and offspring cIMT (Geerts *et al.*, 2008b).

It is important to note that environmental tobacco smoke exposure may also result in increased cIMT in children as observed in a South African study (Viikari *et al.*, 2014). However, in older paediatric studies, no association was found between *in utero* nicotine exposure, nor postnatal environmental tobacco smoke exposure and alterations in cIMT at eight years old (Ayer *et al.*, 2011). Nevertheless, environmental tobacco smoke exposure should be considered as a potential confounder for increased cIMT observed in this population. In South Africa, a prospective longitudinal study has reported lower HDL-cholesterol and higher triglyceride levels in children exposed to smoking during pregnancy (Ayer *et al.*, 2011). Therefore, *in utero* dual exposure to nicotine and alcohol may affect lipid profiles, and thus, cIMT in the offspring.

Henceforth, the need for early detection of dyslipidaemia and atherosclerosis in children, as atherosclerotic plaque formation can begin as early as the fetal and neonatal period (Benedict *et al.*, 2018).

Although increased cIMT is used to detect the early stages of the development of atherosclerosis, cIMT measurements is only an indication of structural alterations in the arterial wall. Therefore, endothelial functional changes should also be assessed to further understand the underlying mechanisms of exposures to nicotine and alcohol during pregnancy. Brachial flow mediated dilation should be considered in follow up studies to assess endothelial dysfunction as this is the earliest identifiable event of the process of atherosclerosis (Harris *et al.*, 2010). Together, these measurements will provide a deeper understanding of the effects of in utero exposure to nicotine and alcohol on structural and functional changes within the cardiovascular system.

The associations between teratogen exposure and high intima-media thickness were only observed with the right carotid artery and not the left carotid artery in the present study. Due to the different anatomical origins between the left and right common carotid arteries, risk factors may have a different impact on the degree of atherosclerotic changes in the left common carotid artery verses the right common carotid artery (Luo *et al.*, 2011). The left common carotid artery branches directly from the arch of the aorta and subjected to hydrostatic pressure from the arch of the aorta, whereas, the right common carotid artery branches from the innominate artery, an extension from the ascending aorta and therefore exposed to high pressure due to the blood flow from the ascending aorta (Luo *et al.*, 2011). This interaction between the blood flow and intima resulting in stress on the right carotid intima has been associated with thicker cIMT and atherosclerotic plaque development (Luo *et al.*, 2011).

Furthermore, RcIMT was significantly associated with adiposity indices such as WC and BMI in both males and females in the nicotine exposed and dual exposed group. Similarly, a study comparing the effect of risk factors on the left cIMT verses the right cIMT observed a positive correlation between RcIMT and BMI as well as RcIMT and WC (Luo *et al.*, 2011). In this study, males had a positive and significant correlation between WC and RcIMT in the dual exposed group. However, this correlation was not significant after the adjustment for confounders such as BMI at five years old. Similarly, females had a significant correlation between WC and RcIMT in the nicotine exposed group. However, the correlation between RcIMT and WC was not significant after the adjustment for BMI at five years old. It is well documented that BMI and increased WC is correlated with cIMT (Łoboz-Rudnicka *et al.*, 2016). A study showed that overweight and obese children were found to have a higher mean cIMT than children with normal BMI measurements (Sajja *et al.,* 2020). Therefore, BMI was treated as a potential confounder in this study.

In children aged 6-11 years with no *in utero* teratogen exposure, BMI was correlated with cIMT, with a stronger correlation in adolescents aged 12-18 years (Gooty *et al.,* 2018). The latter study agrees with the present study where BMI was correlated with RcIMT at five years old in males in the dual exposed group. However, there was no significant correlation after the adjustment for WC at five years old. Likewise, females in the nicotine exposed group had a significant correlation between LcIMT and BMI. However, this correlation was not significant after adjusting for WC at five years old. Also, RcIMT was significantly correlated with BMI in females in the nicotine exposed group which remained significant after the adjustment for WC at five years old.

5.5 *In utero* teratogen exposure and childhood hypertension

Systolic blood pressure was significantly higher in children with *in utero* exposure to nicotine and alcohol, when compared to children with *in utero* exposure to nicotine alone. Similarly,

studies reported significantly higher SBP in both children and adolescents with *in utero* exposure to teratogens such as nicotine when compared to children and adolescents with no exposure (Ayer *et al.*, 2011; Stevens *et al.*, 2018). In addition, females had significantly higher BP measurements than males in the dual exposed group such as SBP, DBP, MAP and HR at five years old. In this study, exposure to teratogens had no significant association with SBP, DBP, prehypertension or hypertension in children at five years old. It is important to note that maternal hypertension and maternal anaemia had no effect on the moderate prevalence of prehypertension and hypertension at five years old in this study as these conditions were not associated with SBP, DBP, prehypertension or hypertension at five years old. However, maternal factors such as psychosocial stress may also influence elevated blood pressure in children, which may be present in this population which is in a low socioeconomic setting as well as in a developing country such as South Africa. Studies have reported an association between maternal psychosocial stress and a 1.5 mmHg increase in SBP, DBP and MAP in children aged 5-7 years old (van Dijk *et al.*, 2012). Therefore, this should be taken into account in future studies to rule out the influence of maternal factors on CVD risk in children.

In the current study, BMI was significantly correlated with SBP and DBP in children of low socioeconomic status at five years old. Although studies reported that prenatal alcohol exposure was associated with a reduced risk of prehypertension, it was confirmed that prenatal nicotine exposure was associated with an increased risk of prehypertension in both children and adolescents with an increased BMI (Arnold *et al.*, 2019). Additional risk factors that are associated with increased BP in children include low socioeconomic status, BMI, maternal BMI, prenatal nicotine exposure, low birth weight, and also increased weight gain during pregnancy (Arnold *et al.*, 2019). In a systematic review, BP was reported to be independently and positively associated with cIMT in children and remained significantly associated after adjusting for CVD risk factors (Day, Park and Kinra, 2017). A study in Portugal reported that

higher BMI is associated with higher SBP and DBP values in children and adolescents, of which proportional numbers of children were selected from both urban and rural environments (Ribeiro *et al.,* 2003). Lastly, RcIMT correlated significantly and negatively with HR of females in the nicotine exposed group and the LcIMT correlated significantly and negatively with HR of males in the dual exposed group.

5.6 Strengths and limitations

The strengths of this study included the large number of participants. The case-control study design minimised the influence of external factors by selecting cases and adjustment for potential confounding variables during statistical analysis. This study also forms part of a prospective study and therefore more advantageous in comparison to a retrospective or cross-sectional study. As the exposure time to conventional cardiovascular risk factors has been rather short due to the paediatric age in this population, it is possible that these exposures have not manifested long enough to cause vascular damage in this population, thus minimizing the effect of conventional risk factors on cardiovascular risk at five years old. Limitations of this study included the self-reporting of smoking and alcohol use during pregnancy which may be under reported. The paediatric group exposed to alcohol only was small in sample size and there were no significant differences detected in the preliminary data analysis. It was therefore not included in this study. This might be attributed to the small number of participants in this group. Additional data could not be collected such as physical activity, dietary habits and family history of CVD as well as environmental tobacco smoke exposure which can be treated as confounders.

5.7 Conclusion

This study observed a significantly higher RcIMT in children with exposure to nicotine and alcohol during pregnancy. As this study hypothesised, there was a significant association between *in utero* exposure to nicotine and alcohol and a high RcIMT, specifically in females

at five years old after the adjustment for confounders. Children exposed to alcohol and nicotine *in utero* were 2.3 times more likely to have an increased RcIMT with a relative risk of 1.9 times greater to children with no exposure. Females also had significantly higher BP measurements in the dual exposed group. Cardiovascular risk factors were modestly present in children of low socioeconomic status and at five years old. However, increased adiposity indices were not observed in this population at five years old and were not associated with teratogen exposures. This may indicate that dual exposure to nicotine and alcohol has a significant effect on the intima-media thickness of the carotid arteries in children, but not necessarily on central and peripheral adiposity at five years old. Furthermore, there were noticeable differences in cardiovascular risk measurements across gender which should also be taken into consideration when studying paediatric populations in low socioeconomic populations. Cardiovascular disease risk factors need to be identified early in childhood, specifically in low socioeconomic regions at risk for CVD.

5.8 Recommendations

It is recommended that future paediatric studies should classify populations according to gender and be studied separately as there were noticeable differences in cardiovascular risk across gender. This may have significant implications in developing prevention strategies in paediatric populations that are gender specific. Prospective studies in the field of DOHaD are scarce in South Africa, and consequently, further research studies should be carried out in low socioeconomic populations, specifically in children with *in utero* exposures to both alcohol and nicotine. Future studies should also study lipid profiles in children exposed to nicotine and alcohol during pregnancy in order to determine if high cIMT may be mediated through dyslipidaemia.

5.9 Summary

In utero exposure to nicotine and alcohol may cause CVD later in life. The development of atherosclerosis can begin in early childhood and therefore should be detected early in order to prevent the development of CVD risk factors in adulthood. This is particularly important in vulnerable populations such as those in low socioeconomic populations, where smoking and alcohol consumption during pregnancy is highly prevalent, as well as external factors such as poverty, poor dietary intake and tobacco smoke exposure. The aim of this study is to compare CVD risk in five-year-old children from a low socio-economic population with dual *in utero* exposure to nicotine and alcohol and in utero nicotine exposure by measuring cIMT, anthropometric measurements (BMI, WC, SFT) and clinical measurements including blood pressure.

The present study observed a significantly higher intima media thickness in the right carotid artery in children with *in utero* dual exposure to nicotine and alcohol during pregnancy and a significantly higher RcIMT in males with *in utero* dual exposure to nicotine and alcohol when compared to females. A significant association was found between *in utero* dual exposure to nicotine and alcohol and a high RcIMT, specifically in females at five years old after the adjustment for confounders. Consequently, females also had significantly higher SBP, DBP, MAP and HR in the dual exposed group. Therefore, children exposed to both nicotine and alcohol during pregnancy may have a higher cardiovascular risk compared to children exposed to nicotine alone. Further research studies in the field of DOHaD should be carried out in low socioeconomic populations in South Africa to further investigate the effects of nicotine and alcohol on cardiovascular risk in paediatric patients.

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