**The effect of maternal exposure to alcohol and nicotine on pancreas and kidney size, aorta and carotid intima thickness and visceral fat in their children.**

by

Juléy Janice Abigail De Smidt

Submitted in partial fulfilment of the requirements for the degree

PhD Medical Biosciences



In the Department of Medical Biosciences
Faculty of Life Sciences
University of the Western Cape
Cape Town
South Africa

November 2019

**Declaration**

I hereby declare that this thesis which I herewith submit at the University of the Western Cape for the award of the PhD degree in Medical Biosciences is my own research and has not been submitted by me for a degree at any other university or institution of higher learning

Juléy Janice Abigail De Smidt

November 2019

Abstract

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**By**

**Juléy Janice Abigail De Smidt**

Supervisor: Prof A Oelofse
Co-Supervisor: Prof HJ Odendaal

Department: Medical Biosciences
Degree: PhD (Medical Biosciences)

**Abstract**

**Background:** *In utero* exposure to teratogens, increasing urbanization, rapid nutritional transition from poverty to affluence, adoption of a Western-style diet and physical inactivity have contributed to the growing obesity epidemic in the low-income countries.

**Aim:** To investigate the associations between *in utero* exposure to alcohol and nicotine on the growth and development of children aged five years from a low-income setting. These effects will be observed in children aged five years as a reduced pancreas and kidney size, higher aorta and carotid intima thickness as well as higher visceral abdominal adiposity measurements**.**

**Methods:** A prospective cohort study of children aged five years from a low-income setting. This is a further follow-up study of children born in the Safe Passage Study. Data was collected from 500 mother-child pairs at antenatal clinic visits, at birth and at the age five years. Maternal data were collected at antenatal clinics in the residential area of Bishop Lavis, Western Cape, when women enrolled for their first antenatal visit. All other assessments were done at follow-up study visits at Tygerberg Academic Hospital in Bellville, South Africa. Dependent variables included: anthropometric measurements at birth, weight (BW), length (BL), mid-upper-arm circumference (MUAC). And, body mass index (BMI), skinfold thickness (SFT) and waist circumference (WC) at age five years. Also, clinical assessments at five years, blood pressure (BP), mean arterial pressure (MAP) and heart rate (HR). And, ultrasound assessments consisted of the aorta and carotid intima media thickness (aIMT and cIMT) and visceral adipose tissue (VAT), kidney and pancreas size. With independent variables: maternal *in utero* exposure to nicotine and alcohol, maternal BMI and MUAC.

**Results:** We observed higher cIMT and lower visceral adipose tissue values as a result of dual *in utero* exposure to alcohol and nicotine in males; maternal adiposity influenced the adiposity measures of their children. Females had higher SBP, DBP and HR values compared to males. Also, females born to overweight mothers had higher SFT values compared to those born to normal weight mothers (OR 1.62, 95 % confidence interval 1.24 – 2.13). Pancreas body and kidney length were associated with *in utero* nicotine exposure [F (3, 485) = 2.86 at p = 0.04] and [F (3, 493) = 2.99 at p = 0.03].

**Conclusions:** *In utero* exposure to alcohol and nicotine had both individual as well as compounding negative effects on the growth and development. Ultimately, higher cIMT and visceral adipose tissue values translate to cardiometabolic risk factors which are present in these five-year-old children from a low-income setting in South Africa.

**Acknowledgements**

***"Success without honour is an unseasoned dish, it will satisfy your hunger, but it wouldn't taste good"***

***Joe Paterno***

I would like to honour a few people who made writing this thesis possible but, I don't really know where to start. Since order is so important in the research field, I'll start with those how contributed in making this thesis possible and I will end off with those who helped me as a person.

Firstly, I would like to thank the patients, the parents or caregivers of the study population for their participation and effort. As a researcher, I realize the value of patients every day. Without patients we don't have anything to research or write about.

Secondly, to my supervisors, to whom I am in great debt. Thank you, André and Hein, for your expertise, your knowledge and experience in your respective fields. I have the utmost respect not only for your work but for you as human beings, fellow researchers and educators. I am immensely grateful to you. Love and respect always!

Thirdly, to all the ladies of the Safe Passage Team, Carlie, Heidi and Lucy. A huge thank you for your contribution with the data collection and compilation of files. To the statisticians, Daan, Theunis and Ria, thank you for all the hours of work and, to educating me in the field of Statistics!

Fourthly, to all my postgraduate students, I have learnt a great deal from each of you and I am eternally grateful.

Lastly, to my family, I would like to express my gratitude and love. To my parents, Stanley and Judy, your continuous support and love make me want to be more, do more and make that difference in people's lives. Thank you for the example that you are. Thank you for instilling and demonstrating hard work and endurance in me. Thank you for so many other things too but most importantly thank you for giving me the gift of life. To my sisters, their husbands and all my nieces and nephews, thank you for filling me heart with love, laughter and so much joy every day. Thank you to Fergill and Rafael, oh, Fire and Snowy (my four-legged children) too, for allowing me to pursue my passion. Being a mother, keeping a household going and writing a thesis are an enormous challenge for sure but, in the end most rewarding.

To my Creator and Father in Heaven, I bring all the glory and praise.

**Table of contents**

# Chapter 1

## General Introduction

Cardiometabolic diseases (CMDs) such as type 2 diabetes mellitus (T2DM), hypertension (HPT), cardiovascular disease (CVD) and ischemic heart disease (IHD), are non-communicable diseases (NCDs) and pose a huge concern for global health and wellness. These diseases are responsible for over 35 million deaths each year, representing nearly two-thirds or 63% of the world's deaths, with more than 80 % of NCD-related deaths occurring in low- and middle-income countries (LMIC) undergoing rapid socioeconomic transition. Furthermore, nearly a third of those deaths occur before age 60 [1 – 4]. Overweight and obesity, associated with numerous adverse health outcomes, are still at the forefront of modifiable risk factors for cardiometabolic risk assessment and management [5]. Globally, in the past 35 years, obesity almost doubled, hypertension is present in about 40% of over 25-year olds and about 700 million people suffer from dysglycaemia including diabetes mellitus and impaired glucose tolerance. For this reason, NCDs are considered among the five leading threats to the worldwide economy[6].



Corresponding, in South Africa childhood obesity doubled from 2008 to 2015, and most prevalent among females according to the birth-to-twenty cohort of Soweto, South Africa [7, 8]. In addition, hypertension is the most prevalent cardiometabolic risk factor in South Africans[9]. According to a South African self-reporting cross-sectional survey done in 2008, individuals over the age of 50 had a NCD prevalence of 51.8%. In 22.5% of these more than two chronic conditions were present. Furthermore, low-income countries battle with both malnutrition and over-nutrition[9]. Compromised foetal growth, a changed body composition and impaired brain development are some of the immediate consequences of maternal under-nutrition[10]. In fact, consequences of maternal malnutrition may result in impaired cognitive development, educational incapacities and eventually lowered work capacity in their children. Other long-term consequences include an altered metabolic phenotype with impaired glucose and lipid metabolism[2, 11] together with hormone and gene dysfunction experienced by generations to follow [12]. These long-term effects place an enormous amount of strain

on the work force capacity[10] and healthcare systems of low-income countries[9]. On the other hand, maternal over-nutrition adds to the growing obesity epidemic. To alleviate the heavy burden of cardiometabolic diseases on the healthcare system and decrease the demand for healthcare services in low-income countries, new research approaches are needed[13].

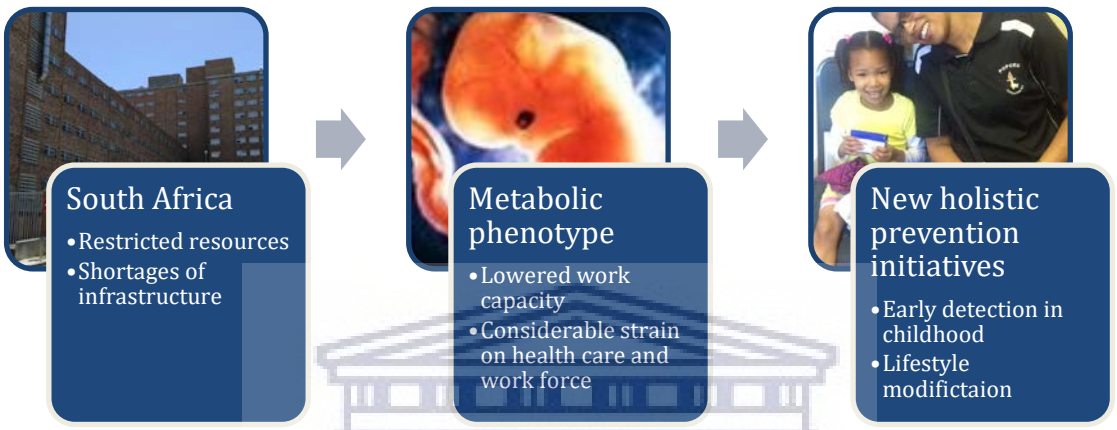


Figure 1.1 LMIC cardiometabolic challenges and solutions

As described, childhood cardiometabolic risk trail into adulthood and the emergence of these risk factors among young individuals is of great concern[7]. In South Africa, 10% of pre-school children, 14.2% of primary school-aged children and between 20-40% of adolescents are overweight[14, 15]. To illustrate, overweight adolescents are twice as likely to develop CVD and have a seven-time greater risk of developing atherosclerosis. These individuals also have greater risk for hypertension, T2DM, stroke and cancer[16]. In short, the thrifty phenotype, hyperphagic and thin-fat hypotheses aim to explain the potential mediators in cardiometabolic disease programming and greater risk experienced by these individuals. Finally, critical time periods have been identified as preconception, *in utero*, early postnatal, pubertal and adolescence. As a result of maternal as well as paternal nutrition, BMI and lifestyle factors, early feeding practices and exposure to environmental toxins, potential role players of cardiometabolic risk, are identified [17, 18, 19].

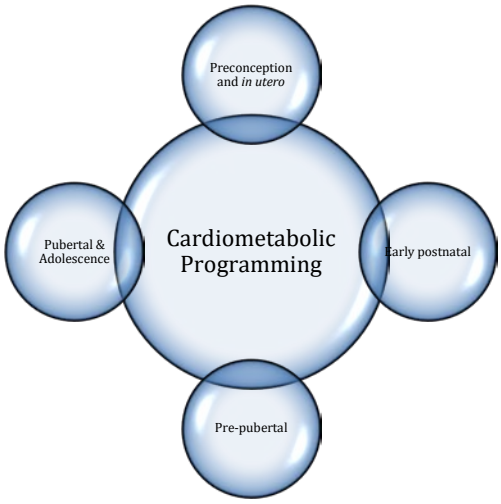


Figure 1.2 Critical time periods for cardiometabolic disease programming

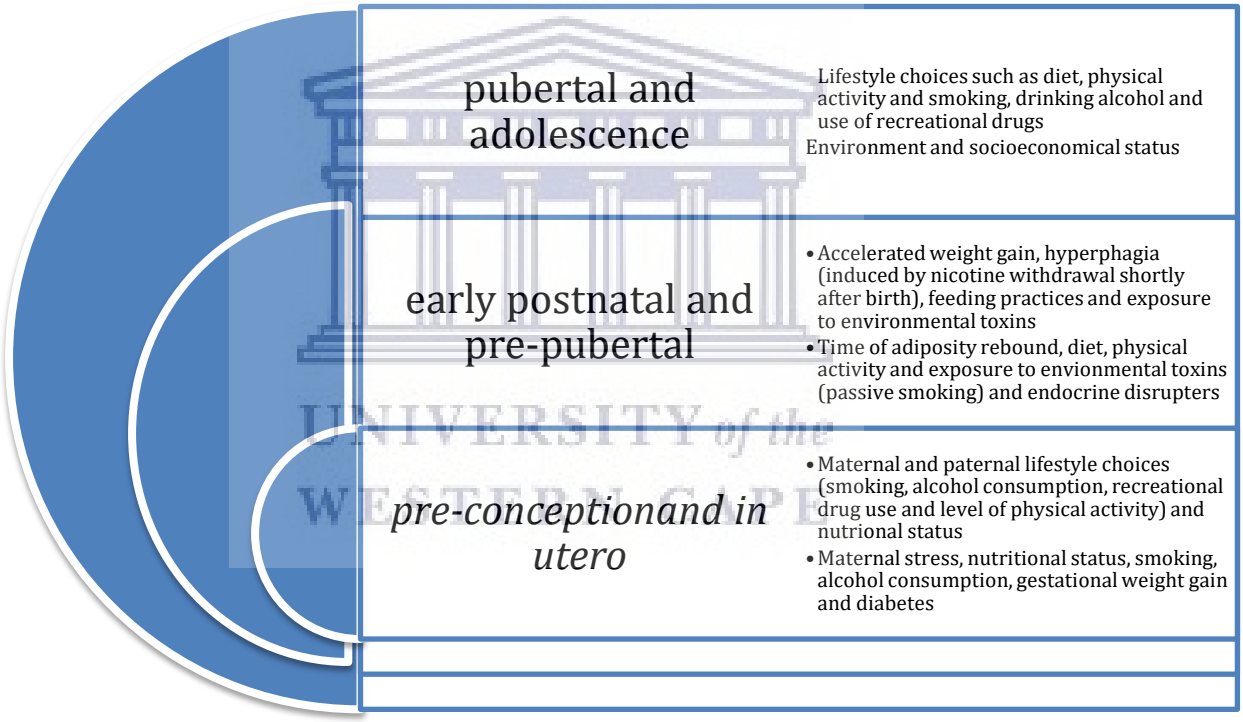


Figure 1.3 Factors associated with Cardiometabolic disease Programming during critical time periods

Metabolic disease programming, often referred to as developmental plasticity of human[20] health, was first observed and described more than 40 years ago in 1975 by Dörner. Consequently, early-life, pre- and postnatal, experiences are thought to induce permanent changes in the individual's physiological function leaving the body at risk of cardiometabolic disorders [21]. Later, Hales et al., 1992 described the link between early nutrition, birth weight and development of cardiometabolic diseases in adult life[11].

According to this hypothesis, the foetus makes adaptations, including reduced somatic growth, in order to survive when exposed to deprived or deranged nutritional conditions. And, in 1994, Dörner et al described the perinatal period and programming of hyperinsulinemia, diabetes, obesity and associated cardiometabolic risk[20]. From a developmental origin of health and disease (DOHaD) point of view, DNA modifications and methylation in critical tissues[22] is the one element that merits attention. For example, gestational hyperglyemia causes DNA methylation in the leptin gene promoter, resulting in reduced expression, in the placenta. Supported by other studies, changes in DNA methylation sequence often result in cardiometabolic risk factors such as obesity and T2DM[23]. Furthermore, the responses to leptin and insulin in association with maternal high fat diets and over-nutrition during pregnancy have gained increase recognition in recent years [6, 24, 25]. Individuals have reduced appetite regulatory responses resulting in hyperphagia, childhood obesity and forecasting adult obesity[25 - 28].

Similarly, both low birth weight (LBW) and high birth weight (HBW) are major drivers associated with adult obesity, especially central obesity[5, 24]. Also, BMI above the 95$^{th}$ percentile for age and sex during infancy, childhood and adolescence predisposes an individual to have higher BMI, skinfold thickness and waist-hip ratio as adults[14, 20, 24]. One other important contributor of adult cardiometabolic risk is rapid nutritional transition, especially in low- and middle-income countries. In addition, exposure to environmental stressors, *in utero* and secondary exposure to alcohol and nicotine, infections[28-30] also, physical inactivity[15] accentuate cardiometabolic disease risk.

Admittedly, individually alcohol and nicotine influence foetal growth and thus programming of obesity and cardiometabolic disease risk negatively[19, 24, 31]. Yet, how exposure to these teratogens affects foetal development and cause epigenetic modifications resulting in low birth weight (LBW), growth restriction, and defective organ development [30] still needs elucidation. Depending on the time period during which nutrient deficiencies and exposure to teratogens occurred, different aspects of organogenesis, lipid-, glucose metabolism and cell differentiation are affected [2, 19, 24, 32]. Supported by animal study observations, these effects of foetal programming are a result of epigenetic changes in regulatory genes, DNA methylation, and hypothalamus-pituitary-adrenal axis involvement. Organs affected by these include the brain, heart,

liver and kidney[30]. Given the above, *in utero* exposure to teratogens has an influence on the development of the vascular structures leading to premature development of HPT and atherosclerosis in the IMT of blood vessels. Also, accelerated infant weight gain together with the foetal programming effect of hyperphagia and impaired satiety responses may lead to obesity and cardiometabolic risk. Lastly, the *in utero* exposure to teratogens may affects organogenesis resulting in decreased organ size, for example, the kidneys and pancreas affecting blood pressure and responses to glucose later in life.

Rationale and Problem statement

The lifestyle of the mother, prior (preconception) and during pregnancy (*in utero*), plays a critical role in the development and manifestation of cardiometabolic disease risk in their children. These manifestations may be observed as increased adiposity and early development of hypertension and diabetes trailing from early childhood into adulthood[18]. In addition, during critical time periods, maternal nutrition, smoking and alcohol consumption may be elements prompting metabolic disease programming. Furthermore, little is known about the dual *in utero* exposure effect of alcohol and nicotine and the development of cardiometabolic risk in children. The impact of these risk factors on a country's healthcare system and economy necessitates new holistic initiatives to prevent cardiometabolic diseases. In this study, we aim to investigate the effect of *in utero* exposure to alcohol and nicotine on cardiometabolic risk five years after birth in children from a low-income setting.



The main objectives of this thesis were to investigate the individual and well as the additive effects of *in utero* exposure to alcohol and nicotine on the size of the pancreas and kidney, the extent of visceral adipose tissue and the vascular wall thickness using ultrasonography in five-year-old children from a low-income setting. Towards this goal, we used antenatal as well as the birth data from 500 mother-child pairs from the Safe Passage Study (SPS), a prospective cohort study from a low-income community in the Western Cape where alcohol consumption and smoking among women are particularly high. At age five years, the selected sample of 500 children born in this cohort was assessed for cardiometabolic risk using anthropometry, blood pressure and ultrasound measurements. The data was analysed according to the alcohol consumption and cigarette smoking history of the mother. The children were grouped as controls or non-exposed if their mothers abstained from drinking alcohol and did not smoke. The

rest of children were grouped as dual exposed if their mothers consumed alcohol and smoked during pregnancy, or alcohol only if their mothers consumed alcohol without smoking and lastly as nicotine only if their mothers only smoked.

Hypothesis

We hypothesised *in utero* nicotine and alcohol not only have individual negative effects on intrauterine growth and development but combined *in utero* exposure to nicotine and alcohol, has greater negative effects. These effects will be observed in children aged five years as a reduced pancreas and kidney size, higher aorta and carotid intima thickness as well as higher visceral abdominal adiposity measurements.

Outline of the thesis

Chapter 2, focusing on developmental origins of health and disease (DOHaD), describes epigenetics, inter-and transgenerational inheritance with regards to adiposity, vascular wall thickness, blood pressure as well as pancreas and kidney size. In this chapter special emphasis is placed on critical time periods and the mechanisms behind the programming of cardiometabolic diseases. We described the relationship between the maternal age, BMI, MUAC and child's SFT and WC using anthropometry, in Chapter 3. The effect of dual *in utero* exposure to alcohol and nicotine on the IMT of the arteries is described in Chapter 4. While in chapter 5, we described the individual and additive effects of *in utero* exposure to alcohol and nicotine on the size of the pancreas, and kidneys with BMI as confounder. Also, we investigated the pancreas and kidney size in children at age five born with LBW. In chapter 6, we describe to *in utero* effects of alcohol and nicotine on visceral abdominal adipose tissue. Lastly, the influence of maternal obesity during pregnancy on the anthropometry of their children five years after birth and, the relationship between SFT. Finally, the main findings, public health implications, suggestions for future research and conclusions are discussed in Chapter 7.

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## Chapter 2

**Title: DOHaD: Early-life incidents and intergenerational or transgenerational inheritance responses related to cardiometabolic risk**

De Smidt JJA[1¶*#a], Odendaal HJ[2¶], Nel DG[3], Nolan H[2&], Du Plessis C[2&], Brink LT[2], Oelofse A[1]

[1] Department of Medical Biosciences, University of the Western Cape.

[2] Department of Obstetrics and Gynecology, Stellenbosch University, South Africa,

[3]Department of Statistics and Actuarial Science, Stellenbosch University, South Africa

*Submitted to the Journal of Maternal, Foetal and Neonatal Health*

*Corresponding author: JJA De Smidt

Email address: jjdesmidt@gmail.com (JJA)

[#a] Current address: University of the Western Cape, Private BagX17, Bellville 7535, South Africa

Keywords: aorta and carotid intima media thickness, blood pressure, beta cell dysfunction, cardiometabolic risk, epigenetics, foetal programming, intergenerational inheritance, *in utero* exposure, kidneys, obesity, pancreas, socioeconomic status, teratogens, transgenerational inheritance, visceral adipose tissue

**Abstract**

**Introduction**: Early-life insults are responsible for metabolic disease programming. An imbalance of nutrients, hormones, or metabolites caused by maternal and infant nutrition, play an important role in DNA methylation, suppressing or enhancing gene expression.

**Aim**: The aim of this review is to evaluate the possible gaps in the field of developmental origins of health and diseases with special emphasis on the epigenetic process and cardiometabolic disease programming.

**Methods**: Database searches included Pubmed and Medline, Google scholar and Elsevier. Recent studies from 2008 to 2019, were included. Both human and animal studies with reference to foetal programming of cardiometabolic risk, epigenetics and inter- and transgenerational inheritance were included.

**Results**: In summary, developmental programming of cardiometabolic risk takes place during foetal development, preconception in oocytes and sperm cells as well as during the early postnatal years. The developmental programming is caused by maternal, paternal and infant nutrition, maternal and paternal smoking and alcohol consumption through DNA methylation process.

**Conclusion**: Longitudinal human studies are needed to map out the trajectories of cardiometabolic risk as a result of epigenetic changes and transgenerational inheritances.

## INTRODUCTION

### Obesity programming

There has been an alarming increase in cardiometabolic risk in young children in South Africa. Firstly, the cardiometabolic risk starts in childhood or adolescent years, trails into adulthood and predicts cardiometabolic disease outcomes in later years[1]. Although research shows unmodifiable genetic factors play an important role in the early onset of cardiometabolic risk, genetics alone appear to be insufficient to explain the increase in obesity rates over the last decades[2]. To summarize, cardiometabolic programming may be best explained by a combination of both unmodifiable genetic and modifiable dietary, physical inactivity plus other lifestyle factors such as alcohol consumption and smoking. Secondly, maternal and infant nutrition play an important role in adiposity programming and ultimately, the cardiometabolic risk experienced [3, 4]. Consequently, an imbalance of nutrients, hormones, or metabolites may trigger changes in the DNA methylation process, suppressing or enhancing gene expression [5 - 8] or affecting the hypothalamus-pituitary-adrenal axis[9]. Similarly, nutrients from dairy protein intake are associated with secretion of insulin growth factor-I (IGF-I), which in turn, is associated with the growth of the foetus and infant. Therefore, the IGF-I concentration of infants influences obesity programming as well as its related cardiometabolic disorders in later life[10]. In addition, untimely weaning and excessive infant formula feeding can also cause epigenetic modifications. Lastly, rapid infant weight gain, especially in the first two years, adds to the development of obesity and related disease risk in later life[4, 11].

Especially *in utero* or during the early postnatal period, these adverse environmental early-life insults can result in unapparent morphological and physiological alterations which translate into cardiometabolic diseases in adulthood[6]. Consequently, these alterations are caused by epigenetic changes taking place in the germ cells of embryos. In addition, impaired glucose tolerance during gestation causes DNA methylation in the leptin gene promoter, alterations in the placenta and resulting in a reduced expression. In fact, DNA methylation in the promoter of the adiponectin gene, on the foetal side of the placenta, is inversely correlated with maternal glucose levels during the second trimester of pregnancy[8]. Not only maternal factors impact on foetal development but also paternal factors. In a study done on male rats, were male rats were placed on a high-fat diet, researchers found impaired glucose tolerance in the female

offspring. This is thought to be as a result of epigenetic modification of sperm DNA [12 - 14]. Ultimately, these physiological changes in the phenotype help the offspring to survive in a predicted deprived environment. Finally, if there is an incompatibility between the offspring former (*in utero*) and its later (postnatal) environment, it leads to cardiometabolic risk.

**Rationale, problem statement and research questions**

An increase in the prevalence of cardiometabolic risk factors in early childhood is distressing and calls for approaches to ensure early detection. For instance, the presence of cardiometabolic risk factors such as high blood pressure (BP) and higher intima media thickness are present in primary school-aged children[15, 16]. Critical research goals within the field of DOHaD research include determining the time frames for cardiometabolic programming, which chemicals and toxins have harmful effects and which tissues are targeted[11, 17]. Also, the inter- and transgenerational inheritance from one generation to the next [18]. In addition, to determine disease risk more precisely, reliable and accurate methods to assess body composition at an early age, are needed [4]. The aim of this narrative review is to evaluate the possible gaps in the field of developmental origins of health and diseases with special emphasis on the epigenetic process of cardiometabolic disease programming and the inter-and transgenerational inheritance thereof.



**METHODS**

Database searches included Pubmed, Google scholar and Elsevier for recent studies published from 2008 to 2019. Both human and animal studies were included. Keywords used included, epigenetics, intergenerational and transgenerational inheritance, foetal programming, cardiovascular disease, diabetes

**RESULTS**

Database searches yielded 34 published articles using relevant keywords. From these 19 articles were included. Developmental programming of cardiometabolic risk takes place during foetal development, preconception in oocytes and sperm cells as well as during the early postnatal years. The developmental programming is caused by

maternal, paternal and infant nutrition, maternal and paternal smoking and alcohol consumption through DNA methylation process (table 2.1).

Table 2.1 Summary of epigenetic, intergenerational and transgenerational studies

| Year Published & Name of the authors | Title of the article | Study design, participants | Main findings and Conclusion |
|---|---|---|---|
| 2008<br>Gicquel N et al | Epigenetic regulation and foetal programming | Animal study | Epigenetic changes in regulatory genes and growth related genes are associated with foetal programming<br>Maternal nutrition during pregnancy influences foetal programming of adult diseases<br>Uncertainty remains about the mechanisms of foetal programming of adult disease |
| [19]2008<br>Grigore D, Ojeda NB, Alexander BT | Sex differences in the foetal programming of CVD | Animal study | Sex differences are observed and suggest sex hormones modulate the<br>activity of regulatory systems<br>Foetal programming of adult disease is multifactorial<br>HPT is seen in both IUGR sexes but only males remain hypertensive. Estrogen may play a protective role after maternal protein restriction during pregnancy[19] |
| [20]2008<br>Grigore D, Ojeda NB, Alexander BT | Developmental programming of HPT: Insights from animal models of nutritional manipulation | Animal study | Sex hormones mediates foetal growth<br>Slow fetal growth leads to alterations in normal regulatory systems involved in the long-term control of BP regulation[20] |
| [21]2008<br>Ojeda NB, Grigore D, Alexander BT | Intrauterine growth restriction: Foetal programming of HPT and kidney disease | Animal models | Maternal nutrient restriction, placental insufficiency and hypoxia are associated with endothelial dysfunction.<br>CVD and kidney disease develop in response to fetal adaptations[21] |
| [22] 2009<br>Pudddu M, Fanos V, Podda F, Zaffanello M | The kidney from prenatal to adult life: Perinatal programming and reduction in numbers of nephrons during development | Animal study | Maternal undernutrition causes a reduction in nephron numbers, altered glomerular structures and ultimately leads to reduced renal function<br>LBW causes low nephron numbers[22] |
| [23]2010<br>Benz K, Amann K | Maternal nutrition, low nephron numbers and arterial hypertension later in life | Human study | Brain and heart development is spared at the expense of kidney and pancreas development during embryology in IUGR individuals.<br>The 16th week of gestations is a vulnerable time – genetic and environmental factors disturb nephron formation and leading to low nephron numbers[23] |
| [24]2010<br>Heerwagen MJR, Miller MR, Barhour LA, Friedman JE | Maternal obesity and fetal programming: a fertile epigenetic soil | Animal study | Maternal obesity is associated with health risk for the developing foetus<br>Fetal exposure to saturated fatty acids can activate inflammation / pro-inflammatory pathways, which could impact substrate metabolism, mitochondrial function and stem cells[24] |
| [25]2011<br>Chen M, Zhang L | Epigenetic mechanisms in developmental programming of adult diseases | Animal study | In males LBW was associated with adult CKD<br>In both males and females, BW was associated with BP<br>Vascular dysfunction was enhanced in only the male nutrient restricted animals.<br>Placental insufficiency was associated with adult HPT in males[25] |
| [26]2011<br>Jones JE, Jurgens JA, Evans SA, Ennis RC, Villar VAM, Jose PA | Mechanisms of fetal programming in Hypertension | Human study | During embryology, nutrients are re-routed from kidneys to vital organs such as the brain<br>IUGR may result in a reduction in the expression of genes involved in nephrogenesis[26] |
| [13] 2012<br>Koleganova N, Benz K, piecha G, Ritz E, Amann K | Renal, cardiovascular and metabolic effects of fetal programming | Animal study | Intrauterine nutrient deprivation leads to programming endocrine systems toward energy saving during foetal life<br>Nutrient restriction is associated with foetal programming of vascular dysfunction, catch-up growth hyperphagia and obesity[13] |

| | | | |
|---|---|---|---|
| [27]2012<br>Vickers MH, Sloboda DM | Leptin as mediator of the effects of developmental programming | Animal study | Post-natal is a critical period whereby leptin treatment can modify the development of neural circuitry<br>Manipulations of leptin concentration can reverse developmental programming[27] |
| [9]2012<br>Xiong F, Zhang L | Role of the hypothalamus-pituitary-adrenal axis in developmental programming of health and disease | Animal study | Epigenetic modification of the GR promoter plays a central role in mediating these programming processes<br>Prenatal stressors including malnutrition, hypoxia and glucocorticoid exposure may lead to resetting of HPA activity and increased susceptibility of disease[9] |
| 2013<br>Lucassen PJ, Naninck EFG, van Goudoever JB, Fitzimons C, Joels M, Korosi A | Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics | Human study | During the critical periods of development, adverse events can affect gene expression. Maternal care, stress hormones, neuropeptides and early nutrition modulate epigenetic influences<br>The hippocampus is greatly receptive during the early-life period. During gestation and lactation, stress can affect the intake of both macro- and micronutrients. Thus, altering the nutrient intake of the offspring leading to alteration in hippocampal structure |
| [8]2014<br>Ge ZJ, Zhang CL, Schatten H,Sun QY | Maternal diabetes mellitus and the origin of NCDs in offspring: The role of epigenetics | Animal study | Diabetic intrauterine environment causes epigenetic alterations in gene expression<br>DNA methylation at the CpG sites leads to abnormal expression in the glucose transporters. Resulting in impaired glucose metabolism in maternal oocytes.<br>Maternal T2DM programs glucose intolerance in future generations[8] |
| [28]2014<br>Pembrey M, Saffery R, Bygren LO<br>Network of Epigen. Epidermiology | Human transgenerational responses to early life experiences: Potential impact on development, health, biomedical research | Human study | Paternal grandfather' food supply during mid-childhood influences mortality rates of men<br>Paternal smoking showed no significant association with BMI of daughters. However reduction in total lean mass is observed[28] |
| [29]2015<br>Li M, Reynolds CM, Segovia SA, Gray C, Vickers MH | Developmental programming of nonalcoholic fatty liver disease: The effect of early life nutrition on susceptibility and disease severity in later life | Animal study (rats) | Both over and under maternal nutrition lead to NAFLD in males Moderate to severe protein restriction leads to hepatic steatosis in offspring[29] |
| [30]2015<br>Monteiro LJ, Norman JE, Rice GE, Illanes SE | Fetal programming and gestational diabetes mellitus | Animal study (rats) | Female offspring from diabetic mothers are at increased risk of obesity and diabetes mellitus as well as abnormal glucose tolerance by the time they reach reproductive age decreased beta cell mass (in rats) in diabetic mothers reduces expression of the IGF-II Placental changes also seen[30] |
| [31]2017<br>Lucendo-Villarin B, Fillis P, Swortwood MJ, Huestis MA, Meseguer- Ripolles J, Cameron K, Iredale JP, O'Shaughnesy PJ, Fowler PA, Hay DC | Modelling fetal exposure to maternal smoking using hepatoblasts from pluripotent stem cells | Animal study | Maternal smoking associated with foetal endocrine signals and in the development of gonads and placenta<br>Sex differences reported: Effects of maternal smoking on male offspring affects pathways regulating liver fibrosis and cirrhosis whereas in female offspring maternal smoking affects glucose metabolism[31] |
| [10]2017<br>Navarro E, Funtikova AN, Fito M, Schroder H | Prenatal nutrition and the risk of adult obesity: Long term effects of nutrition on epigenetic mechanisms regulating gene expression | Animal (mice) models | Intervention with dietary interference using Chromatin Agouti and Axin-fused genes plus IGF II/ H19 locus are susceptible for diet- induced modulation of phenotypic traits (affected by methylation)<br>Maternal malnutrition affects epigenetic regulation of the pancreas development[10] |

## DISCUSSION

### Visceral Adipose Tissue Programming

Central abdominal adiposity and higher waist circumference values are associated with hypertension, insulin resistance, abnormal inflammatory cytokines secretion, all of which are linked to cardiometabolic insults. Secondly, visceral abdominal adipose tissue is thought to have more detrimental effects than generalized obesity. Accordingly, visceral abdominal adiposity is significantly associated with abnormal cytokine secretion and resulting in adverse cardiometabolic risk[32]. Furthermore, an increased adipose tissue deposition programmed *in utero*, as well as hyperinsulinemia are consequences associated with maternal hyperglycaemia and obesity. Similarly, children born with HBW as well as those born to obese and diabetic mothers have a two-fold greater risk to develop cardiometabolic diseases. As a result, infants born to diabetic mothers are at higher risk of becoming obese themselves and develop T2DM at a young age, compared to those born to non-diabetic mothers. Because epigenetic changes via DNA methylation take place in the oocytes of diabetic mother, impaired glucose tolerance is predominantly seen in female offspring[8]. Thus, young women of childbearing years, whose mothers had diabetes during pregnancy, are at greater risk of becoming obese and developing T2DM themselves, perpetuating the cycle.

Conversely, both growth restricted infants and those infants born to obese or diabetic mothers who consume a western diet are distinctly at risk of adult obesity. Between 25-63% of adult T2DM, hypertension (HPT) and CVD in general, can be attributed to low birth weight (LBW) with subsequent accelerated infant to adolescent weight gain. To summarize, introducing suboptimal formula feeding (FF), untimely weaning and introducing solid foods prematurely as well as overfeeding practices during the early postnatal period contribute to rapid infant weight gain[12]. Other factors associated with rapid infant weight gain include maternal smoking, nicotine withdrawal and hyperphagia and stimulation of adipogenesis[33]. In fact, the hyperphagia is caused by maternal and or foetal under- or over-nutrition[27]. In addition, the hyperphagic theory suggests that being overweight does not suppress appetite nor does dietary manipulations override normal regulatory mechanisms. Yet, certain diets encourage irreversible deviations in body fat, and these are not evident from changes in body weight. Also, these hyperphagic individuals are generally less active and have a lower quality diet[7]. Given the above, both extremities of birth weight, low birth weight (LBW)

and those infants with high birth weights (HBW) are equally at risk of childhood and adult obesity.

Furthermore, studies confirmed waist circumference (WC) is the anthropometric index best correlated with blood pressure (BP). For this reason, WC remains the strongest independent predictor of BP after adjusting for confounders such as BMI[34]. Thus, WC may be used as a preventative screening tool for cardiometabolic disease risk in young children[35].

**Transgenerational inheritance of obesity: rapid nutritional transition in low-income settings**

Maternal obesity is the most common health risk for her growing foetus[24]. Also, many animal studies confirmed intergenerational and transgenerational inheritance of cardiometabolic disease risk. In the same way paternal health and lifestyle choices are of importance. Thus, paternal smoking is significantly associated with the programming of the lean body mass and body composition in female offspring[28]. Furthermore, paternal nutrient restriction during mid-childhood years are also associated with mortality rates in male offspring[28].

The first generation, $F_1$ generation (direct exposed offspring) are somatically exposed during development through *in utero* exposure. Consequently, describing intergenerationl inheritance effects. As a result, the $F_3$ is the first generation to represent transgenerational inheritance[18]. While if the $F_0$ generation and their germ cells, $F_1$, are directly exposed *in utero*, the $F_2$ generation, the grandchildren, is the first generation to represent the transgenerational epigenetic inheritance. While transmission to subsequent generations could somewhat be different, elucidating the mechanisms of epigenetic changes will be of significant value[17]. In most low-income countries, such as South African, a combination of intergenerational and transgenerational epigenetic inheritance translates into different profile mothers in the $F_2$ and $F_3$ generation. In addition, rapid nutritional transition from poverty to affluence, result in mismatched adapted states which affects the mother and child[36].

**Foetal programming of vascular wall thickness, hypertension and kidney size**

The functions of the adipose tissue go far beyond just energy storage. In fact, the adipose tissue serves an endocrine function through the secretion of protein molecules adipokines and cytokines, collectively referred to as adipocytokines[37]. These adipocytokines include apelin, adiponectin, visfatin, resistin, omentin, leptin and chemerin. These molecules show extensive regulatory function on glucose and lipid metabolism and are considered a link between obesity and the development of cardiometabolic diseases (CMDs). Futhermore, many adipocytokines exert direct effects on the vascular wall, capable of altering inflammatory responses and ultimately leading to endothelial injury. Subsequently, dysfunction of endothelial cells and development of atherosclerosis. Thus, adipocytokines are considered active participants in cardiometabolic disease development[38]. In addition, arteriosclerosis and arterial damage, describing diffuse thickening and stiffening of mainly large- and medium sized arteries, form the pathological basis for cardiometabolic disease development. Accordingly, increased inflammatory cytokines activity is observed, along with changes in blood vessel formation and cardiometabolic gene expression, in intrauterine growth restriction (IUGR) [26]. Finally, higher carotid intima media thickness (IMT) seen in children from high-risk groups, is strongly related to other markers of target organ disease[39].



Furthermore, vascular endothelial dysfunction programming, observed in IUGR individuals, is associated with LBW, as a result of maternal nutrient restriction and placental insufficiency[13, 25]. *In utero* programmed impaired angiogenesis[40], programmed hypertension in adult life[19, 25, 26, 41]. Furthermore, hypertension as a result of IUGR, was observed in both male and female infants but, only the males remained hypertensive into adulthood[26]. In addition, another study confirmed vascular endothelial dysfunction and hypertension, also in males only or earlier adverse changes in vasculature structures are seen compared to age-matched females[25]. Although HPT is often considered a late-onset chronic disorder, it is now well recognized to affect the young, from neonates to adolescents[26]. In the young, HPT is defined as average systolic or diastolic BP $\geq 95^{th}$ percentile according to age, sex and height on three or more occasions[26]. Consequently, high systolic BP is observed in children born to smoking

mothers[33] and those from alcohol consuming and smoking mothers had a significant higher systolic and diastolic blood pressure[19 -21].

In addition, a multitude of studies indicate a strong association between birth weight (BW) and nephron number, mean glomerular volume and eventually hypertension[19, 20, 26]. As a result, an inverse relationship exists between BW and HPT due to nephron number deficit, caused by maternal under nutrition during foetal programming[22, 23]. Because, in humans, nephrons develop between the 28th and 34th week of gestation and nephrogenesis is not complete until end of 34-36 weeks of gestation. Typically, after this period the individual has a finite number of functioning nephrons for their entire life, an average of 750, 000 per kidney. And, no additional nephrons are formed after this period[22]. However, after birth the nephrons' tubular length and glomerular size increases. Furthermore, the amount of postnatal tubular growth in length and glomerular size varies inversely with nephron number[42]. Consequently, the 16th week period is a very vulnerable period where environmental factors such as maternal diet or medication use result in epigenetic changes in nephron formation[23]. To summarize, low nephron numbers are associated with increased risk of hypertension, proteinuria, and kidney disease in later life. In addition, sodium-dependent hypertension and albuminuria are seen in animal studies[43], while post-mortem studies reveal significantly fewer glomeruli per kidney and greater glomerular volume[44].



In the same way, maternal diabetes possibly affects the renal functional reserve of their offspring. Consequently, a decreased in nephron numbers due to exposure of gestational diabetes is seen. Also, maternal iron restriction during pregnancy leads to lower glomerular number in the offspring. Not only is impaired nephrogenesis associated with maternal hyperuricemia but, the uric acid also interferes with endothelial cell proliferation[23]. Similarly, *in utero* alcohol exposure impairs the embryonic ureteric bud branching resulting in low nephron number. Thus, alcohol consumption is linked to a dose dependent increased risk of prematurity and foetal growth restriction[23, 42]. Importantly, *in utero* alcohol exposure has the least desired effects if consumed in the second trimester[45]. For this reason alcohol consumption during this period may lead to LBW and preterm birth, which in turn is associated with increased arterial stiffness[45], more severe in males compared to females[46].

**Foetal programming of ß-cell dysfunction**

Adipocytokines have also been implicated in β-cell failure, which, when coupled with insulin resistance, it is a key factor in the development of T2DM. Furthermore, changes in circulating levels of adipocytokines form a significant link between excessive adiposity in obesity and β-cell failure. As a result, some adipocytokines have beneficial effects while others have detrimental effects[47]. Consequently, the balance between beneficial adipokines and detrimental ones are paramount in normal homeostasis and imbalances may lead to the development of cardiometabolic diseases. Furthermore, as a result of maternal gestational nutrient restricted diet, a decrease in pancreatic ß-cell mass and associated glucose intolerance in adult offspring, are observed. And, a reduction in pancreatic islet mass and ß-cell proliferation, resulting from possible decreased vascularization are documented[48]. Given the above, ß-cell dysfunction and eventually ß-cell failure are the hallmarks of T2DM. Furthermore, during the critical stages of pancreatic development, *in utero* nutritional insults may damage or weaken the ability of the ß-cells to undergo physiological adaptation when the work load is increased. Finally, initial reduction in ß-cell mass, changes in gene expression and impaired pancreatic development are seen, then later, defective mature ß-cell function may result in progressive ß-cell dysfunction[47, 48].

In addition, other recognized factors involved in reduced pancreatic ß-cell and glucose intolerance are *in utero* exposure to nicotine, prolonged *in utero* exposure to high glucose concentration and gradual loss of insulin gene expression[49]. The mechanism behind long-term sustained *in utero* hyperglycemia, is defective insulin biosynthesis, secretion and ultimately apoptosis activated by stress-induced pathways. Ultimately, the chronic hyperglycemia induces non-immune mediated inflammatory pathways in the pancreatic islets as well as pro-inflammatory signals triggering apoptosis, related to mechanisms associated with oxidative and endoplasmic reticulum (ER) stress[49]. Thus, chronic ER stress leads to ß-cells death and apoptosis[50] caused by the high demand for pro-insulin synthesis associated with the chronic hyperglycemia (Cernea et al., 2013). In addition to glucose, free fatty acids and islet amyloid polypeptides are known triggers of ß-cells ER stress.

## CONCLUSION

It is clear huge strides have been made unravelling the causes of intrauterine growth restriction and its consequences for cardiometabolic diseases, especially in early onset of these risk factors. However, longitudinal human studies are needed to map out the trajectories of foetal programming of cardiometabolic risk. In addition, teratogens causing the epigenetic changes and consequently resulting in inter- and transgenerational inheritances should be clearly elucidated.

Conflict of interest: none

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## Chapter 3

**Title: Tracking adiposity from mother to child: a prospective study**

De Smidt JJA[1], Odendaal HJ[2], Nel DG[3], Nolan H[2], Du Plessis C[2], LT Brink[2], Oelofse A[1]

[1] Department of Medical Biosciences, University of the Western Cape; [2] Department of Obstetrics and Gynaecology, Stellenbosch University, South Africa, [3]Department of Statistics and Actuarial Science, Stellenbosch University, South Africa

*Published as extended abstract for the Perinatal Priorities Conference Proceedings*

Key words: adiposity, cardiometabolic risk factors, maternal obesity, low socioeconomic population, paediatrics, skin fold thickness, waist circumference

Corresponding author: JJA De Smidt

Email address: jjdesmidt@gmail.com

Address: University of the Western Cape, Private BagX17, Bellville 7535, South Africa

**Abstract**

**Background**: Maternal obesity and excessive weight gain during pregnancy are associated with adverse infant birth outcomes such as macrosomia and a higher prevalence of childhood obesity trailing into adolescence and adulthood.

**Aim**: To investigate the associations between maternal BMI, MUAC and child's SFT and WC at age five years in children from a low-income setting. Secondly, to explore whether maternal smoking and alcohol consumption during pregnancy affected male and female offspring differently.

**Methods**: This is a further follow of children born in the Safe Passage Study. Data was collected from 500 mother-child pairs at antenatal clinic visits, at birth and at the age five years. Maternal data was collected at antenatal clinics in the residential area of Bishop Lavis, Western Cape, when women enrolled for their first antenatal visit. All other assessments were done at follow-up study visits at Tygerberg Academic Hospital in Bellville, South Africa. Anthropometric measurements included maternal body mass index (BMI), mid-upper-arm circumference (MUAC) and gestational age at enrolment and birth weight (BW), length (BL) and MUAC of the infant at birth. At age five years BMI, skinfold thickness (SFT) and waist circumference (WC) and blood pressure (BP), mean arterial pressure (MAP) and heart rate (HR) measurements.

**Results**: Maternal BMI and MUAC correlated significantly and positively with triceps, subscapular SFT and WC ($p<0.01$) of their offspring. Weight of the child at five years mediated the effect of maternal MUAC and triceps, subscapular SFT and WC in the control group. Instead, in the exposed group, both maternal MUAC and weight of the child were significant predictor IDVs.

**Conclusion**: Maternal adiposity as well as smoking and drinking during pregnancy influenced the adiposity of their children five years after birth. Weight of the child at age five is independently strongest associated with all variables. Thus, obesity screening especially for abdominal adiposity should be a priority in children of this age group. Our findings may contribute to earlier detection of obesity risk.

**Introduction**

Maternal obesity and excessive weight gain during pregnancy are associated with adverse infant birth outcomes such as macrosomia and a higher prevalence of childhood obesity trailing into adolescence and adulthood [1, 2, 3].

**Aim:** To investigate the associations between maternal BMI, MUAC and child's SFT and WC at the age of five years in a low-income setting [4, 5]. Secondly, to explore whether maternal smoking and alcohol consumption during pregnancy affected male and female offspring differently.

**Methods**

This is a further follow of children born in the Safe Passage Study. Data was collected from 500 mother-child pairs at antenatal clinic visits, at birth and at the age five years. Study design: A further follow-up of children born in the Safe Passage Study. Data was collected from 501 mother-child pairs at antenatal clinic visits, at birth and at the age five years.

Study population and setting: Maternal data was collected at antenatal clinics in the residential area of Bishop Lavis, Western Cape. All other assessments were done at follow-up study visits at Tygerberg Academic Hospital in Bellville, South Africa.



Maternal data were collected at antenatal clinics in the residential area of Bishop Lavis, Western Cape, when women enrolled for their first antenatal visit. All other assessments were done at follow-up study visits at Tygerberg Academic Hospital in Bellville, South Africa. Anthropometric measurements included birth weight (BW), length (BL) and mid-upper-arm circumference (MUAC) and body mass index (BMI), skinfold thickness (SFT) and waist circumference (WC) at age five years[6].

Statistical analysis

SPSS® software (version 21.0 for Windows; SPSS, Inc., Chicago, IL) was used for all analyses. Quantitative data was described as the means along with SD for normally distributed data and medians with 95 % confidence intervals (CI) for skewed data. Intergroup differences were determined using independent samples t-tests to evaluate the interaction between the two groups, exposed and controls and also between male and female sex. Pearson correlation coefficients were used to describe linear

relationships between maternal and child adiposity continuous variables. To explore the possible association of maternal measurements of adiposity (BMI and MUAC) and paediatric anthropometric (SFT and WC) measurements of adiposity, Pearson correlations as well as partial correlations were used. Factorial analysis was perform to simplify data to reduce the number of variables for regression models. Also, to describe the main effect versus the interaction of the IDVs. Subsequently, multiple linear regression analyses were performed separately for the controls, exposed and whole group. For these models the independent variables (IDVs) were, triceps and subscapular SFT and WC and the dependent variables (DVs) included maternal age, MUAC, BMI and weight of the child. Statistical significance was set at $p < 0.05$.

**Results**

The study sample descriptive data is represented in table 3.1. The maternal data was collected at recruitment and the mean gestational age (GA) at recruitment was 135.9 days or 19.4 weeks. As we did not have access to the pre-pregnancy BMI of the mothers, it was difficult to estimate the underweight, overweight and obese percentage.

Table 3.1 Maternal and paediatric characteristics at baseline

| **MATERNAL CHARACTERISTICS** | | | | | |
|---|---|---|---|---|---|
| | **N** | **MEAN** | **SD** | **MIN** | **MAX** |
| Age in years | 501 | 25.0 | 6.0 | 16.0 | 41.0 |
| Height in cm | 492 | 158.7 | 6.6 | 135.2 | 183.3 |
| Weight in kg | 498 | 65.4 | 16.0 | 37.8 | 122.3 |
| BMI at enrolment in kg/m$^2$ | 489 | 25.9 | 6.2 | 16.7 | 49.3 |
| MUAC in cm | 493 | 27.9 | 4.8 | 19.6 | 46.2 |
| GA at enrolment in days | 501 | 135.9 | 47.5 | 45.0 | 269.0 |
| Smoking during pregnancy<br>Yes<br>No | <br>321 (63.7%)<br>147 (29.2%) | | | | |
| Alcohol consumption during pregnancy<br><br>Yes<br>No | <br><br>187 (37.1%)<br>314 (62.3%) | | | | |
| **PAEDIATRIC CHARACTERISTICS** | | | | | |
| Boys | 253 (50.2%) | | | | |
| | **N** | **MEAN** | **SD** | **MIN** | **MAX** |
| Birth weight in kg | 500 | 3.0 | 0.6 | 0.8 | 4.7 |
| Birth length in cm | 415 | 48.7 | 2.2 | 40.0 | 55.2 |
| MUAC in cm | 412 | 10.5 | 1.0 | 8.3 | 13.6 |
| BMI in kg/m$^2$ | 414 | 12.7 | 1.9 | 5.7 | 20.9 |

BMI = body mass index, MUAC = mid-upper arm circumference, GA = gestational age

Maternal characteristics, BMI, MUAC and GA at recruitment differed significantly between the controls and the smoking and alcohol consuming mothers (table 3.2). The smoking and alcohol consuming mothers were significantly smaller with lower mean BMI (27.4 kg/m$^2$) and MUAC (25.3 cm) measurements and they enrolled at a later gestational age (139.9 days) compared to the controls with a BMI of 29.2 kg/m$^2$, MUAC at 27.3 cm and GA at enrolment of 126.2 days. The maternal age between the two groups did not differ significantly (table 3.2). At birth, the males and females did not differ significantly with regards to birth weight (BW), birth length (BL), MUAC, nor gestational age (GA) at delivery. However, BL (p = 0.01) as well as GA at delivery (p = 0.04) were significantly lower for the exposed infants compared to the controls (table 3.2). Furthermore, in this study population, the exposed males had significantly lower mean triceps SFT (8.3 cm) at the age of five years, when compared to the control males, 9.3 cm. The exposed females did not differ significantly from the control females (table 3.3). Furthermore, maternal indicators of adiposity correlated significantly and positively with measures of adiposity (p<0.05) in their offspring. Maternal BMI and MUAC were significantly and positively associated with the weight, length, MUAC or SFT. Maternal age significantly correlated with triceps SFT of their children first five years after birth (table 3.4)

Table 3.2 Maternal and paediatric characteristics according to smoking and alcohol consuming and control groups.



| MATERNAL CHARACTERISTICS | CONTROL GROUP | | | SMOKING AND ALCOHOL GROUP | | | P-VALUE |
|---|---|---|---|---|---|---|---|
| AGE (Y), MEAN (SD), N | 25.6 | 6.1 | 147 | 24.7 | 5.9 | 354 | 0.16 |
| MUAC (CM), MEAN (SD), N | 27.3 | 7.1 | 143 | 25.3 | 5.7 | 346 | <0.01 |
| BMI (KG/M$^2$) | 29.2 | 5.4 | 145 | 27.4 | 4.5 | 348 | <0.01 |
| GA AT ENROLLMENT (DAYS) | 126.2 | 44.7 | 147 | 139.9 | 48.1 | 354 | <0.01 |
| **PEDIATRIC CHARACTERISTICS AT BIRTH** | **Control Group** | | | **Exposed Group** | | | **P-value** |
| WEIGHT (KG) MEAN, SD, N | 3.1 | 0.5 | 147 | 3.0 | 0.6 | 353 | 0.18 |
| LENGTH (CM) MEAN, SD, N | 49.1 | 2.1 | 126 | 48.5 | 2.3 | 289 | 0.01 |
| MUAC (CM) MEAN, SD, N | 10.5 | 0.9 | 124 | 10.5 | 1.0 | 288 | 0.14 |
| GA (DAYS) AT DEL MEAN, SD, N | 274.1 | 13.8 | 147 | 271.2 | 14.8 | 354 | 0.04 |

Y = years, SD = standard deviation, N = sample size, GA = gestational age, Kg = kilograms, CM = centimetres, MUAC = mid-upper-arm circumference

Table 3.3 Paediatric characteristics at age five years comparing male and female control and exposed groups.

| PEDIATRIC CHARACTERISTICS AT AGE FIVE YEARS | MALES | | | FEMALES | | |
|---|---|---|---|---|---|---|
| | Control | Exposed | p-value | Control | Exposed | p-value |
| BMI (KG/M²) MEAN, SD, N | 15.3 (1.7) (77) | 15.1 (1.4) (176) | 0.57 | 15.5 (2.4) (70) | 15.1 (1.5) (178) | 0.29 |
| TRICEPS SFT (CM) MEAN, SD, N | 9.3 (3.4) (77) | 8.6 (2.5) (176) | **0.05** | 11.0 (4.0) (70) | 10.2 (3.0) (178) | 0.07 |
| SUBSCAPULAR SFT (CM) MEAN, SD, N | 7.1 (3.2) (76) | 6.6 (2.5) (176) | 0.14 | 8.3 (5.1) (70) | 7.5 (2.6) (178) | **0.09** |
| WC (CM) MEAN, SD, N | 51.6 (4.5) (67) | 51.0 (3.7) (169) | 0.31 | 52.3 (5.9) (65) | 51.1 (3.8) (169) | 0.15 |

BMI (Kg/m$^2$) = body mass index in kilograms per meter squared, SFT (cm) = skin fold thickness in centimetres, SD = standard deviation, N = sample size, WC (cm) = waist circumference in centimetres

Table 2.4 Correlations between maternal and child measures of adiposity at different time periods expressed as Pearson Correlation Coefficients (r)

| Variable and Time point | Maternal BMI (in kg/m²) | Maternal MUAC (in cm) | Maternal Age (in years) |
|---|---|---|---|
| Child weight, length and MUAC at birth | Weight (**p<0.01**) r=0.25; Length (**p=0.04**) r=0.11, MUAC (**p<0.01**) r=0.21 | Weight (**p=0.00**) r=0.27; Length (**p=0.01**) r=0.14, MUAC (**p<0.01**) r=0.20 | |
| Child weight, length and MUAC at age one month | Weight (**p=0.05**) r=0.14; MUAC (**p=0.04**) r=0.10 | Weight (**p=0.01**) r=0.16; Length (**p=0.05**) r=0.10, MUAC (**p=0.01**) r=0.14 | |
| Child weight, length and MUAC at age one year | Weight (**p=0.04**) r=0.11 | Weight (**p=0.01**) r=0.16; Length (**p=0.05**) r=0.10, MUAC (**p=0.01**) r=0.14 | |
| Child weight and length at age four years | Weight (**p=0.02**) r=0.13; Length (p=0.09) r=0.15 | Weight (**p=0.03**) r=0.17; Length (**p<0.01**) r=0.18 | |
| Child weight, length, WC, SFT and SBP at age five years | Weight (**p=0.01**) r=0.17; Length (**p=0.01**) r=0.16 | Weight (**p<0.01**) r=0.20; Length (**p<0.01**) r=0.18, WC (**p=0.02**) r=0.12 | Triceps SFT (**p=0.05**) r=-0.10; SBP (**p=0.02**) r=0.12 |

BMI = body mass index (kg/m²); MUAC = mid-upper-arm circumference in cm; WC = waist circumference in cm; SFT = skin fold thickness in cm; SBP = systolic blood pressure in mmHg

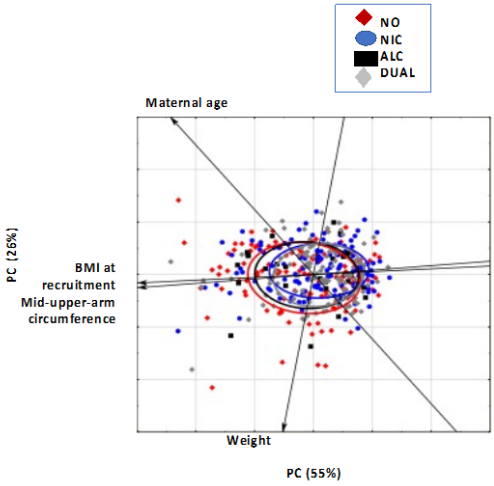


Figure 3.1 Factorial analysis illustrating the child's weight, maternal BMI, and maternal age as IDVs and their interaction with the observed variables.

The main effect as well as the interaction between the IDVs, maternal age, maternal BMI, MUAC at recruitment and the weight of the child at age five year, are illustrated by figure 3.1. The weight of the child, Factor 1, explains 55% of the effect of the outcome variables. And, maternal BMI and MUAC, Factor 2, explain 26% of the effect.

To explain the interaction of the IDVs: maternal BMI, maternal MUAC and *in utero* exposure, on the DVs, child's weight at five years, a separated linear regression analysis was conducted. As a result, only 8% of the variation in the child's weight at age five is explained by the maternal IDVs [F (, 479) = 13.28, $R^2$ = 0.08 at $p < 0.01$] with maternal MUAC (beta = 0.42, $p < 0.01$), exposure to alcohol and nicotine (beta = 0.11, p = 0.01), and maternal BMI (beta = 0.21, p=0.09). Both maternal MUAC and the *in utero* exposure to nicotine and alcohol are significant predictor variables for child's weight at age five years. Subsequently, multiple regression models were constructed to investigate whether child's weight at age five years could potentially be a mediator between the IDVs: maternal age, maternal MUAC, maternal BMI and the individual DVs: triceps, subscapular SFT and WC. These models were constructed separately for the controls, exposed and whole group and reported on in table 3.5.

Table 3.5 Multiple linear regression analysis for Controls versus Exposed to Alcohol and Nicotine Children

| IDV | Controls | | Exposed | |
|---|---|---|---|---|
| | | **Beta coefficients and p-value** | | **Beta coefficients and p-value** |
| Mat Age | **DV = triceps SFT $R^2$ = 0.38 p≤0.01** | -0.18 (-0.09) ***p = 0.05** | **DV = triceps SFT $R^2$ = 0.34 p≤0.01** | -0.14 (-0.07) p = 0.25 |
| Mat MUAC | | -0.16 (-0.10) p = 0.46 | | 0.33 (0.19) p = 0.07 |
| Mat BMI | | 0.16 (0.07) p = 0.44 | | -0.91 (-0.53) ***p = 0.03** |
| Weight of the child | | 0.58 (0.56) * **p ≤ 0.01** | | 0.71 (0.91) * **p ≤ 0.01** |
| Mat Age | **DV = subscapular SFT $R^2$ = 0.27 p≤0.01** | -0.13 (-0.08) @ p = 0.18 | **DV = subscapular SFT $R^2$ = 0.37 p≤0.01** | -0.09 (-0.08) p = 0.57 |
| Mat MUAC | | -0.20 (-0.15) p = 0.40 | | 1.02 (0.83) ***p = 0.02** |
| Mat BMI | | 0.31 (0.11) p = 0.35 | | -1.19 (-0.72) ***p = 0.01** |
| Weight of the child | | 0.50 (0.57) * **p ≤ 0.01** | | 0.67 (0.90) * **p ≤ 0.01** |
| Mat Age | **DV = WC $R^2$ = 0.63 p≤0.01** | -0.10 (-0.08) p = 0.17 | **DV = WC $R^2$ = 0.61 p≤0.01** | 0.01 (0.01) p = 0.90 |
| Mat MUAC | | 0.05 (0.04) p = 0.79 | | 0.22 (0.21) p = 0.19 |
| Mat BMI | | 0.01 (0.01) p = 0.98 | | -0.35 (-0.25) ***p = 0.04** |
| Weight of the child | | 0.78 (1.10) ***p ≤ 0.01** | | 0.78 (1.20) ***p ≤ 0.01** |



For the control group, maternal age (beta = 0.09, p = 0.05) was a predictor variable for triceps SFT of the children but the effect IDVs, maternal MUAC and BMI, on the triceps SFT is mediated via the effect of the weight of the child. However, for the exposed group, both the maternal BMI (beta = 0.53, p < 0.01) and weight of the child at age five years (beta = 0.91, p < 0.01) were predictor IDVs of triceps SFT [F (4, 495) = 35.54, $R^2$ = 0.34 at p < 0.01]. Instead, effect on triceps SFT, is not mediated by the weight of child for the exposed group (table 3.5). Likewise for the other DVs, subscapular SFT and WC. The exposed to *in utero* alcohol and nicotine, both the maternal IDVs and weight of the child are predictor variables for subscapular SFT and WC, However, in the control group, triceps, subscapular SFT nor WC were no longer with maternal BMI. Instead, weight of the child (beta = 0.57 and 1.10, p < 0.01) was acting as an independent predictor variable on the outcome, subscapular

SFT ($R^2$ = 0.27 at $p < 0.0$) and WC ($R^2$ = 0.63 at $p < 0.01$). Therefore indicating the effect of maternal BMI and MUAC on the child's triceps SFT is mediated via the effect of the weight of the child.

**Conclusion**

Maternal MUAC and BMI during pregnancy correlated with weight, BMI, SFT and WC of their children at different time periods over the first five years of life [2, 3]. In addition, *in utero* exposure to alcohol and nicotine was a significantly associated with lower triceps SFT, an indication of peripheral adiposity, in male offspring[4, 5, 8]. Children from this low-income setting [1,4],[9], where high rates smoking and alcohol consumption [8,9] are common, should be closely monitored for the development of cardiometabolic risk factors [9, 10]. The weight of the child at the age of five years was identified as a potential mediator for the outcome variables: triceps SFT, subscapular SFT and WC in the control group. And maternal age, BMI and MUAC are predictor IDVs for either triceps or subscapular SFT or WC. Therefore, findings from this study suggest close monitoring of maternal BMI and MUAC at antenatal clinic visits to reduce the health risk of their children in later life. Furthermore, findings from this study may allow early detection of obesity development among primary school-aged children by using easy and effective tools such as SFT and WC measurements to identify at-risk children, using WHO reference values[2] for males and females. Finally, findings recommend smoking cessation programmes [3] as well as health education at antenatal primary healthcare clinics should be strengthen.



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## Chapter 4

**Title: *In utero* teratogen exposure and cardiometabolic risk in five-year-old children: a prospective paediatric study**

De Smidt JJA[1¶*#a], Odendaal HJ[2¶], Nel DG[3], Nolan H[2&], Du Plessis C[2&], Brink LT[2], Oelofse A[1]

[1] Department of Medical Biosciences, University of the Western Cape;

[2] Department of Obstetrics and Gynaecology, Stellenbosch University, South Africa,

[3]Department of Statistics and Actuarial Science, Stellenbosch University, South Africa

*Published in the Journal of Maternal, Foetal and Neonatal Health November 2019*

DOI: 10.1080/14767058.2019.1692387

*Corresponding author: JJA De Smidt

Email address: jjdesmidt@gmail.com (JJA)

[#a] Current address: University of the Western Cape, Private BagX17, Bellville 7535, South Africa



Key words: anthropometry, cardiometabolic risk factors, intima media thickness, intrauterine teratogen exposure, paediatrics

**Abstract**

**Background:** Aorta and carotid intima-media thickness (IMT) is a measure of subclinical atherosclerosis and useful to assess cardiometabolic risk in the young. The *in utero* milieu may involve cardiometabolic programming and the development of cardiometabolic risk factors in children. Maternal smoking, alcohol consumption and micronutrient deficiencies during pregnancy influence the development of the cardiovascular system through a process of DNA methylation.

**Aim:** To explore an association between maternal smoking and alcohol consumption during pregnancy and intima media thickness in five-year-old children for a low-income setting.

**Methods:** Data was collected from 500 mother-child pairs at antenatal clinic visit, at birth, and at age five years. Anthropometric measurements were collected at birth and again at age five years. As well as clinical and ultrasound measurements at age five years. Clinical measurements, at age five years, included blood pressure, mean arterial pressure and heart rate. Ultrasound measurements of the aorta and carotid arteries IMT were performed at age five years. Main outcome of interest was effect of dual teratogen exposure on the ultrasound measures IMT as indication of cardiometabolic risk.

**Results:** cIMT was significantly higher in children exposed to both alcohol and nicotine during pregnancy compared to those not exposed (p=0.008). In separate linear models, dual *in utero* exposure (beta = 0.12; p=0.01) and male sex (beta = 0.14; p=0.01) were associated with higher right cIMT values [F (6,445) = 5.20; $R^2$= 0.07, p<0.01]; male sex (beta = 0.13; p=0.01) and birth weight (beta =0.07; p=0.01) with higher left cIMT value [F (4; 491) = 4.49; $R^2$=0.04; p=0.01]; and males sex (beta = 0.11; p=0.02) with higher aorta IMT [F (6,459) = 5.63; $R^2$ = 0.07; p<0.01]. Significant positive correlations between maternal measures of adiposity, maternal MUAC (r=0.10; p=0.03), and maternal BMI (r=0.12; p<0.01) and right cIMT measurements adjusted for the BMI of the child at age five years as covariate. Blood pressure measurements at age five years were not significantly associated with IMT. Instead, BP correlated significantly and positively with the BMI of the child at age five years (p<0.01).

**Conclusion:** Children exposed to both maternal smoking and alcohol consumption during pregnancy presented with cardiometabolic risk factors five years after birth. In

addition, maternal adiposity, male sex and low birth weight were associated with higher IMT at age five years.

## INTRODUCTION

Cardiometabolic diseases are associated with significant morbidity, mortality, and decreased quality of life. The management of these diseases and the associated complications place considerable financial strain on health systems, necessitating early intervention to mitigate the burden and decrease healthcare costs[1, 2]. In many cases, end-organ vascular complications including myocardial infarction and stroke occur years or even decades following the establishment of a formal diagnosis or clinical recognition of the associated risk factors[3-5]. In addition, there is growing recognition of cardiometabolic risk as an important clinical issue in the paediatric population[6]. In this context, longitudinal studies support an association between cardiometabolic risk factors present during childhood and the development of vascular insults or complications during adulthood[7].

The developing world is facing a dual pandemic of obesity and malnutrition[8]. On the one hand, poverty and food insecurity are associated with underweight status and nutritional deficiencies in adults and children[9]. On the other, low levels of education and unemployment are associated with consumption of cheaper, less nutrient-dense foods, which in turn increase risk for development of obesity and obesity related illnesses such as type-2 diabetes mellitus (T2DM) and CVD[10-13]. In addition, maternal obesity and excessive weight gain during pregnancy have been associated with higher birth weights and adiposity in female offspring[14] and predisposition of insulin resistance during childhood[15].

Moreover, developing countries struggle with high rates of *in utero* alcohol and nicotine exposure which are associated with micronutrient deficiencies and mechanisms such as endothelial dysfunction[16, 17] and abnormal foetal angiogenesis implicated in the development of cardiometabolic diseases later in life[18, 13]. The combined deleterious effects of *in utero* exposure to both alcohol and nicotine awn foetal growth are more severe than the effects of either smoking or drinking alone[19, 20]. *In utero* teratogen exposure may be further compounded by poor maternal nutrition during pregnancy[8, 20]. In this context, maternal antenatal health may predispose the

child to development of cardiometabolic diseases later in life[16] particularly if compounded by poor childhood diet and sedentary lifestyle [6]. These findings support the importance of both maternal health and early life insults as determinants of cardiometabolic risk in the paediatric population.

In South Africa, there is paucity of prospective studies investigating the effects of *in utero* teratogen exposure on the longitudinal development and progression of cardiometabolic risk factors during early childhood. In addition, studies to date have been limited to adult studies as well as underrepresentation of diverse ethnic groups, which warrants investigation given the proposed genetic underpinnings of cardiometabolic risk. In a previous study (unpublished data), we demonstrated increase adiposity values and cardiometabolic risk factors in a cohort of 500 children from a low-income South African community. In the present study, we sought to build on these existing findings by exploring the effects of *in utero* alcohol and nicotine exposure on aorta and carotid IMT over the first five years of life in the same cohort of 500 children. In addition, we aimed to investigate to what extent maternal adiposity contributed to the development of these risk factors in children during early life. In particular, we focused on ultrasonographic aorta and carotid IMT measurements associated with adiposity indices.



## MATERIALS AND METHODS

### Ethical approval

Ethics approval for the present study was obtained from the Health and Research Ethics Committee (HREC) of Stellenbosch University (SU) and Biomedical Research Ethics Committee (BMREC) of the University of the Western Cape (UWC). Voluntary written informed consent was obtained from the mother or caregiver of the child. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki[21].

### Study design

The present sub-study drew on longitudinal data collected as part of the larger descriptive Safe Passage Study (SPS), designed to obtain more information on the role of antenatal exposure to alcohol in stillbirths and sudden unexplained infant

deaths[22]. In the present analysis, we used maternal information obtained prospectively for the SPS during antenatal clinic visits, as well as prospective data on paediatric health obtained from assessment of the child at birth and again at five years of age.

Selection of study participants

The present study was conducted at Stellenbosch University Obstetrics and Gynaecology SPS Unit at Tygerberg Hospital, Cape Town over an 18-month period (June 2016 to December 2017). Pregnant women were recruited from the Belhar antenatal clinic or Bishop Lavis Midwife Obstetric Unit (MOU) and had prenatal research follow-up at Tygerberg Hospital. Data from antenatal visits were obtained from pregnant women who attended Bishop Lavis antenatal clinic enrolled for the SPS. All pregnant women booking for antenatal care were invited to be part of the study. For infant follow up we excluded twins and children with congenital abnormalities at birth. Children age five years with severe features of foetal alcohol syndrome as well as other forms of severe metal restriction were excluded from the study.

Participant assessments

Maternal assessments

A study questionnaire was used to document socio-demographic information during antenatal visits, as well as data on nutrition, pregnancy history, and use of alcohol as well as nicotine[22]. Body mass index (BMI) was calculated from measurements collected at the first antenatal visit and calculated as the body weight in kilograms divided by the height in meters squared. Mid-upper arm circumference (MUAC) was also measured as the circumference of the right upper arm measured at the midpoint between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium) using a tape measure.

Paediatric assessments

BMI was measured at birth and age five. Weight was measured using an electronic Mellerware, Munich scale to the nearest 0.01 kg. Height was measured without shoes using a mechanical Panamedic stadiometer fixed to a wall. MUAC was measured from children at birth, one month, and at one year of age. Triceps (tSFT) and subscapular skin fold thickness (sSFT) were measured in millimetres using a Holtain

calliper. Subscapular SFT was measured from the left side of the body to the nearest 0.1 mm while the fingers continued to hold the skinfold. The actual measurement was read from the calliper about 3 seconds after the calliper tension was released. Measurements were taken at the following sites: (a) triceps, halfway between the acromion process and the olecranon process and (b) subscapular, approximately 20 mm below the tip of the scapula, at an angle of 45° to the lateral side of the body. Waist circumference was calculated at year five as measured around the waist at the midpoint between the last rib and the iliac crest using a tape measure. Each child was measured three times and mean values were obtained from the three measurements. Systolic and diastolic blood pressure, mean arterial pressure and heart rate were measured at five years of age. Blood pressure was measured in the right upper arm in a sitting position using a validated CAS Medical Systems, Inc, USA, 740 MAXNIBP automated digital sphygmomanometer. A size-appropriate blood pressure cuff was used and all measurements were repeated three times.

Ultrasonography was performed on children at five years of age using a Voluson E8 ultrasound system (GE Healthcare, Kretz Ultrasound, Zipf, Austria) equipped with a RAB4-8D (3.1 – 8 MHz) convex 3D/4D transabdominal transducer and a 9L-D (3.1 – 10 MHz) linear array transducer specific for vascular and paediatric application. Left and right carotid (cIMT) as well as aorta intimal-medial thickness were measured at age five using existing recommended international protocols such as the Mannheim Consensus[23]. The decision to measure cIMT was motivated by superficial location of the carotid in the neck and easily visualized by non-invasive quality of ultrasound[24].



Statistical analysis

SPSS® software (version 21.0 for Windows; SPSS, Inc, Chicago, IL) was used for all analyses. Quantitative data was described as the means along with SD for normally distributed data and medians with 95 % confidence intervals (CI) for skewed data. Categorical variables were presented as percentages. Independent samples t-tests and analyses of variance (ANOVA) were used for comparisons between groups. To explore the effects of alcohol and nicotine exposure on outcome variables, comparisons were performed between four groups, i.e. 1) mothers who did not use alcohol or nicotine during pregnancy (33%), 2) mothers who used both alcohol and nicotine during pregnancy (28%), mothers who used alcohol but not nicotine during

pregnancy (9%), and 4) mothers who used nicotine but not alcohol during pregnancy (29%). Post-hoc LSD, Tukey or Bonferroni tests were used to indicate differences between exposure groups. Pearson correlation coefficients were used to describe linear relationships between continuous variables. Separate linear regression models were constructed to illustrate the independent association between *in utero* exposure to alcohol and nicotine and cIMT measurements with adjustment for BMI, sex of the child. Statistical significance was set at $p < 0.05$.

**RESULTS**

The characteristics of the study group are described in Table 4.1 and 4.2. Baseline maternal and neonatal characteristics are presented according to the alcohol consuming and smoking status of the mother (Table 4.1) and paediatric characteristics at end-point, age five years, are described separately (Table 4.2) and compared between the four exposure groups, controls (n=146), dual exposed to alcohol and nicotine (n=154), alcohol only exposed (n=33) and nicotine only exposed (n=167).

Table 3.1 Maternal and neonatal baseline characteristics according to maternal smoking and alcohol consumption. Data is expressed as mean (SD) and comparison between smoking and alcohol consuming mothers and controls.



**Maternal Characteristics at enrolment**

| | Age (years) | MUAC (cm) | BMI (kg/m$^2$) | GA at enrolment (days) |
|---|---|---|---|---|
| Control Group | N= 147<br>25.6 (6.1) | N=143<br>27.3 (7.1) | N=145<br>29.2 (5.4) | N=147<br>126.2 (44.7) |
| Smoking and Alcohol Group | N=354<br>24.7 (5.9) | N=346<br>25.3 (5.7) | N=348<br>27.4 (4.5) | N=354<br>139.9 (48.1) |
| p-value | 0.16 | ***<0.01** | ***<0.01** | ***<0.01** |

**Paediatric neonatal Characteristics at birth**

| | Weight (kg) | Length (cm) | MUAC (cm) | GA at delivery (days) |
|---|---|---|---|---|
| Control Group | N= 147<br>3.1 (0.5) | N=126<br>49.1 (2.1) | N=124<br>10.5 (0.9) | N=147<br>274.1 (13.8) |
| Smoking and Alcohol Group | N=353<br>3.0 (0.6) | N=289<br>48.5 (2.3) | N=288<br>10.5 (1.0) | N=354<br>271.2 (14.8) |
| p-value | 0.18 | ***0.01** | 0.14 | ***0.04** |

MUAC = mid-upper-arm circumference, BMI = body mass index, GA = gestational age, *significance at p<0.05

Maternal age did not differ significantly between the controls and the smoking and alcohol consuming mothers. However, MUAC, BMI and GA at enrolment were significantly lower and later for the smoking, alcohol consuming group (MUAC: 25.3 ± 5.7 cm, p<0.01; BMI: 27.4 ± 4.5 kg/m$^2$, p<0.01 and GA at enrolment: 139.9 ± 48.1 days) compared to the control group (MUAC: 27.3 ± 7.1 cm, p<0.01; BMI: 29.2 ± 5.4 kg/m$^2$, p<0.01 and GA at enrolment: 126.2 ± 44.7 days) (Table 4.1). At birth, infants born to smoking and alcohol consuming mothers had significantly lower body length (48.5 ± 2.3 cm, p=0.01) compared to controls (49.1 ± 2.1 cm, p=0.01). The infants from smoking, alcohol consuming mothers were also delivered at a significantly earlier GA (271.2 ± 14.8 days, p=0.04) compared to controls (274.1 ± 13.8 days, p=0.04). Body weight and MUAC at birth did not differ significantly between infants born to smoking, alcohol consuming mothers and controls (Table 4.1). In addition, the mothers of LBW infants had significantly lower maternal MUAC (26.5 ± 4.2 cm; p<0.01) and BMI (24.2 ± 5.3 kg/m$^2$; p<0.01) at enrolment when compared to the mothers of NBW infants (MUAC: 28.2 ± 4.9 cm and BMI: 26.3 ± 6.3 kg/m$^2$). Furthermore, BW also differed significantly between the four maternal exposure groups, with lowest BW noted for the nicotine and dual exposure groups (2.98 ± 0.59 kg and 2.98 ± 0.57 kg; p=0.04) compared to controls (3.06 ± 0.52 kg).

Table 4.2 Paediatric End Point characteristics at age five years according to the four exposure groups. Data is expressed as mean (SD) and comparisons between smoking and alcohol consuming mothers, alcohol only, nicotine only and controls.

| | | Controls | Dual exposed | Alcohol exposed | Smoking exposed | P=value | F | df |
|---|---|---|---|---|---|---|---|---|
| | | N=146 | N=154 | N=33 | N=167 | | | |
| Anthropometric measurements | BMI ($kg/m^2$) | 15.4 ± 2.1 | 15.2 ± 1.5 | 15.1 ± 1.7 | 15.1 ± 1.4 | 0.48 | 0.82 | 3, 497 |
| | tSFT (cm) | 10.1 ± 3.8 | 9.4 ± 2.7 | 9.6 ± 4.4 | 9.3 ± 2.7 | 0.11 | 2.03 | 3, 497 |
| | sSFT (cm) | 7.7 ± 4.2 | 7.0 ± 1.9 | 7.6 ± 3.9 | 6.9 ± 2.3 | 0.12 | 1.97 | 3, 496 |
| | WC (cm) | 51.9 ± 5.2 | 51.4 ± 3.9 | 51.2 ± 3.7 | 50.7 ± 3.5 | 0.08 | 2.27 | 3, 466 |
| Clinical measurements | SBP (mmHg) | 106.5 ± 10.7 | 107.1 ± 10.0 | 105.8 ± 11.7 | 104.3 ± 9.3 | 0.07 | 2.41 | 3, 496 |
| | DBP (mmHg) | 65.3 ± 9.1 | 65.7 ± 9.1 | 65.6 ± 11.4 | 63.9 ± 9.0 | 0.29 | 1.24 | 3, 496 |
| | MAP | 78.8 ± 9.5 | 79.4 ± 9.3 | 79.3 ± 11.3 | 77.3 ± 9.3 | 0.20 | 1.55 | 3, 496 |
| | HR (b/min) | 91.7 ± 12.4 | 92.5 ± 14.4 | 91.8 ± 8.7 | 91.4 ± 13.3 | 0.90 | 0.20 | 3, 496 |
| Ultrasound measurements | Left cIMT (mm) | 0.35 ± 0.05 | 0.36 ± 0.05 | 0.35 ± 0.04 | 0.35 ± 0.05 | 0.52 | 0.76 | 3, 493 |
| | Right cIMT (mm) | 0.34 ± 0.04 | 0.36 ± 0.05 | 0.33 ± 0.04 | 0.34 ± 0.04 | ***＜0.01** | 5.92 | 3, 494 |

BMI = body mass index, tSFT = triceps skin fold thickness, sSFT = subscapular skin fold thickness, WC = waist circumference, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, RT CIMT = right carotid intima-media thickness, LT CIMT = left carotid intima-media thickness, * significance at $p<0.05$

Anthropometric, clinical, and ultrasound measurement at age five years did not differ significantly between the four exposure groups except for the ultrasound measurement of the right cIMT. The right cIMT measurements for the dual exposed groups were significantly higher (0.36 ± 0.05 mm) compared to controls (0.34 ± 0.04

mm) F (3,494) = 5.92; p<0.01 (Table 4.2 and Figure 4.1). The left cIMT and aorta IMT measurements did not differ significantly between the four exposure groups F (3,493) = 0.76; p = 0.52 and F (3, 493) = 0.08; p = 0.97. However, at age five years, the left cIMT measurements were significantly lower for the females (0.35 ± 0.05 mm) compared to males (0.36 ± 0.05 mm) and t (495) = 3.07; p<0.01 (Figure 4.2).

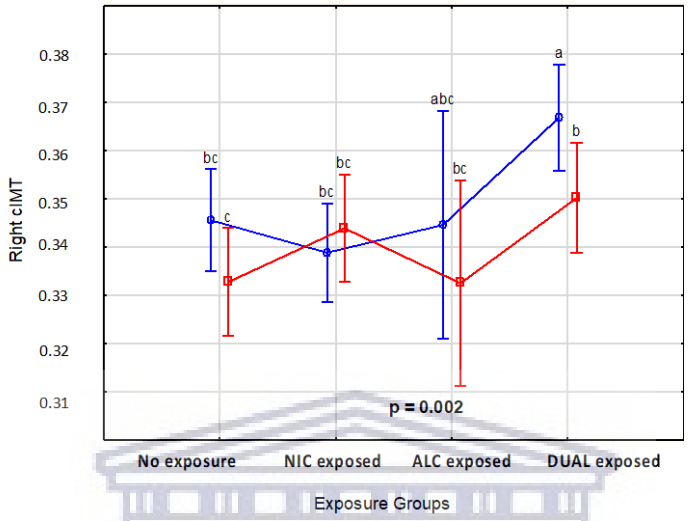


Figure 4.1 Mean values for Right carotid intima-media thickness (mm) according to the four exposure groups (p<0.01) and compared between males and females at age five years as measured by ultrasound

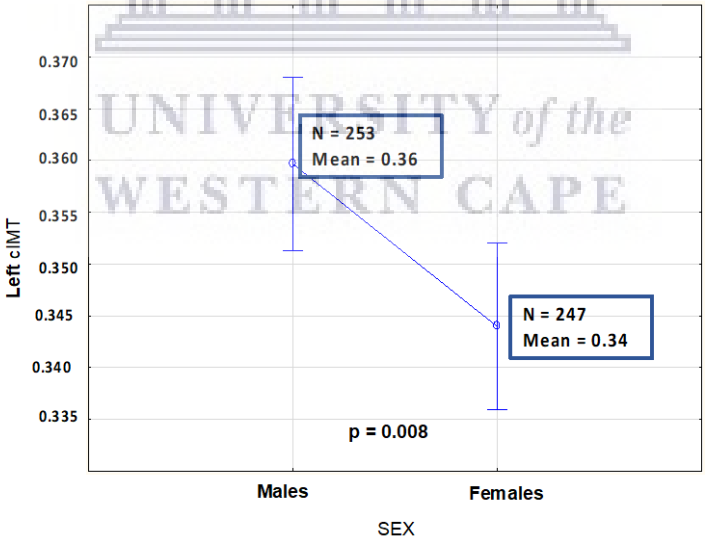


Figure 4.2: Mean values for Left carotid intima-media thickness (mm) compared between males and females (p<0.01) at age five years as measured by ultrasound

In addition, blood pressure, MAP and HR did not differ significantly between the four exposure groups. For systolic blood pressure (SBP), [F (3,496) = 2.41 at p=0.07],

diastolic blood pressure (DBP) [F (3,496) = 1.24 at p=0.29], mean arterial pressure (MAP) [F (3, 496) = 1.55 at p=0.20] and heart rate (HR) [F (3,496) = 0.20 at p=0.90]. Furthermore, right cIMT correlated significantly and positively with maternal MUAC (r=0.11; p=0.02), maternal BMI (r=0.11; p=0.02), body weight of the child at 5 years (r=0.12; p=0.01), BMI of the child at five years (r=0.10; p=0.04) and WC (0.12; p=0.01) at age five years. After controlling for the BMI of the child at five years, maternal MUAC (r=0.10; p=0.03) and maternal BMI (r=0.12; p<0.01) correlated significantly and positively with right cIMT. However, after controlling for the BMI of the child at age five years, the relationship between right cIMT and body weight and WC of the child was no longer significant. Left cIMT correlated significantly and positively with birth weight (r=0.10; p=0.02) and weight of the child at age five years (r=0.09; p=0.05). Aorta IMT correlated significantly and positively with body weight (r=0.21; p<0.01), BMI (r=0.21; p<0.01), subscapular SFT (r=0.10; p=0.03) and WC (r=0.13; p=0.01) of the child at age five years (Table 4.3). After controlling for the BMI of the child, the relationship between body weight of the child (r=0.10; p=0.03) and aIMT at age five was still significant but not with subscapular SFT (r=0.01; p=0.87) or WC (r=0.08; p=0.08).

Table 4.3 Associations between Maternal and Child adiposity indices and Left and Right cIMT expressed as Pearson Correlation Coefficients (r)

| | **Left cIMT** | | | **Right cIMT** | | | **Aorta IMT** | | |
|---|---|---|---|---|---|---|---|---|---|
| | r | p | n | r | p | n | r | p | n |
| **Maternal MUAC** | 0.05 | 0.27 | 489 | 0.11 | ***0.02** | 490 | 0.07 | 0.10 | 489 |
| **Maternal BMI** | 0.06 | 0.18 | 485 | 0.11 | ***0.02** | 486 | 0.07 | 0.14 | 485 |
| **BW** | 0.10 | ***0.02** | 496 | 0.04 | 0.37 | 497 | 0.05 | 0.28 | 495 |
| **Weight at 5 yrs** | 0.09 | ***0.05** | 497 | 0.12 | ***0.01** | 497 | 0.21 | ***<0.01** | 496 |
| **BMI at 5 yrs** | 0.07 | 0.12 | 497 | 0.10 | ***0.04** | 497 | 0.21 | ***<0.01** | 496 |
| **Triceps SFT** | 0.01 | 0.85 | 498 | -0.05 | 0.31 | 499 | 0.06 | 0.19 | 498 |
| **Subscap SFT** | -0.04 | 0.40 | 497 | -0.01 | 0.77 | 498 | 0.10 | ***0.03** | 497 |
| **WC** | 0.07 | 0.12 | 466 | 0.12 | 0.01 | 467 | 0.13 | 0.01 | 466 |

MUAC = mid-upper-arm circumference, BMI = body mass index, BW= infant weight at birth, SFT = skin fold thickness, WC = waist circumference, cIMT = carotid intima media thickness, IMT = intima media thickness, * significance at p<0.05

Left and right cIMT correlated significantly and negatively with HR (r=-0.09; p=0.05 and r=-0.10; p=0.03), but not with SBP, DBP or MAP. Aorta IMT did not correlate

significantly with any of the BP measurements, nor with HR (r=0.05; p=0.31) (Table 4.4). BMI at age five years correlated significantly and positively with SBP (r=0.16; p<0.01), DBP (r=0.18; p<0.01) and MAP (r=0.17; p<0.01) but not with HR (r=0.03; p=0.48) (Table 4.5). Furthermore, after controlling for the BMI of the child, these negative relationships between left and right cIMT and HR were still significant (r=-0.10; p=0.04 and r=-0.10; p=0.02).

Table 4.4 Associations between blood pressure measurements and Left, Right cIMT and aIMT at age five years expressed as Pearson Correlation Coefficients (r)

| | **Left cIMT** | | | **Right cIMT** | | | **Aorta IMT** | | |
|---|---|---|---|---|---|---|---|---|---|
| | r | p | n | r | p | n | r | p | n |
| **SBP** | 0.02 | 0.61 | 496 | 0.04 | 0.37 | 497 | 0.04 | 0.35 | 495 |
| **DBP** | -0.02 | 0.73 | 496 | 0.01 | 0.78 | 497 | 0.04 | 0.33 | 495 |
| **MAP** | 0.03 | 0.45 | 496 | 0.05 | 0.31 | 497 | 0.03 | 0.51 | 495 |
| **HR** | -0.09 | ***0.05** | 496 | -0.10 | ***0.03** | 497 | 0.05 | 0.31 | 495 |

SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, cIMT = carotid intima media thickness, aIMT = aorta intima media thickness, * significance at p<0.05

Table 4.4 Associations between BMI at age five years and blood pressure measurements expressed as Pearson Correlation Coefficients (r)

| | **BMI (kg/m$^2$)** | | |
|---|---|---|---|
| | **r** | **p** | **n** |
| SBP (mmHg) | 0.16 | *<**0.01** | 496 |
| DBP (mmHg) | 0.18 | *<**0.01** | 496 |
| MAP (mmHg) | 0.17 | *<**0.01** | 496 |
| HR (beats per minute) | 0.03 | 0.48 | 500 |

SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, BMI = body mass index, * significance at p<0.05

In a separate linear models, adjusting for maternal MUAC, maternal BMI, current body weight and WC of the child at age five years, *in utero* exposure to alcohol and nicotine (0.12; p=0.01), and sex of the child (0.14; p=0.01) were significantly associated with right cIMT measurements [F (6, 445) = 5.20, $R^2$ = 0.07, p<0.01]. In a second linear model, adjusting for current body weight of the child at age five years and *in utero* exposure to alcohol and nicotine, birth weight (0.07, p= 0.01), and sex of the child (0.13, p=0.01), were significantly associated with left cIMT measurements

[F (4, 491) = 4.49, $R^2$ = 0.04, p=0.01]. In a third linear model, [F (6, 459) = 5.63, $R^2$ = 0.07, p<0.01] adjusting for current body weight, BMI, WC, and subscapular SFT of the child at age five years, sex of the child (0.11, p=0.02) was significantly and independently associated with aorta IMT measurements (Table 4.6).

Table 4.5 Linear Models for Aorta, Right and Left carotid intima media thickness incorporating *in utero* exposure, sex, maternal MUAC, maternal BMI, weight, WC, subscapular skin fold thickness and BMI of the child at age five years

| | **F** | **df** | **p-value** | **$R^2$** | **beta** | **p-value** |
|---|---|---|---|---|---|---|
| **Model: Rt cIMT** | 5.20 | 6, 445 | ***<0.01** | 0.07 | | |
| **IDV**: Exposure | | | | | 0.12 | ***0.01** |
| sex | | | | | 0.14 | ***0.01** |
| Mat. MUAC | | | | | -0.10 | 0.44 |
| Mat. BMI | | | | | 0.21 | 0.10 |
| Weight at 5 yrs | | | | | 0.12 | 0.24 |
| WC at 5 yrs | | | | | 0.02 | 0.81 |
| | **F** | **df** | **p-value** | **$R^2$** | **beta** | **p-value** |
| **Model: Lt cIMT** | 4.49 | 4, 491 | ***0.01** | 0.09 | | |
| **IDV**: sex | | | | | -0.13 | ***<0.01** |
| BW | | | | | 0.07 | ***0.01** |
| Exposure | | | | | 0.06 | 0.19 |
| Weight at 5 yrs | | | | | 0.07 | 0.13 |



| | **F** | **df** | **p-value** | **$R^2$** | **beta** | **p-value** |
|---|---|---|---|---|---|---|
| **Model: aorta IMT** | 5.63 | 6, 459 | ***<0.01** | 0.07 | | |
| **IDV**: sSFT at 5 yr | | | | | 0.02 | 0.74 |
| Sex | | | | | -0.11 | ***0.02** |
| Exposure | | | | | -0.03 | 0.59 |
| BMI at 5 yrs | | | | | 0.02 | 0.86 |
| Weight at 5 yrs | | | | | 0.13 | 0.21 |
| WC at 5 yrs | | | | | 0.09 | 0.47 |

Rt cIMT = right carotid intima-media thickness, Lt cIMT = left carotid intima media thickness, BMI = body mass index of the child at age five years, yrs = years, WC = waist circumference, IMT = intima media thickness, SSC SFT – subscapular skin fold thickness, * significance at p<0.05

In the present study, 352 children were exposed to maternal smoking and alcohol consumption during pregnancy and 146 were not exposed (controls). From the 124

children who had right cIMT measurements above 0.365 mm, above the 75$^{th}$ percentile, ninety-eight (79%) were exposed to smoking and alcohol during gestation. The odds of having a higher than 0.365 mm right cIMT was 1.78 times greater for an exposed child compared to controls with 95% CI: 1.1 – 2.9 and significant at p=0.02.

**DISCUSSION**

The main finding from this study was dual alcohol and nicotine exposure during pregnancy, as well as maternal MUAC and male sex were associated with significantly higher right cIMT values in children five years of age. In contrast, *in utero* alcohol and nicotine exposure was not significantly associated with either higher left cIMT or aorta IMT values in children aged five. However, male sex and birth weight were significantly associated with higher left cIMT values in children aged five years. Male sex was significantly and independently associated with higher aorta IMT values at aged five years. Secondary findings included, maternal adiposity indicators, MUAC and BMI, as well as adiposity indicators of the child were related to higher cIMT values, irrespective of the *in utero* exposure to alcohol and nicotine. Our results are in agreement with previous studies which demonstrated a positive correlation between higher cIMT and LSE status. Liu et al., 2017 was the first to demonstrate an association between children from LSE communities and higher cIMT values in mid-childhood. In the present study, higher cIMT values were observed in a younger study population. Findings from the present study are in line with those of many other studies utilizing the measurement of IMT as an early marker of vascular changes due to the atherosclerosis process and fatty deposits [25-27]. Furthermore, our findings were in agreement with findings by Geerts et al., 2008 where higher cIMT measurements are associated with maternal smoking during pregnancy[28]. Our findings suggest, in addition to possible other pathogenic mechanisms, *in utero* exposure to both alcohol and nicotine might have a compounding effect on cIMT in contrast to nicotine alone. Moreover, animal as well as human studies describe enhanced vascular dysfunction caused by nutrient restriction, placental insufficiency as well as male sex as risk for cardiometabolic disease later in life[29, 30]. Nutrient deficiencies during pregnancy, leading to endothelial dysfunction, might be the underpinning mechanism resulting in higher cIMT values in five-year-old children from low-income settings. Our study population, being one from a low-income setting, experiences high rates of nutrient deficiencies [31]. In addition, maternal adiposity

markers (MUAC and BMI) were not only related to child adiposity (BMI, WC, SFT) but also with higher right cIMT values in their five-year-old offspring. These findings are consistent with those of studies by Cornelius et al., 2000 and Victoria et al., 2008. These authors demonstrated a strong positive association between maternal BMI and child BMI. Also, findings by Park et al., 2015 concluded strong associations between adiposity and cIMT measurements. Although our findings are in agreement with several of these studies, these studies were conducted mostly in Western Europe and the US [32] and, primarily older age groups were utilized. As a result, effects of confounders such as smoking and a sedentary lifestyle which are normally associated with older study populations, should be taken in consideration. Instead, in the present study we opt for younger study population.

In the present study, using the maternal BMI and MUAC at enrolment, smoking mothers were significant shorter and smaller compared to the mothers of the control group. The shorter smoking mother might be a type of mother who tends to have shorter children. More importantly, a mother who smokes and consumes alcohol and who continues to smoke and consume alcohol during her pregnancy, differs from a mother who abstains[20]. The association between maternal smoking during pregnancy and LBW infants was first reported[19] in 1957, subsequently Butler et al., 1972 and Perkin et al., 1997 also described these effect of *in utero* nicotine exposure and LBW. In the present study, exclusive alcohol consumption without smoking during pregnancy was not related to LBW or lower BL. These findings are consistent with those of other studies were BW was not affected by *in utero* alcohol exposure[36, 37]. However, findings from the present study, are consistent with the findings of Butler et al., 1972 and Perkin et al., 1997. Furthermore, in the present study, BP measurements were not significantly associated with higher IMT values. Instead, BP measurements correlated significantly with the child's BMI, indicating an association between BP and adiposity. This finding is in agreement with findings by other studies[35]. Edstedt Bonamy et al., 2008 described an association between *in utero* exposure to nicotine as well as male sex and higher IMT values in an adolescent population but also no association with BP.

Furthermore, in the present study, the LBW children remained smaller and shorter at age five years. They had significantly lower weight, height, skin fold thickness and WC as well as left cIMT values at age five years. Although weak, a significant

positive correlation between subscapular SFT and aorta IMT values at age five years, was observed. This finding not only illustrates the importance of adiposity but also suggests body composition[19, 38] might be more sensitive measurement than BMI alone in cardiometabolic risk assessment.

Furthermore, maternal smoking during pregnancy is related to preterm birth and IUGR which in turn is associated to higher IMT in offspring[35, 39-41]. Several other studies[26,42,27] describe the association between higher IMT values and a high cardiometabolic risk profile including HPT, DM type I and hypercholesterolemia in children.

Interestingly, the present study found dual in *utero exposure* was significantly associated with higher right cIMT, but not left cIMT values. Anatomically, the left carotid artery arises directly from the aortic arch and the expected pressure on the left side is higher compared to the right carotid artery which arises from the brachiocephalic artery and the expected pressure might be lower[43]. This lower pressure might contribute to development of atherosclerosis more easily in the right carotid artery. Contrasting, according to several studies[43], the left carotid artery is more vulnerable for the development of atherosclerosis because of its anatomy and hemodynamics. Furthermore, heart rate (HR) measurements at age five correlated correlated significantly and negatively with higher cIMT values.

Pereira et al., 2017 also found an association between carotid atherosclerosis and impaired cardiac autonomic control, leading to decreased heart rate, in high risk individuals[44].



Strength and Limitations

Some of the limitations need to be addressed. Firstly, maternal pre-pregnancy weight as well as weight gain during pregnancy were not recorded. Secondly, data on fathers were not available. Although other studies confirm cardiovascular risk factors in offspring to be positively associated with risk factors from both mother and father, this disputes against *in utero* exposure and more in favour of a genetic predisposition of cardiovascular risk factors in offspring which was not the scope of this study. Lastly, cardiovascular function was not tested and food frequency questionnaire, physical activity of the children as well as information on exposure to environmental endocrine disrupters were not recorded. Several studies have examined the effects of passive smoking and endocrine disrupters to impact on the development of obesity, in

addition to *in utero* exposure to nicotine. Strengths of this study are the sample size, availability of prospective data from the Safe Passage Study and the high follow up and good compliance rate.

**CONCLUSIONS**

In summary, our findings suggest that dual alcohol and nicotine exposure, as well as the sex of the child and maternal adiposity (MUAC and BMI), are associated with higher cIMT values in paediatric patients, of our study population, at five years of age. From converging lines of evidence, our study confirms the detrimental effects of *in utero* alcohol and nicotine exposure on the development of early atherosclerotic changes in high-risk children from a low-income setting.

Acknowledgements

We would to thank all the participants and the mothers / caregivers of the research study for their participation.

Sources of funding

The Safe Passage Study was funded by the National Institute on Alcohol Abuse and Alcoholism, Eunice Kennedy Shriver National Institute of Child Health and Human Development, and National Institute of Deafness and Other Communication Disorders: U01 HD055154, U01 HD045935, U01 HD055155, U01 HD045991, and U01 AA016501. The present study was partly funded by the South African Sugar Association. We would also like to acknowledge Dr. Paulson for the donation received.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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## Chapter 5

**Title: The effects of *in utero* exposure to teratogens on organ size: a prospective paediatric study**

De Smidt JJA[1], Odendaal HJ[2], Nel DG[3], Nolan H[2], Du Plessis C[2], LT Brink[2], Oelofse A[1]

[1] Department of Medical Biosciences, University of the Western Cape; [2] Department of Obstetrics and Gynaecology, Stellenbosch University, South Africa, [3]Department of Statistics and Actuarial Science, Stellenbosch University, South Africa

Key words: alcohol, body mass index, intrauterine teratogen exposure, kidney size, low-income setting, nicotine, pancreas size, paediatrics, waist circumference

Corresponding author: JJA De Smidt

Email address: jjdesmidt@gmail.com

Address: University of the Western Cape, Private BagX17, Bellville 7535, South Africa

Abstract

**Background**: In low-income countries prospective data on combined effects of *in utero* teratogen exposure are lacking and necessitates new research.

The **aim** of the present study was to explore the effect of *in utero* teratogen exposure on the size of the kidneys and pancreas five years after birth in a low-income paediatric population.

**Methods**: Data was collected from 500 mother-child pairs from a low-income setting. Anthropometric measurements included body weight, body height, mid-upper arm and waist circumference. Clinical measurements included blood pressure, mean arterial pressure and heart rate. Ultrasound measurements included pancreas, and kidney measurements at age five years. Main outcome of interest was the effect of maternal smoking and alcohol consumption on ultrasound measurements of organ size at age five years. **Results**: Left and right kidney length measurements were significantly lower in smoking exposed children compared to controls (p=0.04 and p=0.03).Pancreas body measurements were significantly lower in smoking exposed children (p=0.04). Multiple regression analyses were used to examine the independent associations between the independent variables (IDVs), maternal age, BMI, MUAC and body weight of the child, on the dependent variables (kidney lengths and kidney volumes). Also, the association between IDV, in *utero* exposure to alcohol and nicotine and DVs, pancreas measurements. Waist circumference (WC) was strongest (r=0.28; p<0.01) associated with pancreas head [F (4, 454) = 13.44; $R^2$ = 0.11; p<0.01] and tail (r = 0.30; p<0.01) measurements at age five years, with *in utero* exposure, sex of the child and BMI as covariates.

**Conclusion**: Kidney length and pancreas body measurements are affected by *in utero* exposure to nicotine at age five years and might contribute to cardiometabolic risk in

later life. Also, findings from this study report on ultrasound reference values for kidney and pancreas measurements of children at age five years from a low-income setting

## INTRODUCTION

High prevalence of alcohol consumption and cigarette smoking during pregnancy in association with low socioeconomic status (SES) and poverty, may result in poor maternal nutritional status and magnify the cardiometabolic risk experienced in early childhood through to adulthood[1 -5]. Both maternal alcohol consumption and cigarette smoking during pregnancy are teratogens and two of the leading preventable causes of birth defects and impaired foetal development. These teratogens constitute micronutrient deficiencies, which is especially evident in females of reproductive age, and impairs optimal foetal development[6 - 8]. Maternal smoking or exposure to secondary smoking during pregnancy is strongly associated with foetal growth restriction through two possible mechanisms. One, the nicotine in cigarette smoking causes vasoconstriction of the utero-placental blood vessels leading to a decrease in blood flow to the placenta which will result in a decreased delivery of oxygen and micronutrients to the foetus. Two, the foetal circulation is also compromised as the nicotine concentration increases to higher levels in the foetal circulation compared to the mother's circulation. In addition, the carbon monoxide caused by maternal smoking influences the development of the placenta and it leads to an increase in foetal carboxyhaemoglobinaemia with a subsequent decrease in oxygenation of the developing organs and tissue[9]. Ultimately the impaired foetal blood flow to the abdomen of the foetus may lead to smaller kidneys [10]. Excessive maternal alcohol consumption during pregnancy has similar somatic growth restriction effects on the foetus. In excess, alcohol affects foetal growth and development through DNA methylation and by interfering with folate metabolism and availability and accordingly affects organ development in the foetus. The intricacies of *in utero* teratogen exposure in low-income countries and the association with cardiometabolic diseases in later life remain to be elucidated.

Both alcohol and nicotine in cigarette smoking are teratogens associated with epigenetic responses in the foetus[11, 12]. These agents affect the gene expression with or

without directly affecting the gene sequence of DNA. Teratogenic effects may depend on several factors such as the timing and duration of the exposure, the distribution across the placenta, the concentration amount in the amniotic fluid, as well as the ability of the teratogenic agent to hinder the specific developmental processes and organogenesis[4, 6, 11]. Given the above, *in utero* exposure to teratogens impacts on foetal growth through epigenetic alteration of DNA methylation processes[13, 14] and may result in adult cardiometabolic diseases[15]. Children exposed to maternal cigarette smoking during pregnancy are not only shorter in body length (BL) but animal and human studies confirm they may be predisposed to abnormal glucose tolerance, hypertension and dyslipidaemia by the time they reach reproductive age[7, 11]. Other effects of maternal smoking are higher adiposity[6], lower fat free mass[16] and increased appetite as part of hypothalamus-pituitary-adrenal axis involvement and associated obesity in later life[6, 12, 17].

Thus, this constellation of teratogenic effects on various organs not only affect an individual exposed to it but also a society, communities, health systems as well as the well-being of future generations [4,18,19]. Therefore, early childhood and post-puberty are critical time periods to start addressing cardiometabolic risk factors, especially in females [20].

The *in utero* effects of alcohol consumption and cigarette smoking exposure are most commonly reported on in low-and-middle income countries (LMIC) [8, 21] but, scarcity of prospective data charting cardiometabolic risk, in relation to the *in utero* teratogen exposure from childhood into adulthood, necessitate future research. To map the trajectory of these metabolic risk factors, such as diet, passive smoking exposure, and physical inactivity in children needs consideration earlier rather than later. Determining those cardiometabolic risk factors present in school-aged children, and to map the trajectories of these risk factors are essential [22]. Also, through regular and early screening and intervening the burden of cardiometabolic complications experienced in adult life, be might lessen [20]. We hypothesized the effect of *in utero* exposure are associated with smaller visceral organ size i.e. kidneys and pancreas at age five years and thus associated with cardiometabolic risk. The aim of the present study was to explore the effect of *in utero* teratogen exposure on the size of the kidneys and pancreas five years after birth. Towards this aim we included 500 children from a low-income setting and we compared the *in utero* exposure effects

between controls and exposed children. Anthropometric measurements at birth, anthropometric measurements at age five years, blood pressure measurements and ultrasound measurements of the kidneys and pancreas at age five years were compared between controls and exposed children.

## MATERIALS AND METHODS

### Ethical approval

For the present study ethics approval was obtained from the Health and Research Ethics Committee (HREC) of Stellenbosch University (SU) and Biomedical Research Ethics Committee (BMREC) of the University of the Western Cape (UWC). Voluntary, written informed consent was obtained from the mother or caregiver of the child.

Study design

The present study was conducted at Stellenbosch University Obstetrics and Gynaecology SPS Unit at Tygerberg Hospital, Cape Town over an 18-month period (June 2016 to December 2017). It was a follow-up study of 500 of the children born to the Safe Passage Study, a prospective longitudinal study, to investigate the role of exposure to alcohol during pregnancy on stillbirths and sudden infant deaths [23]. Maternal data was documented for the SPS during dedicated antenatal clinic visits. A modified timeline follow-back method was used to assess alcohol intake during pregnancy and group-bases trajectories were used to categorise smoking and drinking patterns during pregnancy [23]. Information on paediatric health was obtained from assessments at birth and again at five years of age.

Selection of study participants

Pregnant women were recruited from the Belhar antenatal clinic or Bishop Lavis Midwife Obstetric Unit (MOU) and had prenatal follow-up visits at Tygerberg Hospital. All pregnant women booking for antenatal care with their children born from June 2011 through to December 2012 were invited to be part of the study. Five hundred mother-child pairs were selected for this sub-study. For infant follow-up, twins and children with congenital abnormalities at birth were excluded from the present study[23] and 500 infants born to these mothers were included in the study.

Participant assessments

Maternal assessments

Socio-demographic information including data on nutrition, pregnancy history, as well as alcohol and tobacco use was documented using a study questionnaire. Body mass index (BMI) was calculated from measurements collected at the first antenatal visit and calculated as the body weight in kilograms divided by the height in meters squared. Mid-upper arm circumference (MUAC) was measured as the circumference of the right upper arm measured at the midpoint between the tip of the shoulder and the tip of the elbow (olecranon process and the acromion) using a tape measure. Gestational age (GA) at enrolment, as determinant by an early ultrasound examination, was recorded.



Paediatric assessments

Measurements at birth included weight, length, and MUAC. Body weight and length was used to calculate BMI. Gestational age (GA) at delivery was recorded. At year five, body weight, length and BMI calculation were measured again in all children.

Body weight was measured using an electronic scale to the nearest 0.01 kg. For body height, children removed their shoes and were measured using a mechanical stadiometer fixed to a wall. Waist circumference (WC) was measured using a tape measure around the waist at the midpoint between the last rib and the iliac crest. Each child was measured three times and mean values were obtained from the three measurements. Systolic and diastolic blood pressure, and heart rate were measured at five years of age. Blood pressure was measured from the right upper arm in a sitting position using a validated CAS 740 MAXNIBP automated digital sphygmomanometer. A size-appropriate blood pressure cuff was used and all measurements were repeated three times.

Ultrasound measurements were obtained from children at age five years using a Voluson E8 ultrasound machine (GE Healthcare). Measurements were taken with the child in a supine position and fasting approximately 4 hours prior to the examination. Some children were asked to turn into an oblique or prone position in cases with poor visualization of the kidneys. Imaging of the pancreas and kidneys were attained on held inspiration or in some cases with the abdomen extended/pushed out. For the measurement of the pancreas, the transducer was placed transversely in the midline of the upper abdomen (high in the epigastrium). The pancreas was visualized in a coronal section. The transducer was angled heel-toe for optimal visualization of the maximal pancreas length. The head of the pancreas was measured in the mediolaterally and anteroposteriorly whereas the body and tail was measured anteroposteriorly.

The kidneys were visualized in the longitudinal and transverse views. The right liver lobe and spleen were used as acoustic windows, respectively. The transducer was

placed perpendicular, just inferior to the lateral edge of the right costal margin in the sagittal plane and moved medially until the kidney was optimally visualized in the coronal plane (long axis). The maximum length of the kidney was measured from the upper pole to the lower pole of the kidney. The transducer was rotated 90° into the transverse plane, the maximum measurements of the transverse (W) and anteroposterior (T) dimensions of the kidney was taken. The same was repeated on the left side. The kidney volumes were calculated using V=LxWxTx(∏/6) [24, 25].

Statistical analysis

Statistical analysis was performed using SPSS® software (version 21.0 for Windows; SPSS, Inc, Chicago, IL). Quantitative data was described as the means along with SD, minimum and maximum values with 95 % confidence intervals (CI). Categorical variables were presented as percentages. Intergroup differences were determined using independent samples t-tests and one-way analyses of variance (ANOVA) to evaluate the differences among *in utero* exposure groups. Post hoc Tukey comparisons for in-between group differences. Pearson correlation coefficients were used to describe linear relationships between continuous variables. Chi square analysis was conducted for categorical variables to explore the likelihood of mothers with gestational hypertension to fall in the exposure groups. Partial correlations were conducted to determine the relationship between kidney, pancreas measurements and BP, waist circumference (WC) whilst controlling for the BMI of the child. Multiple linear regression analyses were performed to illustrate the independent association between independent variables (IDVs), *in utero* exposure to alcohol and nicotine, maternal age, maternal BMI, maternal MUAC, or sex, weight and WC of the child and dependent variables (DVs), kidney and pancreas measurements. Data was

analysed per exposure group. The control group constitute of the children born to non-smoking mothers who abstained from consuming alcohol, the ALCNIC group was formed by the children born to mothers who both smoked cigarettes and consumed alcohol during pregnancy. The ALC only group was formed by children born to alcohol consuming mothers but no smoking and the NIC only group was formed by the children born to smoking mothers who did not consume alcohol. Statistical significance was set at $p < 0.05$.

**RESULTS**

Maternal and Birth outcomes of infants

The participant characteristics at baseline are described in Table 5.1and 5.2. Demographic characteristics for the mothers forming the control group differed significantly from the mothers forming the exposure groups, both alcohol and nicotine (ALCNIC), alcohol only (ALC), and nicotine only (NIC) (Table 5.1). Maternal age was the not significantly different ($p=0.50$) but, maternal weight was significantly lower for ALCNIC (63.7 ± 14.5 kg) and NIC (63.5 ± 14.8 kg) compared to the controls (68.9 ± 18.3 kg) at $p<0.01$. In addition, maternal BMI and MUAC were significantly lower for ALCNIC mothers (25.1 ± 5.6 kg/m$^2$, 27.2 ± 4.4 cm) and NIC mothers (25.1 ± 5.6 kg/m$^2$ and 27.4 ± 4.6 cm) compared to controls (27.4 ± 7.1 kg/m$^2$ and 27.2 ± 4.4 cm) at $p<0.01$. Gestational age (GA) at enrolment was also significantly ($p<0.01$) later for ALCNIC (142.7 ± 49.4 days) and for ALC mothers (154.5 ± 49.1 days) compared to controls (126.2 ± 44.7 days) (Table 5.1). Gestational diabetes (GDM) was observed in only 1.4% of mothers, hypertension during pregnancy affected 10.7% and anaemia during pregnancy affected 41.7% of mothers.

Neither maternal hypertension nor anaemia during pregnancy affected birth or five year outcomes.

A total of 15.9% of children were born with low birth weight (LBW), 70.2% of children were exposed to alcohol, nicotine or both and 64.1 % exposed to cigarette smoking during pregnancy. Paediatric neonatal data at birth showed significantly lower gestational age (GA) at delivery for NIC compared to the controls (p = 0.03). All other measurements at birth did not differ significantly between the four exposure groups (Table 5.2).

Table 5.1 Maternal characteristics at baseline according to the four groups

| | **Controls** | | | **ALCNIC** | | | **ALC only** | | | **NIC only** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal Characteristic** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95% CI** | **N** | **M (SD)** | **95% CI** |
| Maternal Age in years | 147 | 25.6 (6.1) | 24.6 – 26.6 | 154 | 24.6 (5.9) | 23.6 – 25.5 | 33 | 24.5 (5.0) | 22.7 – 26.3 | 167 | 24.9 (6.2) | 24.0 – 25.8 |
| Body weight in kg | 145 | ***68.9 (18.3)** | 65.9 – 71.9 | 154 | 63.7 (14.5) | 61.4 – 66.0 | 33 | 67.2 (15.6) | 61.6 – 72.7 | 166 | ***63.5 (14.8)** | 61.2 – 65.8 |
| Body Length in cm | 146 | 158.2 (6.9) | 157.1 – 159.4 | 150 | 159.6 (6.5) | 158.6 – 160.7 | 31 | 156.4 (7.2) | 153.8 – 159.1 | 165 | 158.7 (6.2) | 157.8 – 159.7 |
| BMI in kg / $m^2$ | 143 | ***27.4 (7.1)** | 26.3 – 28.6 | 150 | ***25.1 (5.6)** | 24.2 – 26.0 | 31 | 27.3 (6.0) | 25.1 – 29.6 | 165 | ***25.1 (5.6)** | 24.3 – 26.0 |
| MUAC in cm | 145 | ***29.2 (5.4)** | 28.3 – 30.1 | 152 | ***27.2 (4.4)** | 26.5 – 27.9 | 32 | 28.2 (4.5) | 26.5 – 29.8 | 164 | ***27.4 (4.6)** | 26.7 – 28.1 |
| GA at birth in days | 147 | ***126.2 (44.7)** | 119.0 – 133.5 | 154 | ***142.7 (49.4)** | 134.8 – 150.5 | 33 | ***154.5 (49.1)** | 137.0 – 171.9 | 167 | 134.6 (46.2) | 127.5 – 141.6 |

BMI = body mass index, MUAC = mid-upper-arm circumference, GA = gestational age, *significance p<0.05

Table 5.2 Neonatal characteristics at baseline according to the four groups

| | Controls | | | ALCNIC | | | ALC only | | | NIC only | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Characteristic** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95% CI** | **N** | **M (SD)** | **95% CI** |
| Birth weight in kg | 147 | 3.06 (0.52) | 2.98 – 3.14 | 154 | 2.98 (0.57) | 2.89 – 3.07 | 33 | 3.06 (0.59) | 2.85 – 3.27 | 166 | 2.98 (0.59) | 2.89 – 3.07 |
| Birth Length in cm | 126 | 49.06 (2.09) | 48.69 – 49.43 | 125 | 48.31 (2.41) | 47.88 – 48.73 | 24 | 48.50 (2.16) | 47.59 – 49.41 | 140 | 48.62 (2.12) | 48.44 – 48.87 |
| Birth BMI in kg / $m^2$ | 126 | 12.60 (1.94) | 12.26 – 12.95 | 125 | 12.62 (1.81) | 12.30 – 12.94 | 24 | 12.87 (1.81) | 12.10 – 13.63 | 139 | 12.69 (2.07) | 12.35 – 13.04 |
| MUAC in cm | 124 | 10.50 (0.95) | 10.34 – 10.67 | 124 | 10.40 (1.03) | 10.22 – 10.59 | 24 | 10.62 (0.97) | 10.21 – 11.03 | 140 | 10.56 (0.96) | 10.40 – 10.72 |
| GA at birth in days | 147 | ***274.14 (13.78)** | 271.90 – 276.39 | 154 | 271.73 (14.16) | 269.48 – 273.99 | 33 | 275.76 (13.15) | 271.10 – 280.42 | 167 | ***269.87 (15.47)** | 267.51 – 272.24 |

BMI = body mass index, MUAC = mid-upper-arm circumference, GA = gestational age, *significance p<0.05

Paediatric outcomes at age five years

Paediatric participant characteristics at end-point are described in in table 5.3. When paediatric characteristics were compared between the four exposure groups, significantly lower mean body weight (BW) and body length (BL) values were observed between NIC group (17.3 ± 2.3 kg and 108.2 ± 4.5 cm respectively, p=0.03) and controls (18.7 ± 3.6 kg and 110.0 ± 5.2 cm respectively, p=0.01) (Table 5.3). For the kidney measurements, mean left and right kidney length measurements were significantly lower for the children from NIC only group (72.6 ± 5.6 mm and 71.5 ± 5.7 mm) compared to controls (74.2 ± 6.1 mm and 73.3 ± 5.9 mm) at p =0.04 and p=0.03 respectively (figure 5.1). However, all other kidney measurements including width, height and volume, did not differ significantly between the four exposure groups.

Table 5.3 Paediatric characteristics at end-point (five years) according to the four groups

| | Controls | | | ALCNIC | | | ALC only | | | NIC only | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Characteristic** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95% CI** | **N** | **M (SD)** | **95% CI** |
| Body weight in kg | 147 | ***18.7 (3.6)** | 18.1 – 18.3 | 154 | 18.2 (2.7) | 17.7 – 18.6 | 33 | 17.8 (2.7) | 16.9 – 18.8 | 167 | ***17.7 (2.3)** | 17.3 – 18.0 |
| Body Length in cm | 147 | ***110.0 (5.2)** | 10.9.2 – 110.9 | 154 | 109.0 (5.2) | 108.2 – 109.8 | 33 | 108.5 (4.6) | 106.9 – 110.1 | 167 | ***108.2 (4.5)** | 107.5 – 108.9 |
| BMI in kg / $m^2$ | 147 | 15.4 (2.1) | 15.0 – 15.7 | 154 | 15.2 (1.5) | 15.0 – 15.4 | 33 | 15.1 (1.7) | 14.5 – 15.7 | 167 | 15.1 (1.4) | 14.9 – 15.3 |
| Waist circumference in cm | 132 | 51.0 (5.2) | 51.0 – 52.8 | 150 | 51.4 (3.9) | 50.8 - 52.1 | 30 | 51.2 (3.7) | 49.8 – 52.6 | 158 | 50.7 (3.5) | 50.1 – 51.2 |
| Systolic blood pressure in mmHg | 146 | 106.5 (10.7) | 104.7 – 108.2 | 154 | 107.1 (10.0) | 105.6 – 108.7 | 33 | 105.8 (11.7) | 101.6 – 109.9 | 167 | 104.3 (9.3) | 102.8 – 105.7 |
| Diastolic blood pressure in mmHg | 146 | 65.3 (9.1) | 63.8 – 66.7 | 154 | 65.7 (9.1) | 64.3 – 67.2 | 33 | 65.6 (11.4) | 61.6 – 69.7 | 167 | 63.9 (9.0) | 62.5 – 65.3 |
| Mean arterial pressure in mmHg | 146 | 78.8 (9.5) | 77.3 – 80.4 | 154 | 79.4 (9.3) | 78.0 – 80.9 | 33 | 79.3 (11.3) | 75.3 – 83.3 | 167 | 77.3 (9.3) | 75.9 – 78.7 |
| Heart rate in beats per minute | 146 | 91.7 (12.4) | 89.7 – 93.8 | 154 | 92.5 (14.4) | 90.2 – 94.8 | 33 | 91.8 (8.7) | 88.7 – 94.9 | 167 | 91.4 (13.3) | 89.3 – 93.4 |
| LT K Length in mm | 146 | ***74.2 (6.1)** | 73.2 – 75.2 | 153 | 72.7 (5.8) | 71.7 – 73.6 | 33 | 72.9 (4.9) | 71.2 – 74.7 | 165 | ***72.6 (5.6)** | 71.8 – 73.5 |
| LT K Width in mm | 146 | 35.0 (3.8) | 34.4 – 35.7 | 153 | 35.4 (3.8) | 34.8 – 36.0 | 33 | 35.1 (3.7) | 33.8 – 36.5 | 165 | 34.5 (3.6) | 33.9 – 35.0 |
| LT K Height in mm | 146 | 35.5 (3.7) | 34.9 – 36.1 | 153 | 35.4 (3.8) | 34.8 – 36.0 | 33 | 35.5 (3.6) | 34.2 – 36.8 | 165 | 35.1 (3.7) | 34.6 – 35.7 |
| LT K Volume in $mm^3$ | 146 | 48697.1 (10102.6) | 47044.6 – 50349.6 | 153 | 48116.3 (10731.5) | 46402.2 – 49830.4 | 33 | 47954.8 (9824.4) | 44471.2 – 51438.4 | 165 | 46493.7 (10449.0) | 44887.5 – 48099.9 |
| RT K Length in mm | 146 | ***73.3 (5.9)** | 72.4 – 74.3 | 153 | 72.0 (5.6) | 71.1 – 72.9 | 33 | 73.2 (4.6) | 71.5 – 74.8 | 165 | ***71.5 (5.7)** | 70.6 – 72.4 |
| RT K Width in mm | 146 | 32.1 (3.4) | 31.6 – 32.7 | 153 | 33.1 (3.7) | 32.5 – 33.7 | 33 | 31.5 (2.2) | 30.8 – 32.3 | 165 | 32.3 (3.2) | 31.8 – 32.8 |
| RT K Height in mm | 146 | 38.4 (3.9) | 37.7 – 39.0 | 153 | 38.7 (4.4) | 37.9 – 39.4 | 33 | 38.0 (3.4) | 36.8 – 39.2 | 165 | 37.8 (4.1) | 37.1 – 38.4 |
| RT K Volume in $mm^3$ | 146 | 47629.7 (9546.0) | 46068.3 – 49191.2 | 153 | 48829.3 (11451.9) | 47000.1 – 50658.5 | 33 | 46099.3 (6980.8) | 43624.0 – 48574.6 | 165 | 46127.6 (10132.7) | 44570.0 – 47685.2 |
| Pancreas head in mm | 141 | 18.3 (2.6) | 17.8 – 18.7 | 151 | 17.8 (2.5) | 17.4 – 18.3 | 33 | 18.4 (2.8) | 17.5 – 19.4 | 164 | 17.6 (2.5) | 17.2 – 18.0 |
| Pancreas body in mm | 141 | ***7.2 (1.6)** | 6.9 – 7.4 | 151 | 7.0 (1.6) | 6.8 – 7.3 | 33 | 7.2 (1.7) | 6.6 – 7.8 | 164 | ***6.7 (1.5)** | 6.5 – 6.9 |
| Pancreas tail in mm | 141 | 12.8 (2.2) | 12.5 – 13.2 | 151 | 13.0 (2.1) | 12.7 – 13.3 | 33 | 13.3 (3.0) | 12.3 – 14.4 | 164 | 12.7 (2.2) | 12.3 – 13.0 |

BMI = body mass index, MUAC = mid-upper-arm circumference, GA = gestational age, *differences between groups, *significance at $p<0.05$

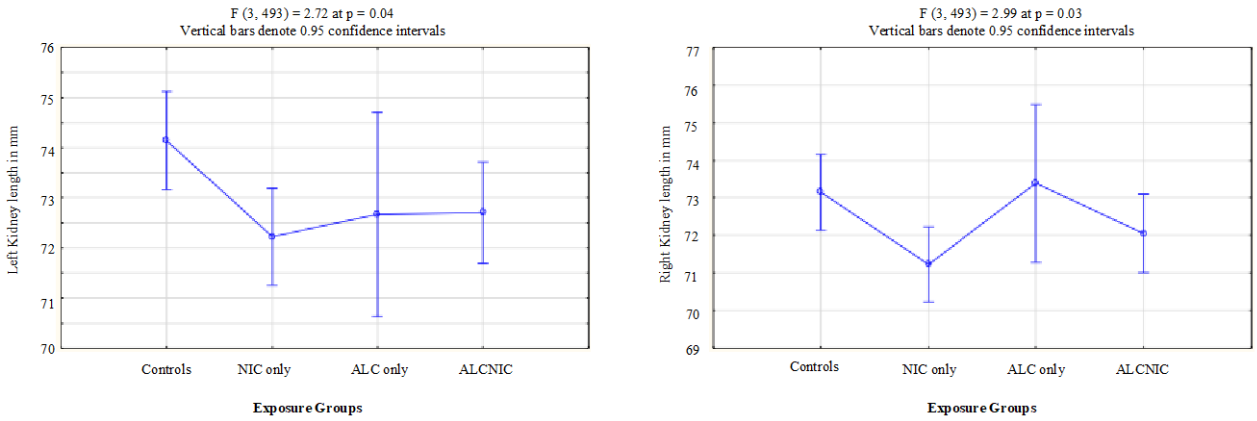


Figure 5.1 Right and Left Kidney values for five year-old children

Also mean pancreas body measurements were significantly lower for NIC (6.7 ± 1.5 mm) compared to controls (7.2 ± 1.6 mm) at p=0.04 (Table 5.3 and figure 5.2). Mean values for the pancreas head (p = 0.08) and tail (p = 0.33) for the children in the exposure groups did not differ significantly from the controls.

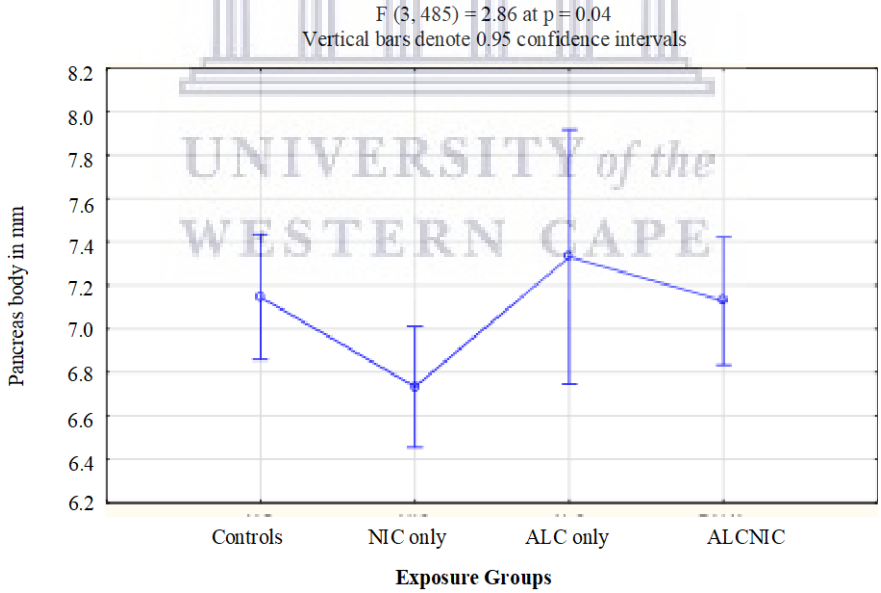


Figure 5.2 Pancreas body values for the four exposure groups

Furthermore, significant positive correlations were found between all anthropometric measurements including BW, BL and WC with all kidney measurements for both the controls and the exposure groups except for left kidney width of the controls (Table

5.4 and 5.5). Significant positive correlations were also found between anthropometric measurements and pancreas measurements. After controlling for the BMI of the child, the relationship between WC and the pancreas head and tail still existed (r=0.13, p<0.01 and r=0.24, p<0.01 respectively) but not for pancreas body.

Table 5.4 Correlation between mean kidney values and anthropometric measurements of the controls

| **Controls N 146** | **Body weight (kg)** | | **Body length (cm)** | | **BMI (kg/m$^2$)** | | **WC (cm)** | |
|---|---|---|---|---|---|---|---|---|
| | **r** | **p** | **r** | **p** | **r** | **p** | **r** | **p** |
| Lt K Length | 0.45 | ***<0.01** | 0.51 | ***<0.01** | 0.26 | ***<0.01** | 0.35 | ***<0.01** |
| RT K Length | 0.41 | ***<0.01** | 0.48 | ***<0.01** | 0.24 | ***<0.01** | 0.31 | ***<0.01** |
| Lt K Width | 0.15 | 0.07 | 0.21 | ***0.01** | 0.06 | 0.49 | 0.09 | 0.29 |
| RT K Width | 0.20 | ***0.02** | 0.26 | ***<0.01** | 0.10 | 0.23 | 0.19 | ***0.03** |
| Lt K Height | 0.25 | ***<0.01** | 0.31 | ***<0.01** | 0.13 | 0.11 | 0.19 | ***0.03** |
| RT K Height | 0.34 | ***<0.01** | 0.29 | ***<0.01** | 0.27 | ***0.01** | 0.29 | ***<0.01** |
| Lt K Volume | 0.38 | ***<0.01** | 0.46 | ***<0.01** | 0.20 | ***0.02** | 0.28 | ***0.01** |
| RT K Volume | 0.45 | ***<0.01** | 0.47 | ***<0.01** | 0.30 | ***<0.01** | 0.38 | ***<0.01** |

LT = left, RT = right, K = kidney, BMI = body mass index, WC = waist circumference, *significance at p<0.05



Table 5.5 Correlation between mean kidney values and anthropometric measurements of the exposed group

| **Exposed N 354** | **Body weight (kg)** | | **Body length (cm)** | | **BMI (kg/m$^2$)** | | **WC (cm)** | |
|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p |
| Lt K Length | 0.45 | ***<0.01** | 0.45 | ***<0.01** | 0.26 | ***<0.01** | 0.34 | ***<0.01** |
| RT K Length | 0.42 | ***<0.01** | 0.43 | ***<0.01** | 0.23 | ***<0.01** | 0.31 | ***<0.01** |
| Lt K Width | 0.28 | ***<0.01** | 0.22 | ***<0.01** | 0.22 | ***<0.01** | 0.26 | ***<0.01** |
| RT K Width | 0.28 | ***<0.01** | 0.19 | ***<0.01** | 0.24 | ***<0.01** | 0.29 | ***<0.01** |
| Lt K Height | 0.33 | ***<0.01** | 0.27 | ***<0.01** | 0.23 | ***<0.01** | 0.28 | ***<0.01** |
| RT K Height | 0.39 | ***<0.01** | 0.30 | ***<0.01** | 0.30 | ***<0.01** | 0.32 | ***<0.01** |
| Lt K Volume | 0.45 | ***<0.01** | 0.39 | ***<0.01** | 0.31 | ***<0.01** | 0.38 | ***<0.01** |
| RT K Volume | 0.47 | ***<0.01** | 0.38 | ***<0.01** | 0.35 | ***<0.01** | 0.41 | ***<0.01** |

LT = left, RT = right, K = kidney, BMI = body mass index, WC = waist circumference, *significance at p<0.05

Table 5.6 summarises the correlations between blood pressure measurements, SBP, DBP, MAP HR and kidney length, kidney volume as well as BMI. Blood pressure was not significantly associated with kidney length nor volume at this age (p >0.01) instead, BP significantly and positively correlated with BMI (p < 0.01). Heart rate correlated significantly but negatively with right kidney volume (r = -0.10 at p = 0.02).

**Table 5.6: Associations between kidney measurements, BMI and blood pressure measurements at age five years expressed as Pearson Correlation Coefficients (r)**

| | Lt K Length | | | Lt K Volume | | | Rt K Length | | | Rt K Volume | | | BMI (kg/m$^2$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **r** | **p** | **n** | **r** | **p** | **n** | **r** | **p** | **n** | **r** | **p** | **n** | **r** | **p** | **n** |
| **SBP** | 0.01 | 0.92 | 496 | -0.03 | 0.45 | 496 | -0.05 | 0.26 | 496 | -0.06 | 0.17 | 496 | 0.16 | <**0.01** | 496 |
| **DBP** | 0.62 | 0.75 | 496 | -0.01 | 0.88 | 496 | -0.04 | 0.35 | 496 | -0.05 | 0.29 | 496 | 0.18 | <**0.01** | 496 |
| **MAP** | 0.01 | 0.84 | 496 | -0.04 | 0.40 | 496 | -0.05 | 0.29 | 496 | -0.07 | 0.14 | 496 | 0.17 | <**0.01** | 496 |
| **HR** | 0.01 | 0.87 | 496 | -0.04 | 0.40 | 496 | -0.03 | 0.57 | 496 | -0.10 | ***0.02** | 496 | 0.03 | 0.48 | 500 |

SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, Lt K Length = left kidney length, Lt K Volume = left kidney volume, Rt K Length = right kidney length, Rt K volume = right kidney volume, BMI = body mass index, * significance at p-value <0.05



To explore the independent associations of IDVs, maternal age, maternal BMI, maternal MUAC as well as the weight of the child at age five years, with DVs, right and left kidney lengths and volumes, we used multiple linear regression analyses (table 5.7). Also for the IDVs, in *utero* exposure to alcohol and nicotine, sex of the child, BMI and WC and DVs, pancreas measurements, multiple linear regression models were constructed (table 5.7). For all the exposure groups, body weight of the child played a significant role. Strong associations (p<0.01) with the DVs, left and right kidney length and volume, with maternal age, maternal BMI and maternal MUAC as covariates. In addition, for the NIC group, maternal age (beta=0.18 at p

=0.03) was significantly associated with left kidney volume with all the maternal factors as covariates.

For pancreas head, WC (beta=0.28, p<0.01) and sex of the child (beta=0.13, p<0.01) were significantly associated with pancreas head values [F (4,454) = 13.4; p<0.01, $R^2$ = 0.11] with *in utero* exposure and BMI as covariates. For pancreas tail, WC (beta=0.31, p<0.01) was significantly and independently associated with pancreas tail values [F (4,545) = 6.60; p<0.01, $R^2$ = 0.06] with *in utero* exposure, sex of the child and BMI as covariates (Table 5.8).

**Table 5.7: Linear Models incorporating maternal age, BMI, MUAC as well as the body weight of the child in association with kidney lengths and volumes at age five years**

| DEPENDENT VARIABLES | CONTROLS N147 | ALCNIC N154 | ALC only N33 | NIC only N164 |
|---|---|---|---|---|
| **Lt K length** | ***p <0.01** | ***p <0.01** | p = 0.25 | ***p <0.01** |
| | $R^2$ = 0.17 | $R^2$ = 0.25 | | $R^2$ = 0.19 |
| | IDV: weight of the child beta = 0.40, ***p < 0.01** | IDV: weight of the child beta = 0.47, ***p < 0.01** | | IDV: weight of the child beta = 0.39, ***p < 0.01** |
| | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | | Covariates: Mat. Age, Mat. BMI, Mat. MUAC |
| **Lt K volume** | ***p <0.01** | ***p <0.01** | p = 0.08 | ***p <0.01** |
| | $R^2$ = 0.11 | $R^2$ = 0.30 | | $R^2$ = 0.24 |
| | IDV: weight of the child beta=0.30 at ***p <0.01** | IDV: weight of the child beta = 0.53 at ***p <0.01** | | IDV: Mat Age beta = -0.18 at ***p =0.03**; Weight of the child beta = 0.45 at ***p <0.01** |
| | Covariates: Mat. Age, Mat. BMI, mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | | Covariates: Mat. BMI and Mat. MUAC |
| **Rt K length** | ***p <0.01** | ***p <0.01** | ***p =0.02** | ***p <0.01** |
| | $R^2$ = 0.24 | $R^2$ = 0.13 | $R^2$ = 0.39 | $R^2$ = 0.15 |
| | IDV: Mat. MUAC beta = 0.63 at ***p =0.01**; | IDV: weight of the child beta | IDV: Weight of the child beta = 0.45 at ***p = 0.02** | IDV: Weight of the child beta = 0.39 at ***p <0.01** |

| | | | | |
|---|---|---|---|---|
| | Mat. BMI beta = -0.49 at ***p =0.04**; weight of the child beta = 0.40 at ***p <0.01** | =0.35 at ***p <0.01** | | |
| | Covariates: Mat. Age | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC |
| **Rt K volume** | ***p <0.01** | ***p <0.01** | p =0.19 | ***p <0.01** |
| | $R^2$ = 0.23 | $R^2$ = 0.34 | $R^2$ = 0.23 | $R^2$ = 0.14 |
| | IDV: Weight of the child beta = 0.46 at ***p <0.01** | IDV: weight of the child beta = 0.56 at ***p <0.01** | IDV: weight of the child beta = 0.41 at ***p = 0.04** | IDV: weight of the child beta = 0.37 at ***p <0.01** |
| | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC | Covariates: Mat. Age, Mat. BMI, Mat. MUAC |

Lt K length = left kidney length, Lt K Volume = left kidney volume, Rt K length = right kidney length, Rt K volume = right kidney volume, Mat. Age = maternal age, Mat. BMI = maternal body mass index, Mat. MUAC = maternal mid-upper-arm circumference, ALCNIC = group of children exposed to both alcohol and nicotine during pregnancy, ALC only = group of children exposed to alcohol but no smoking during pregnancy, NIC only = group of children exposed to nicotine but no alcohol during pregnancy, * significance at p-value <0.05

**Table 5.8: Linear Models incorporating *in utero* exposure, sex, BMI and WC in association with Pancreas measurements at age 5 years**

| | **F** | **df** | **p-value** | **$R^2$** | **beta** | **p-value** |
|---|---|---|---|---|---|---|
| Model 1: PANCREAS HEAD | 13.44 | 4, 454 | *<0.01 | 0.11 | | |
| **IDV: Exposure** | | | | | -0.05 | 0.28 |
| **sex** | | | | | -0.13 | ***<0.01** |
| **WC** | | | | | 0.28 | ***<0.01** |
| **BMI** | | | | | 0.02 | 0.83 |
| Model 2: PANCREAS BODY | 3.56 | 4, 454 | *0.01 | 0.03 | | |
| **IDV: Exposure** | | | | | -0.07 | 0.12 |
| **sex** | | | | | -0.03 | 0.58 |
| **WC** | | | | | 0.15 | 0.13 |
| **BMI** | | | | | -0.01 | 0.98 |
| Model 3: PANCREAS TAIL | 6.60 | 4, 454 | *<0.01 | 0.06 | | |
| **IDV: Exposure** | | | | | 0.04 | 0.42 |
| **sex** | | | | | -0.06 | 0.22 |
| **WC** | | | | | 0.31 | ***<0.01** |
| **BMI** | | | | | -0.10 | 0.34 |

BMI = body mass index, WC = waist circumference, * significance at p-value <0.05

## DISCUSSION

In five-year-old children exposed to *in utero* alcohol and nicotine, the nicotine exposed children had significantly lower body weights and body lengths compared to the controls or the other two exposure groups. However, BMI for the nicotine exposed children was not significantly lower. Furthermore, the smoking mothers were also significantly lighter in body weight compared to the control mothers. Consequently, smaller children born to smaller mothers are expected although these smoking exposed children were not significantly smaller than the controls at birth. The main results from the present study are significantly lower values for both left and right kidney length for the *in utero* nicotine exposed children, compared to controls. Also, significantly lower pancreas body measurements were observed for *in utero* nicotine exposed children compared to controls. In contrast, in the present study, kidney width, height and volume as well as pancreas head and tail measurements were not significantly lower among the *in utero* nicotine exposed children. These findings might be explained by the smaller body size of the nicotine exposed children and the fact that they are born to smaller mother when compared to the controls. The body weight to organ size ratios were the same for all the exposure groups but it does not explain why only the kidney length and pancreas body measurements were significantly lower. A possible explanation might be kidney length and pancreas body are regions within those organs most sensitive to *in utero* teratogen exposure in this population. In the present study, smoking mothers were significantly smaller, shorter stature and lower BMI, compared to the controls or alcohol only consuming mothers. Short maternal stature might be a result of LBW and the childhood environment of the mother[15, 26]. Heavy smoking mothers may consume less nutrient rich diets compared to controls and the compounds of cigarette smoking may interfere with the bioavailability of micronutrients to the developing baby[19, 27]. These finding are supported by those of other studies where smoking mothers were smaller[5, 28]. As described, in the nicotine exposed children smaller body weight, body length as well as organ size might be expected [19]. Confirmed by results from animal studies, maternal nicotine exposure predisposes an increased appetite, increased adipose tissue, as well as impaired glucose metabolism in their offspring in later life [6, 29, 30]. The child's diet during the postnatal life plays an equally important role. Accelerated weight gain and high BMIs early in life are detrimental to the individual's adult health[31, 32]. High fat combined with high carbohydrate and low protein diet is

associated with the higher BMI and development of obesity related cardiometabolic diseases[11, 27, 32 – 34].

According to the literature, smaller pancreas size is associated with T2DM[35, 36] through β-cell destruction and an increased pancreas size is caused by fat infiltration [37] due to obesity. Accordingly, we found significant positive correlations between pancreas measurements and WC, a strong indication of abdominal adiposity, was observed. After controlling for the BMI of the child, WC remained significantly associated with all pancreas measurements. Because WC is a fairly easy measurement to perform, it may be helpful to identify prepubertal children at risk of cardiometabolic diseases[38]. Furthermore, literature confirm WC, which is a reflection of central obesity, is an important cardiometabolic risk indicator[39]. In the present study, maternal BMI and MUAC were also significantly associated with the pancreas head and body measurements, indicating a genetic association with adiposity. However, after controlling for the BMI and WC of the child, these associations were no longer significant. Illustrating the important contribution of postnatal weight gain, BMI and WC compared to the genetic contribution of maternal adiposity[14]. WC remained the strongest independent indicator for pancreatic head and tail as well as kidney measurements. In the present study, using a novel comprehensive assessment of visceral organs size (kidneys and pancreas) by ultrasound in association with WC, we found significant positive correlation between WC and kidney and pancreas size measurements. These findings are supported by previous studies using ultrasound measurements of abdominal adipose tissue[40]. Ultimately, findings from this present study, are in line with other studies from low-income countries where maternal age, education level and SES remained the strongest independent predictors of health and well-being for both mother and child[41, 42]. Females exposed to *in utero* nicotine are more likely to develop gestational diabetes and obesity [6, 30], and have higher BMI and central adiposity[20, 31, 39] in later life. Furthermore, LBW, associated with maternal smoking during pregnancy, is an important driver of cardiometabolic risk in low-income settings[13, 18, 19, 43 - 45].

In this paediatric population, an in-normal-range mean BMI of 15.2 kg/m$^2$ was observed and might be explained by the age of the children as well as the current living conditions and nutritional status of these children. Literature report overweight and obesity, modifiable risk factor, as high as 32% and 8.1 % respectively among

primary school-aged children, adding to the cardiometabolic risk experienced in later life [20, 31]. Contrasting to other South African low-income settings where high obesity rates are due to nutritional transition from poverty to affluence[26]. Moreover, the age of adiposity rebound (AR), the lowest BMI values just before an increase in BMI, is usually observed after the age of five years [22, 46, 47]. Children from our study population might very well be experiencing an adiposity rebound, which explain the in-normal-range BMI. Challenging living conditions, extreme poverty and suboptimal diet may also explain the normal mean BMI at this age. Other factors such as passive smoking, physical activity, and genetics might be equally important explanatory factors.

However, BMI might not be the most sensitive measurement of adiposity [39]. Instead, WC measurement is easy to perform and a more sensitive measurement of central obesity compared to BMI and should ideally be done routinely for paediatric patients as part of screening for cardiometabolic risk[38, 39, 48].

Also, in the present study, blood pressure (BP) was not significantly associated with kidney length nor kidney volume at this age. Confirmed by results from various other studies, BP is not associated with kidney size at age five years[10, 49]. Instead, in the present study, blood pressure correlated significantly with BMI at age five years. Authors from the Leningrad siege study found similar results confirming the association of BP and obesity instead of an association between BP and *in utero* malnutrition [15]. Other literature confirms a stronger association between early development of adiposity and elevated BP [31]. Foetal programming studies confirm an association of elevated BP and intrauterine growth restriction (IUGR) in both sexes but, only males remained hypertensive in later life[44, 50]. *In utero* malnutrition was also associated with the development of obesity related hypertension[11, 13, 15]. However, in the present study, as in other studies, kidney size correlated significantly with anthropometric measurements[51]. Other authors found an association between LBW, smaller kidney size, low nephron numbers and development of hypertension[10, 13, 44, 45]. In a study done on young adults born LBW, hypertension was associated with smaller kidney size [52].

In a previous study of the same 500 mother-child pairs from this low-income community, we demonstrated the role of dual *in utero* exposure of alcohol and nicotine and the association with higher intima media thickness (IMT) in children aged five years[53]. In the present study, we sought to build on these existing findings by exploring the effects of maternal alcohol consumption and smoking during pregnancy and the association with kidney and pancreas size as part of the temporal evolution of cardiometabolic risk factors in children over the first five years of life.

This prospective follow-up study of 500 mother-child pairs, extends to the existing body of evidence of maternal lifestyle choices including smoking and alcohol consumption during pregnancy impacting on a population's cardiometabolic health [5, 6, 11, 14]. From a public health perspective, the novel approach of measuring organ size, using ultrasound, in conjunction with routine WC measurement, for central obesity screening, in primary school-aged children may be used as a preventative tool.

**Strength and Limitations**

The present study had a few strengths and limitations worth mentioning. Perhaps the main limitation of the present study was the inability to measure pancreatic mass and compare it with measures of pancreatic beta cell functioning i.e. insulin sensitivity. However, measurement of pancreas and kidney functions was not part of the scope of the present study. Also, our main aim of the study was to assess *in utero* exposure to alcohol and nicotine on the growth and development of visceral organs such as the pancreas and kidneys in a low-income setting paediatric population, we opted for the non-invasive, radiation free and relative ease of ultrasound. Therefore, a huge strength of the present study was the ability to obtain reference values for pancreas and kidney parameters measured by ultrasound. These values obtained by ultrasound may serve as a screening tool to identify and follow-up at risk children from similar low-income settings in South Africa.

## CONCLUSIONS

*In utero* exposure to alcohol and nicotine was significantly associated with lower values for both left and right kidney length. WC, an indicator of abdominal adiposity, was independently associated with pancreas and kidney size. Perhaps, the most important conclusion of the present study is the correlation between WC and all the pancreas measurements, independently of the weight, height or sex of the child. This study confirms the harmful effects of *in utero* teratogen exposure on visceral organ size in a paediatric population five years after birth from a low-income setting in South Africa. Pancreas and kidney size by ultrasound could be used as a screening tool for diabetes and hypertension risk development.

Acknowledgements

We would to thank all the participants and the mothers and caregivers of the research study for their participation. Secondly, we would like to thank the sonographer, Heidi Nolan, for the collecting the ultrasound data, Carlie Du Plessis for the anthropometrics, and Lucy Brink, for data management.



Sources of funding

South African Sugar Association

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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## Chapter 6

**Title: A paediatric study of adiposity using anthropometry and ultrasound: in association with *in utero* exposure to teratogens**

De Smidt JJA[1], Odendaal HJ[2], Nel DG[3], Nolan H[2], Du Plessis C[2], LT Brink[2], Oelofse A[1]

[1] Department of Medical Biosciences, University of the Western Cape; [2] Department of Obstetrics and Gynaecology, Stellenbosch University, South Africa, [3]Department of Statistics and Actuarial Science, Stellenbosch University, South Africa

*Submitted to Journal of Maternal and Child Health November 2019*

Key words: abdominal adiposity, cardiometabolic risk, *in utero*, teratogen exposure, maternal adiposity, low-income setting

Corresponding author: JJA De Smidt

Address: University of the Western Cape, Private BagX17, Bellville 7535, South Africa

Email address: jjdesmidt@gmail.com

**Abstract**

**Background:** Increasing urbanization, rapid nutritional transition from poverty to affluence, adoption of a Western-style diet and physical inactivity have contributed to the growing obesity epidemic in the low-income countries.

**Aim:** To investigate the effects of *in utero* exposure to smoking and alcohol on abdominal adipose tissue five years after birth, using conventional anthropometry in comparison with ultrasound techniques.

**Subjects and methods:** A prospective cohort study of children aged five years from a low-income setting. This is a further follow-up study of children born in the Safe Passage Study. Data was collected from 500 mother-child pairs at antenatal clinic visits, at birth and at the age five years. Maternal data were collected at antenatal clinics in the residential area of Bishop Lavis, Western Cape, when women enrolled for their first antenatal visit. All other assessments were done at follow-up study visits at Tygerberg Academic Hospital in Bellville, South Africa. Dependent variables include: anthropometric measurements included body weight (BW), body length (BL) skinfold thickness (SFT) and waist circumference (WC) at age five years. Also, ultrasound assessments of the visceral adipose tissue (VAT) at age five years. Independent variables included *in utero* exposure to nicotine and alcohol and maternal adiposity measurements (Body Mass Index and Mid-Upper-Arm Circumference at enrolment of the study).

**Results:** *in utero* exposure to alcohol and nicotine (r=0.11 at p=0.01) was a significant predicator IDV for visceral adiposity, measured by ultrasound. Maternal anthropometric measurements, BMI (r = 0.20 at p = 0.04) and MUAC (r = 0.20 at p = 0.05), significantly and positively correlated with anthropometric measurements, triceps, subscapular SFT and WC, of their children ($p<0.05$). Conventional anthropometric measurements did not correlate with visceral adipose tissue. Odds ratio (OR) of higher SFT was increased among females who were born to overweight mothers (OR 1.62, 95 % confidence interval 1.24 – 2.13).

**Conclusion:** *In utero* exposure to teratogens was a predictor IDV for ultrasound measures of visceral abdominal adipose tissue but not with anthropometric measures of adiposity. Overweight and obese mothers have children with higher adiposity measures compared to normal weight mothers. Males born to smoking and alcohol consuming mothers had lower SFT measurements compared to female counterparts.

## INTRODUCTION

In recent decades, increasing urbanization, adoption of a Western-style diet and physical inactivity have contributed to the growing obesity epidemic in low-income countries [1, 2]. There is also increasing recognition that obesity is not only highly prevalent among adults in low-income countries, but also affects children and adolescents [3, 4]. Multiple peri- and post-natal factors acting upon a genetic risk backdrop may contribute to the development of obesity and cardiometabolic diseases in later life[5]. For example, maternal obesity and type II diabetes mellitus (T2DM) are well-known risk factors for having high birth weight (HBW) offspring who are in turn are predisposed towards developing obesity as adults [6-9]. In addition, *in utero* exposure to teratogens, poor maternal diet, malnutrition and dietary deficiencies may explain increased cardiometabolic risk in early childhood [10-12].

Indeed, maternal and childhood indices of obesity are related and may be traced from childhood into adolescence and finally adulthood[13, 14]. Female sex is particularly vulnerable to maternal gestational weight gain and pre-pregnancy maternal BMI [15]. Especially, higher abdominal visceral adiposity seems to be more detrimental to cardiometabolic health than generalised adiposity[16]. In addition, low birth weight (LBW) infants are also predisposed towards rapid weight gain during the first two years of life, partly attributable to programmed hyperphagia resulting from an impaired response to leptin and insulin[17, 18]. However, the role of *in utero* exposure to teratogens and maternal obesity on the development of cardiometabolic risk factors in males versus females remains incompletely explained. In particular, the effects of *in utero* alcohol and nicotine exposure on the visceral adiposity and the higher cardiometabolic risk in children from low-income settings should be elucidated.

In a previous study of 500 children from the same low-income community from the Western Cape province of South Africa, we demonstrated the important role of maternal MUAC measurements as a predictor IDV of child's weight at five years of age. In the present study, we sought to build on these existing findings by exploring the effects of maternal smoking and alcohol consumption during pregnancy in association with visceral abdominal adiposity and generalized adiposity at age five years. Also, to what extend does maternal indices of adiposity influences that of their children and the temporal evolution of cardiometabolic risk factors in the children

over the first five years of life. Towards this goal, anthropometric indices of maternal adiposity such as mid-upper arm circumference (MUAC) and body mass index (BMI) as well as alcohol consumption and smoking during pregnancy were obtained from 500 mothers during routine antenatal visits when they were participating in the Safe Passage Study (SPS[19]. These measures were then correlated with anthropometric and ultrasonographic assessments of adipose tissue at the age of five years (BMI, skin fold thickness and waist circumference). In this study we wish to investigate the effects of *in utero* exposure to smoking and alcohol on abdominal adipose tissue five years after birth using anthropometry and ultrasonography. Secondly, to determine if *in utero* exposure to smoking and alcohol has the same effects in both males and females at age five years.

## MATERIALS AND METHODS

### Ethics approval

Ethics approval was obtained from the Health and Research Ethics Committee (HREC) of Stellenbosch University (SU) and Biomedical Research Ethics Committee (BMREC) of the University of the Western Cape (UWC). Voluntary, written informed consent was obtained from the mother or caregiver of the child.



### Study design

The study was conducted at Stellenbosch University SPS Unit in the Department of Obstetrics and Gynaecology at Tygerberg Hospital, Cape Town over an 18-month period (June 2016 to December 2017). Maternal data were collected earlier during antenatal clinic visits for the SPS. Information on paediatric health was obtained from assessments at birth for SPS and then later at five years of age.

### Selection of study participants

The SPS was a large prospective study to determine the role of exposure to alcohol during pregnancy on Sudden Infant Death (SIDS) and stillbirths[19]. Pregnant women were recruited from the Belhar antenatal clinic or Bishop Lavis Midwife Obstetric Unit (MOU) and had prenatal follow-up assessments at Tygerberg Hospital. All pregnant women booking for antenatal care were invited to be part of the study. A modified timeline follow back method was used to assess exposure to al during

pregnancy[20]. For this study, we selected five hundred children born to mothers in the SPS. These children, selected for different exposure to cigarette smoking and alcohol consumption during pregnancy, were examined for the present study, excluding twins and children with congenital abnormalities at birth.

Participant assessments

Maternal assessments

Socio-demographic information including data on nutrition, pregnancy history, as well as alcohol and tobacco use was documented using the study questionnaire. Body mass index (BMI) was calculated from measurements collected at the first antenatal visit and calculated as the body weight in kilograms divided by the height in meters squared. Mid-upper arm circumference (MUAC) was measured as the circumference of the right upper arm measured at the midpoint between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium) using a tape measure[19].

Paediatric assessments

BMI was measured at birth and again at five years of age. Body weight (BW) was measured using an electronic scale to the nearest 0.01 kg. For body length (BL), children removed their shoes and were measured using a mechanical stadiometer fixed to a wall. Triceps (tSFT) and subscapular skin fold thickness (sSFT) were measured in millimetres using a Holtain caliper. Subscapular SFT was measured from the left side of the body to the nearest 0.1 mm while the fingers continued to hold the skinfold. The actual measurement was read from the caliper about 3 seconds after the caliper tension was released. Measurements were taken at the following sites: (a) triceps, halfway between the acromion process of the scapula and the olecranon process of the elbow and (b) subscapular, approximately 20 mm below the tip of the scapula, at an angle of 45 ° to the lateral side of the body. Waist circumference (WC) was calculated at year five as measured around the waist at the midpoint between the last rib and the iliac crest using a tape measure. Each child was measured three times and mean values were obtained from the three measurements.

Ultrasonography to measure abdominal adiposity was performed on children at five years of age using a Voluson E8 ultrasound machine (GE Healthcare, Kretz Ultrasound, Zipf, Austria) as this method compared favourably with computed tomography (Ferrozzi et al., 1999). All images were recorded at the end of inspiration

with the linear transducer in the midline, perpendicular and parallel to the *linea alba* from the xiphoid process to the umbilicus. Preperitoneal maximum ($P_{max}$) thickness was used as indication of visceral adipose tissue (VAT) (Jaddoe et al., 2012). $P_{max}$ thickness was measured as the distance (mm) from the posterior border of the *linea alba* to the peritoneum anterior to the liver. The subcutaneous minimum ($S_{min}$) fat thickness was measured as the distance (mm) between the inner surface of subcutaneous tissue below the skin to the anterior surface of the *linea alba*. The P/S ratio was calculated from the maximum thickness of the preperitoneal fat layer ($P_{max}$) and minimum thickness of subcutaneous fat layer ($S_{min}$) and refers to the abdominal wall thickness (AWT). With the transducer rotated 90° from the midsagittal plane and perpendicular to the *linea alba*, then moved superiorly from the umbilicus to the xiphoid, the thickest subcutaneous fat layer ($SC_{max}$) was measured.

Statistical analysis

SPSS® software (version 21.0 for Windows; SPSS, Inc., Chicago, IL) was used for all analyses. Quantitative data was described as the means along with SD for normally distributed data and medians with 95 % confidence intervals (CI) for skewed data. Categorical variables were presented as percentages. Intergroup differences were determined using independent samples t-tests and one-way analyses of variance (ANOVA) to evaluate the interaction between the independent variable (IDV), *in utero* exposure. Pearson correlation coefficients were used to describe linear relationships between continuous variables and chi square analysis for categorical variable. Partial correlations were conducted to determine the relationship between anthropometric adipose tissue measurement and ultrasound measurements of adipose tissue whilst controlling for either current BW or BMI of the child. To explore the possible association of maternal measurements of adiposity (BMI and MUAC) and paediatric anthropometric (BW, SFT and WC) and ultrasound measurements of adiposity (VAT, AWT), linear regression analyses were performed with adjustment for either the *in utero* exposure to alcohol and nicotine, maternal MUAC, or the current BW of the child at age five years. Odds ratios were calculated to explore the probability of increased adiposity (being overweight or having an overweight mother) and the probability of increased SFT measurements in females. Statistical significance was set at $p < 0.05$.

## RESULTS

The characteristics of the study population are described in Table 6.1, maternal characteristics at baseline, and table 6.2, paediatric characteristics at end-point. The mothers who smoked and consumed alcohol (ALCNIC), those who consumed alcohol only (ALC only) and those who smoked only (NIC only) during pregnancy had significantly lower body weights ($p<0.01$), BMI ($p<0.01$), MUACs ($p<0.01$) and enrolled at a later gestational age (GA) ($p<0.01$) into the study compared to controls (Table 6.1). Maternal age and body length did not differ significantly among the mothers in the four groups. Maternal body weight, [$F(3, 494) = 3.90$ at $p<0.01$], was significantly lower for the ALCNIC (63.7 ± 14.5 kg) and the NIC only (63.5 ± 14.8 kg) groups compared to the controls (68.9 ± 18.3 kg). Also for maternal BMI, [$F(3, 494) = 5.38$ at ($p<0.01$)] and maternal MUAC, [$F(3, 494) = 5.32$ at ($p<0.01$)]. For gestational age (GA) at enrolment, $F(3, 494) = 5.00$ at $p<0.01$, the mothers in ALCNIC and ALC only groups entered later compared to control and NIC only groups (Table 6.1).

Table 6.1 Maternal characteristics according to exposure groups: controls, smoking and alcohol consuming, smoking only, alcohol only groups. Data expressed as mean (SD (N)) and 95% Confidence interval (CI)

| | **Group 0 Controls** | | | **Group 1 ALCNIC** | | | **Group 2 ALC only** | | | **Group 3 NIC only** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal Characteristic** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95 % CI** | **N** | **M (SD)** | **95% CI** | **N** | **M (SD)** | **95% CI** |
| Mat Age in years | 147 | 25.6 (6.1) | 24.6 – 26.6 | 154 | 24.6 (5.9) | 23.6 – 25.5 | 33 | 24.5 (5.0) | 22.7 – 26.3 | 167 | 24.9 (6.2) | 24.0 – 25.8 |
| BW in kg | 145 | ***68.9 (18.3)** | 65.9 – 71.9 | 154 | ***63.7 (14.5)** | 61.4 – 66.0 | 33 | 67.2 (15.6) | 61.6 – 72.7 | 166 | ***63.5 (14.8)** | 61.2 – 65.8 |
| BL in cm | 146 | 158.2 (6.9) | 157.1 – 159.4 | 150 | 159.6 (6.5) | 158.6 – 160.7 | 31 | 156.4 (7.2) | 153.8 – 159.1 | 165 | 158.7 (6.2) | 157.8 – 159.7 |
| BMI in kg / $m^2$ | 143 | ***27.4 (7.1)** | 26.3 – 28.6 | 150 | ***25.1 (5.6)** | 24.2 – 26.0 | 31 | 27.3 (6.0) | 25.1 – 29.6 | 165 | ***25.1 (5.6)** | 24.3 – 26.0 |
| MUAC in cm | 145 | ***29.2 (5.4)** | 28.3 – 30.1 | 152 | ***27.2 (4.4)** | 26.5 – 27.9 | 32 | 28.2 (4.5) | 26.5 – 29.8 | 164 | ***27.4 (4.6)** | 26.7 – 28.1 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GA days | 147 | ***126.2 (44.7)** | 119.0 – 133.5 | 154 | ***142.7 (49.4)** | 134.8 – 150.5 | 33 | ***154.5 (49.1)** | 137.0 – 171.9 | 167 | 134.6 (46.2) | 127.5 – 141.6 |

Mat. Age = maternal age, BW = body weight, BL = body length, BMI = body mass index, MUAC = mid-upper-arm circumference, GA = gestational age at recruitment, ALCNIC = alcohol consuming and smoking group, ALC only = alcohol consuming group, NIC only = smoking group, * significance at p<**0.01**

At age five years, anthropometric measurements, BMI (p = 0.48), tSFT (p = 0.11), sSFT (p = 0.12) nor WC (p = 0.08) did not differ significantly among the children from the four exposure groups. However, the ultrasound measurement of visceral abdominal wall as an indication of visceral adiposity, was significantly less for the children born to smoking and alcohol consuming mothers, F (3, 494) = 2.84 at p = 0.04 (table 6.2).

Table 6.2 Paediatric characteristics at end-point, age five years, according to exposure groups: smoking and alcohol consuming group, smoking only groups and control group. Data is expressed as mean (SD) and (N)

| **Groups** | | **Controls** | **ALCNIC** | **ALC only** | **NIC only** | **P value** | **F** | **df** |
|---|---|---|---|---|---|---|---|---|
| | | N 146 | N 154 | N 33 | N 167 | | | |
| **Anthropometric measurements** | BW in kg | ***18.7 ± 3.6** | 18.2 ± 2.7 | 17.8 ± 2.7 | ***17.7 ± 2.3** | ***0.02** | 3.30 | 3, 497 |
| | BL in cm | ***110.0 ± 5.2** | 109.0 ± 5.2 | 108.5 ± 4.6 | ***108.2 ± 4.5** | ***0.01** | 3.70 | 3, 497 |
| | BMI ($kg/m^2$) | 15.4 ± 2.1 | 15.2 ± 1.5 | 15.1 ± 1.7 | 15.1 ± 1.4 | 0.48 | 0.82 | 3, 497 |
| | tSFT (cm) | 10.1 ± 3.8 | 9.4 ± 2.7 | 9.6 ± 4.4 | 9.3 ± 2.7 | 0.11 | 2.03 | 3, 497 |
| | sSFT (cm) | 7.7 ± 4.2 | 7.0 ± 1.9 | 7.6 ± 3.9 | 6.9 ± 2.3 | 0.12 | 1.97 | 3, 496 |
| | WC (cm) | 51.9 ± 5.2 | 51.4 ± 3.9 | 51.2 ± 3.7 | 50.7 ± 3.5 | 0.08 | 2.27 | 3, 466 |
| **Ultrasound measurements** | Pmax (mm) | ***3.89 ± 1.47** | ***3.48 ± 1.35** | 3.42 ± 1.29 | 3.57 ± 1.23 | ***0.04** | 2.84 | 3, 494 |
| | Smin (mm) | 1.97 ± 2.28 | 1.82 ± 1.86 | 1.91 ± 1.99 | 1.86 ± 1.68 | 0.93 | 0.15 | 3, 494 |
| | P/S ratio (mm) | 0.48 ± 0.41 | 0.54 ± 0.44 | 0.57 ± 0.59 | 0.55 ± 0.48 | 0.53 | 0.73 | 3, 494 |
| | SCmax (mm) | 3.43 ± 3.94 | 2.81 ± 2.81 | 2.99 ± 2.88 | 2.88 ± 2.68 | 0.32 | 1.16 | 3, 494 |

BMI = body mass index, tSFT = triceps skin fold thickness, sSFT = subscapular skin fold thickness, WC = waist circumference, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, HR = heart rate, Pmax = periperitoneal maximum thickness, Smin = subcutaneous minimum value, P/S ratio = ratio of the, SCmax = subcutaneous maximum measurement value. *Significance at p<0.05

Maternal anthropometric measures, BMI and MUAC, did not correlate significantly with visceral adiposity of their children at age five years. However, anthropometric measurements, BMI, SFT and WC, of the child at age five years correlated significantly and positively with ultrasound measures of visceral abdominal adiposity (table 6.3). After controlling for the BMI of the child, these relationships between anthropometric and ultrasound measures of adiposity, were still significant (table 6.4). Furthermore, body weight of the child at age five years, on its own, had a stronger association with the ultrasound measures of adiposity (table 6.5). Hence, conducting linear regression analyses were included in the results to examine the influence of the body weight of the child at age five years on the DVs, visceral adiposity and anthropometric measures of adiposity in addition to the maternal IDVs, *in utero* exposure and maternal BMI and MUAC (table 6.6).

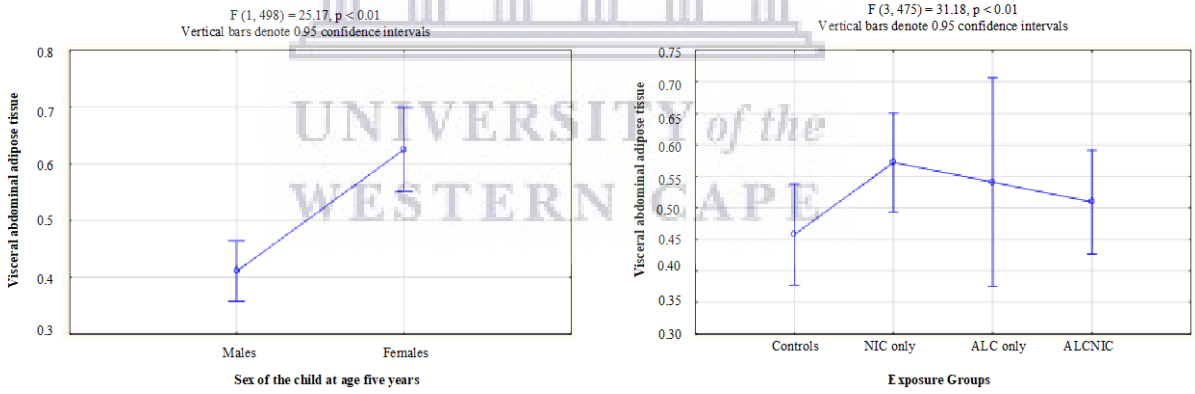


Figure 6.1 Visceral abdominal adipose tissue measured in mm between males and females and also according to the four exposure groups

Table 6.3 Correlations between maternal, child anthropometric measures of adiposity and ultrasound measures of adiposity expressed as Pearson Correlation Coefficients (r)

| | **P/S** | | | **$P_{max}$** | | | **$S_{min}$** | | | **$SC_{max}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | n | r | p | n | r | p | n | r | p | n |
| Maternal MUAC | 0.02 | 0.69 | 490 | 0.14 | ***<0.01** | 490 | 0.13 | ***<0.01** | 490 | 0.18 | ***<0.01** | 490 |
| Maternal BMI | 0.00 | 1,0 | 486 | 0.11 | ***0.01** | 486 | 0.12 | ***0.01** | 486 | 0.18 | ***<0.01** | 486 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI at 5 yrs | 0.53 | ***<0.01** | 498 | 0.42 | ***<0.01** | 498 | 0.75 | ***<0.01** | 498 | 0.77 | ***<0.01** | 498 |
| TSFT | 0.55 | ***<0.01** | 499 | 0.30 | ***<0.01** | 499 | 0.68 | ***<0.01** | 499 | 0.68 | ***<0.01** | 499 |
| SSFT | 0.58 | ***<0.01** | 498 | 0.36 | ***<0.01** | 499 | 0.76 | ***<0.01** | 498 | 0.75 | ***<0.01** | 498 |
| WC | 0.51 | ***<0.01** | 467 | 0.44 | ***<0.01** | 467 | 0.74 | ***<0.01** | 467 | 0.77 | ***<0.01** | 467 |

MUAC = mid-upper-arm circumference, TSFT = triceps skin fold thickness, SSFT = subscapular skin fold thickness, BMI = body mass index, WC = waist circumference, P/S = pre-peritoneal maximum / subcutaneous minimum (indication of abdominal wall thickness), $P_{max}$ = preperitoneal maximum (indication of visceral adipose tissue), $S_{min}$ = subcutaneous minimum, $SC_{max}$ = subcutaneous maximum, * significance at p<**0.01**

Table 6.4 Partial Correlations between anthropometry and ultrasound measures of adiposity expressed as Pearson Correlation Coefficients (r) and controlling for BMI of the child

| | **$P_{max}$** | | | **$S_{min}$** | | | **P/S** | | | **$SC_{max}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | df | r | p | df | r | p | df | r | p | df |
| TSFT | 0.32 | ***<0.01** | 455 | 0.73 | ***<0.01** | 455 | 0.57 | ***<0.01** | 455 | 0.74 | ***<0.01** | 455 |
| SSFT | 0.39 | ***<0.01** | 455 | 0.84 | ***<0.01** | 455 | 0.61 | ***<0.01** | 455 | 0.83 | ***<0.01** | 455 |
| WC | 0.42 | ***<0.01** | 455 | 0.73 | ***<0.01** | 455 | 0.51 | ***<0.01** | 455 | 0.75 | ***<0.01** | 455 |
| Body weight | 0.44 | ***<0.01** | 455 | 0.65 | ***<0.01** | 455 | 0.44 | ***<0.01** | 455 | 0.67 | ***<0.01** | 455 |

TSFT = triceps skin fold thickness, SSFT = subscapular skin fold thickness, WC = waist circumference, P/S = pre-peritoneal maximum / subcutaneous minimum (indication of abdominal wall thickness), $P_{max}$ = preperitoneal maximum (indication of visceral adipose tissue), $S_{min}$ = subcutaneous minimum, $SC_{max}$ = subcutaneous maximum, * significance at p<**0.01**



Table 6.5 Partial Correlations between anthropometry and ultrasound measures of adiposity expressed as Pearson Correlation Coefficients (r) and controlling for weight of the child

| | **$P_{max}$** | | | **$S_{min}$** | | | **P/S** | | | **$SC_{max}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | df | r | p | df | r | p | df | r | p | df |
| TSFT | 0.06 | 0.21 | 464 | 0.46 | ***<0.01** | 464 | 0.38 | ***<0.01** | 464 | 0.46 | ***<0.01** | 464 |
| SSFT | 0.14 | ***<0.01** | 464 | 0.60 | ***<0.01** | 464 | 0.43 | ***<0.01** | 464 | 0.57 | ***<0.01** | 464 |
| WC | 0.09 | ***0.05** | 464 | 0.44 | ***<0.01** | 464 | 0.30 | ***<0.01** | 464 | 0.47 | ***<0.01** | 464 |

TSFT = triceps skin fold thickness, SSFT = subscapular skin fold thickness, WC = waist circumference, P/S = pre-peritoneal maximum / subcutaneous minimum (indication of abdominal wall thickness), $P_{max}$ = preperitoneal maximum (indication of visceral adipose tissue), $S_{min}$ = subcutaneous minimum, $SC_{max}$ = subcutaneous maximum, * significance at p<**0.01**

In linear model 1 [F (3,476) = 3.33; p = 0.02, $R^2$ = 0.02], collectively, the independent variables (IDVs), *in utero* exposure, maternal BMI or maternal MUAC, had a significant association with the DV, visceral abdominal adipose tissue. However, none of them had individual effects on the dependent variable (DV), visceral abdominal adipose tissue. Alternatively, in linear model 2 [F (3,479) = 13.28, p<0.01, $R^2$ = 0.08] adjusting for maternal BMI, maternal MUAC (beta = 0.42, p<0.01) and *in utero* exposure (beta = 0.11, p<0.01) were significantly associated with body weight, anthropometric adiposity measurement, at age five years. When adding body weight of the child at age five years as an additional IDV, linear model 3 [F (4,475) =31.18, p<0.01, $R^2$ =0.21], the IDVs, maternal MUAC and BMI were no longer significant. The body weight of the child (beta=0.47, p<0.01 has mediated the effect of maternal MUAC and BMI. However, the *in utero* exposure effect (r=0.11, p = 0.01) was significant and not mediated via the effect of the child's body weight at age five years. In linear model 4 [F (4,478) = 83.07, p<0.01, $R^2$ = 0.41] *in utero* exposure was not a predictor IDV for triceps SFT. Instead, maternal BMI (beta = -0.20, p = 0.04) MUAC (beta=0.20, p= 0.05) and body weight of the child at age five years (beta =0.63, p<0.01) were significantly predictor IDVs for triceps SFT at age five years. In the same way, the effect of IDVs, maternal BMI, MUAC was mediated by the child's body weight (beta =0.65, p<0.01 and beta=0.88, p<0.01respectively) in linear model 5 [F (4,477) = 89.10, p<0.01, $R^2$ = 0.43], with DV subscapular SFT and linear model 6 [F (4, 450) = 382.53, p<0.01, $R^2$ = 0.77] with DV, WC at age five years (Table 6.6).



Table 6.6 Linear Models incorporating dual *in utero* exposure, maternal MUAC and BMI as IDV, adding Body weight as IDV and DV as well as anthropometric and ultrasound measurements of adiposity at age 5 years

| | **F** | **df** | **p-value** | **$R^2$** | **beta** | **p-value** |
|---|---|---|---|---|---|---|
| **Model 1: VAT** | **3.33** | **3, 476** | ***0.02** | **0.02** | | |
| IDV: *in utero* exp | | | | | -0.06 | 0.18 |
| Mat. BMI | | | | | -0.03 | 0.83 |
| Mat. MUAC | | | | | 0.15 | 0.24 |
| **Model 2: BW** | **13.28** | **3, 474** | ***<0.01** | **0.08** | | |
| IDV: *in utero* exp | | | | | -0.11 | ***0.01** |
| Mat. BMI | | | | | -0.21 | 0.09 |
| Mat. MUAC | | | | | 0.42 | ***<0.01** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Model 3: VAT** | **31.18** | **4, 475** | ***<0.01** | **0.21** | | |
| IDV: *in utero* exp | | | | | 0.11 | ***0.01** |
| Mat. BMI | | | | | -0.06 | 0.58 |
| Mat. MUAC | | | | | -0.02 | 0.85 |
| BW at 5 yrs | | | | | 0.47 | ***<0.01** |
| **Model 4: tSFT** | **83.07** | **4, 478** | ***<0.01** | **0.41** | | |
| IDV: *in utero* exp | | | | | 0.01 | 0.95 |
| Mat. BMI | | | | | -0.20 | ***0.04** |
| Mat. MUAC | | | | | 0.20 | ***0.05** |
| BW at 5 yrs | | | | | 0.63 | ***<0.01** |
| **Model 5: sSFT** | **89.10** | **4, 477** | ***<0.01** | **0.43** | | |
| IDV: *in utero* exp | | | | | 0.01 | 0.81 |
| Mat. BMI | | | | | 0.07 | 0.44 |
| Mat. MUAC | | | | | -0.05 | 0.63 |
| BW at 5 yrs | | | | | 0.65 | ***<0.01** |
| **Model 6: WC** | **382.53** | **4, 450** | ***<0.01** | **0.77** | | |
| IDV: *in utero* exp | | | | | 0.01 | 0.69 |
| Mat. BMI | | | | | 0.03 | 0.61 |
| Mat. MUAC | | | | | -0.03 | 0.64 |
| BW at 5 yrs | | | | | 0.88 | ***<0.01** |

VAT = visceral abdominal adipose tissue, BW = body weight, Mat. MUAC = maternal mid-upper-arm circumference, Mat. BMI = body mass index, tSFT = triceps skin fold thickness, sSFT = subscapular skin fold thickness, WC = waist circumference, exp = exposure, yrs = years, DV = dependent variable, IDV = independent variable, * significance at p<**0.01**

Lastly, an odds ratio was calculated using maternal overweight, defined as a maternal BMI above 26 kg/m$^2$) and females with higher than 75$^{th}$ percentile SFT values. The odds ratios (OR) of higher SFT were increased among females who were born to overweight mothers (OR 1.62, 95 % confidence interval 1.24 – 2.13).

## DISCUSSION

Our main finding was a significant positive correlation between anthropometric and ultrasound measures of adiposity, BMI, WC and SFT in children aged five years. The

*in utero* exposure to alcohol and nicotine was a predictor IDV for visceral abdominal adiposity, measured by ultrasound. In this study, a novel comprehensive assessment of abdominal adipose tissue by ultrasound was used. And, *in utero* exposure to these teratogens had a significant effect on the anthropometry of males but not females. Instead, maternal obesity influenced the higher skin fold thickness measurements in females. And, maternal anthropometric measurements, BMI and MUAC, were significantly associated with anthropometric triceps SFT measurements. Our findings add to the existing body of effective use of ultrasound as a measuring tool for abdominal adipose tissue[21-23]. In addition, our findings around *in utero* exposure to teratogens affecting males and females differently with males exhibiting more detrimental effects on lower weight gain, fat free muscle mass and foetal growth are supported by findings of Zarén et al 2000 and Cornelius et al 2000[24, 25]. These studies concluded, male foetuses have higher vulnerability to teratogen exposure compared to females *in utero*, possibly through the mechanisms of impaired blood circulation delivering less oxygen for foetal growth [24.] Because the male foetus has a higher growth velocity compared to the female foetus, the male foetus seems to be more affected by the nicotine exposure. In addition, in low-income populations the male offspring are more affected by exposure to environmental hazards and have higher rates of disease incidence, morbidity and mortality [26]. Furthermore, in our study population, females had significantly higher triceps and subscapular SFT values when compared to the males. These findings are supported by previous studies from Siani et al 2002, Lundeen et al 2015, Monteiro et al., 2016 and Munthali et al., 2016 who concluded: higher incidence of obesity amongst women of childbearing age compared to their male counterparts[27, 15, 28, 29]. Even during childhood, females show higher incidence of prepubertal overweight and obesity than age-matched males [27, 15]. Contrasting, in the present study, we observed overweight and obesity only among 4.6% of the study population although nearly 50% of the mothers fell in the overweight category. The low prevalence of overweight among the paediatric population might be explained by the age of the children. The age of adiposity rebound, the lowest BMI values just before an increase in BMI, is usually observed after the age of five years[30]. In addition, studies reported on the trajectories of overweight of prepubertal females[15, 29] into adulthood and the association with cardiometabolic disease development. Although maternal genetics might partially explain the development of obesity and increased adipose tissue in their female

offspring[15], other factors such as infant and childhood nutrition and feeding practices during the postnatal period[10, 11], passive smoking, physical inactivity, age of adiposity rebound and how these constitute overweight and obesity might be of equal clinical importance.

In the present study, smoking and alcohol consuming mothers were significantly smaller than the controls, illustrating the difference in profile of a mother from a low-income community who continue to smoke and consume alcohol throughout her pregnancy. Other studies confirmed these findings of smaller smoking mothers[3, 12]. In these exposed children an expected higher incidence of overweight and obesity is linked to postnatal nicotine withdrawal, hyperphagia and increased weight gain[31]. Confirmed by animal studies, these considerations lead to higher adiposity, body weight, BP as well as impaired glucose metabolism[31], further accentuated by the postnatal high fat – high carbohydrate and low protein and fresh vegetables and fruit diet[32].

The female offspring from this low-income setting were twice as likely as male counterparts to have higher SFT at age five years. These findings are in line with other studies of low- and middle income countries where maternal education level and SES remain the strongest independent predictors of health and well-being of their offspring[13] and where females exposed to *in utero* nicotine exposure are more likely to develop gestational diabetes and obesity[31], and have higher BMI and central adiposity[16]. Also, smoking during pregnancy is indeed more prevalent in low-income settings[2] and results in lower BMI. Yet, from our results it is important to note that although the nicotine exposed children were smaller, they had the highest mean visceral adipose tissue values (figure 6.1).

This prospective follow-up study of 500 mother-child pairs, extends the existing body of evidence of higher adiposity among females compared to males even in early childhood. Furthermore, maternal lifestyle such as smoking, alcohol consumption, plus poor prenatal care, especially micronutrient deficiencies during pregnancy impact more severely on the male foetus *in utero*[12, 24].

**Strength and Limitations**

Perhaps the main limitation of the present study was the inability to measure lean muscle mass and compare it with measures of adiposity. However, in this age group, we opted for the non-invasive, radiation free and relative ease of ultrasound instead of MRI. Another limitation might be underreporting of smoking during pregnancy using a self-reporting method. Lastly, maternal pre-pregnancy weight as well as weight gain during pregnancy were not recorded. Strengths of this study are the large sample size, availability of prospective data from the Safe Passage Study and the high follow up and good compliance rate.

## CONCLUSIONS

We were able to demonstrate the detrimental association between maternal smoking and alcohol consumption during pregnancy and visceral abdominal adiposity measured by ultrasound at age five years. Also, ultrasound measuring technique might be a more sensitive measuring tool for visceral abdominal adiposity compared to conventional anthropometry. Nonetheless, anthropometric measurement of adiposity, such as SFT, is a sensitive tool to use in peripheral adiposity measurement and it is easy and cost effective. Furthermore, female offspring from overweight and obese mothers had higher adiposity measures, SFT, compared to females from normal weight mothers. And, male offspring anthropometric measurements of adiposity were affected by the *in utero* exposure compared to female offspring. Measures of maternal adiposity (MUAC and BMI) during pregnancy, constitute predictors of higher adiposity (SFT) but not WC or ultrasound measures of abdominal adiposity in their children at five years of age. Our study confirms the detrimental effects of *in utero* exposure to teratogens and higher maternal adiposity on temporal evolution of early cardiometabolic risk in a paediatric population from a low-income setting.

Acknowledgements

We would to thank all the participants and the mothers and caregivers of the research study for their participation.

Sources of funding

The South African Sugar Association partially funded this study.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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# Chapter 7

## General Discussion

This thesis describes the investigations of maternal smoking and alcohol consumption during pregnancy on outcomes of their children at age five years. We investigated maternal alcohol consumption and smoking and the associated individual as well as the additive effects on the specific outcome variables which translate as cardiometabolic risk at age five years. In addition, the influence of maternal anthropometry on the underlying physiological mechanism for associations with cardiometabolic risk factors outcome variables were investigated. This chapter summarizes the main findings and discusses the possible health implications for these children in their later life. General conclusions and recommendations for future research are grounded on this discussion.

The main findings from the studies described in this thesis are summarized in Table 7.1.

Table 7.1 Summary of main findings

| **Chapter** | **Main finding** | **Health implication** |
|---|---|---|
| Chapter 3 | Maternal MUAC, BMI as well as exposure to alcohol and nicotine have effects on BW, SFT, and WC of their children. Also, for the non-exposed children, BW mediates the influence of maternal MUAC on child's SFT and WC | Maternal anthropometry in the form of pre-pregnancy BMI and MUAC together with gestation weight gain are important risk factors or predicators of the child's health outcomes. Consequently, should be monitored closely during antenatal visits. |
| Chapter 4 | Dual / additive effect of *in utero* exposure to maternal smoking and alcohol consumption is associated with higher right cIMT values in male offspring | Primary school-aged boys should possibly be screened regularly (where possible) and health promotion at schools with regards to healthy eating habits and physical exercise can be strengthen. |
| Chapter 5 | Individual effects of *in utero* exposure to maternal smoking is associated with smaller kidney size. | Maternal smoking cessation should be encouraged and reinforced in pregnant women at primary health care settings. |

| | | |
|---|---|---|
| Chapter 5 | Individual effects of *in utero* exposure to maternal smoking is associated with smaller pancreas size. Waist circumference is independently the strongest associated with organ size. | Maternal smoking cessation should be encouraged and reinforced in pregnant women at primary health care settings.<br>WC is an easy and effective tool to use in pre-pubertal children. |
| Chapter 6 | The *in utero* exposure to maternal smoking is associated with lower BMI and weight gain in males five years after birth. Female offspring born to overweight and obese mothers had higher SFT values compared to male offspring. Ultrasound is an effective measuring tool for visceral abdominal adipose tissue. | Smoking cessation should be encouraged among pregnant women. Male offspring from smoking and alcohol consuming mothers should be closely monitored for impaired growth and weight. Female offspring from overweight and obese mothers should be closely monitored for obesity development. |

**Maternal smoking, alcohol consumption and poverty - The Study Population**

In our study population, 67 % of the mothers consumed alcohol, smoked or both during their pregnancies. According to data from SANHANES and Reddy et al., in 2015, just over forty percent of adult smokers in South Africa were from mixed ancestral background while the Western Cape had a smoking prevalence of 32.9 %. In addition to high smoking rates among women of childbearing age, poverty leads to food insecurity, hypoglycemia and eventually diabetes according to findings by a study conducted by Booysen et al., 2015. Furthermore, poverty limits access to proper nutrition during pregnancy and hence influence micronutrient delivery to the foetus and healthy weight gain[1-3]. Also, micronutrient deficiencies caused by nutrient-poor diets in these young children are a common observation. According to a report from SANHANES in 2014, most females in their reproductive years who are from low- and middle income settings, were Vitamin A and iron deficient. Correspondingly, other studies too have demonstrated a deleterious effect of *in utero* exposure to micronutrient deficiencies and teratogens on the foetal organ development[4]. Perhaps, poverty or socioeconomic status (SES) may be the single most important explanatory factor contributing to the cardiometabolic risk experienced by this study population, which is one of the poorest low-income settings in the Western Cape, South Africa. This study population is predominantly from a mixed ancestral background and nearly 50% of households have a low monthly income of R3 200 (<$250) or less [3]. Very

often low-income settings experience social problems caused by unemployment, crime, violence, alcohol and drug abuse[3]. In addition to alcohol and smoking, the use of other recreational drugs, among women of childbearing age in these low-income settings is particularly high.

Table 7.2 Possible Confounding and Mediating factors associated with Cardiometabolic risk outcomes

| Confounding or Mediating factor | Outcome | Supporting literature |
|---|---|---|
| Socio-economic status (SES) | Poverty influences food choices | Vorster et al., 2007, Boyer et al., 2015 |
| Maternal age | Younger maternal age is associated with lower education and increased health risks | Fall et al., 2015 |
| Maternal anthropometry (pre-pregnancy BMI) and Gestational weight gain | LBW is associated with low maternal BMI. HBW is associated with excessive gestational weight gain and high maternal BMI | Berends et al., 2012, Hochner et al., 2012, Gaillard et al., 2014 |
| Maternal diet and nutritional status | MUAC is an indication of maternal nutritional status (>24 cm). Maternal high fat diets are associated with epigenetic changes | Stanner et al., 2007, May et al., 2016 |
| Maternal education | Linked to maternal age but on its own also influences food choices and health outcomes if their children | Fall et al., 2015 |
| Maternal lifestyle choices: smoking, alcohol consumption and physical activity | Smoking, excessive alcohol consumption and physical inactivity are associated with less favourable health outcomes | May et al., 2016<br>Nakhoul et al., 2017 |
| Paternal diet | Famines studies demonstrated an associated between paternal diet and cardiometabolic risk in their children in later life (adult) | Nakhoul et al., 2017 |
| Paternal lifestyle choices: smoking and alcohol consumption and physical activity | Smoking, alcohol consumption and physical inactivity induce epigenetic changes in sperm / germ cells which affect the offspring's health | Gaillard et al., 2014 |
| Offspring weight (BMI) | Accelerated weight gain in early life (birth to two years) has detrimental effects on adult health outcomes and increases cardiometabolic risk | Monteiro et al., 2016, Lundeen et al., 2015, Muntuli et al., 2016, Lucendo-Villarian et al., 2017 |
| Offspring diet / nutritional status | Under – and over nutrition are associated with obesity and adult health outcomes | Vickers et al., 2012 |
| Exposure to environmental toxins | Secondary smoking and other environmental toxins have harmful effects on cardiometabolic health | Forray et al., 2016 |

**Maternal smoking, alcohol consumption and *in utero* adiposity programming**

*At birth (anthropometry)*

Micronutrient insufficiency as well as unhealthy weight gain during pregnancy pose treats to foetal growth and development. Both of these are challenges faced by LMIC. Furthermore, foetal growth restriction and placental insufficiency are commonly associated with maternal smoking during pregnancy[4]. The low birth weight rate (LBWR) of this population of 16.4 % is similar to the national LBWR of 15.4% in South Africa. According to a 2003 survey, low birth weight rates in urban and rural setting are 17.6 % and 13.3% respectively. In a more recent survey of 2017, the LBWR showed a decreased of 1.4 % in the Western Cape[20]. Furthermore, within the Western Cape, the LBWR differed in communities with different ethnicity. In Khayelitsha, a predominantly African community, the LBWR is 7.6% compared to Mitchell's Plain, a community of mixed ancestry, with a LBWR of 10.2%. Possibly as a result of the higher smoking rate among the women from mixed ancestry but also possibly due to the genetic profile of a smoking mother [21, 22]. In our study population, the exposed infants had slightly lower birth weights (BW) compared to the controls but, this difference did not reach significance[23]. More importantly the children born from smoking mothers had lower body weights and lower body lengths at the age of five years. Thus, results reported in this thesis are in accordance with those of other studies where adverse birth outcomes are associated with *in utero* exposure to teratogens [22 - 24]. However, these deleterious outcomes of especially *in utero* nicotine exposure is prominent five years after birth independent of body size [25, 26]. In 1957, Simpson was the first to describe low birth weight rates as a result of maternal smoking during pregnancy. Subsequently, in 1972, Butler et al. and in 1997, Perkin et al. demonstrated the dose effect of smoking during pregnancy. Butler et al. concluded a smoking mother who continues to smoke during her pregnancy had a different profile than a mother who didn't smoke or ceased smoking upon pregnancy confirmation. These mothers tended to have smaller children[21] as a result of impaired maternal appetite, oxygen deprivation and hypoxia in the developing foetus[22]. On the one hand the *in utero* effects of maternal smoking result in intrauterine growth restriction due to placental insufficiency[4], consequently leading to LBW infant. On the other hand, these infants are predisposed towards rapid weight gain over their first

two years, partly attributable to nicotine withdrawal, associated with the maternal smoking during pregnancy, and increased appetite stimulation shortly after birth[27]. Long-term consequences of maternal smoking during pregnancy includes overweight and obesity due to impaired responses to leptin on the satiety centre of the hypothalamus [27, 28,29]. Moreover, the accelerated weight gain may result in fatty infiltration of the visceral organs leading to cardiometabolic risk experienced at younger ages than anticipated.

*At age five years (anthropometry)*

In our study population, the children born LBW demonstrated a significant greater change in BMI and weight gain compared to the children born NBW. These LBW children showed accelerated growth over the first five years of life. Furthermore, overweight and obesity, at the age of five years, were observed in a low 4.6% for the children born NBW and 0% in the children born LBW. One possible explanation, these children are experiencing a BMI nadir[30] before the adiposity rebound occurs, usually after the fifth year, as reported by Koyama et al., 2013.On the contrary, other South African studies, using different study populations, observed overweight in 10-14 % of pre- and primary school children [31, 32]. Another explanation for the relatively low overweight seen at five years could be the evolutional effect of smoking on birth weight in this low-income setting. The true effects may be masked by transgenerational and intergenerational inheritance on the programming of increased adiposity [33] and lower lean muscle mass [34, 35]. Yet another explanation, the transition from poverty and nutritional deprivation to affluence usually display a high prevalence of obesity[15]. In LMIC we often see these mismatch environments from childhood poverty in rural areas and the transition to affluence in urban areas in later life [36 -38]. However, this “mismatch” phenomenon was not evident in our study population possibly due to the severe poverty and vast environmental insults still experienced by the study population. Other reasons for low overweight percentage seen among the children of our study population could include, the availability of food, food choices, exposure to secondary smoking, the mothers’ age and educational level are some of the factors impacting on the post-natal development of the child and the resulting cardiometabolic risk [7, 10]. Even within a low-income setting, clear disparities are expected in different households of these children[20]. Data on SES

within our study population was not collected[1, 20]. Therefore, we relied on data collected by researchers for the SANHANES report of 2014 as well as the South African Health Review of 2017 to provide an indication of the average household income in this population. Although the majority of the households had an income of R3200 ($215 or €19.5) or less, substantial variation in education, household usage and prestige might be expected. Higher income is associated with healthier, more nourish meals with higher variety compared to lower income, even within a low-income setting[39].

*Waist circumference (anthropometry) and visceral adipose tissue (ultrasound)*

From our findings, dual *in utero* exposure to alcohol and nicotine was also significantly associated with lower abdominal visceral adiposity in males. However, *in utero* nicotine exposure was associated with higher visceral abdominal adiposity values irrespective of the smaller body size of these children. According to Després et al, higher visceral abdominal adipose tissue is most harmful and significantly link to cardiometabolic disease development [40]. In addition to *in utero* exposure, body weight as well as the sex of the child at age five years were also strongly association with visceral abdominal adiposity. However, the lower adiposity values observed in the exposed children might be explained by the dietary intake, food choices and environment of the control group children which differed from that of the children in the exposed groups. Within this low-income setting there might be different levels of food insecurity experienced by individuals of the different exposure groups. Smoking and alcohol consuming mothers might have less money to spend on food than the mothers of the children in the control group. They may also have a less healthy approach to the diet of their children. All the visceral adipose tissue and abdominal wall thickness measurements at age five years, measured using ultrasound, were lower for the children exposed to *in utero* teratogens compared to those not exposed. Furthermore, the exposed children in this study population did not display increased weight gain or BMI, still smaller than the non-exposed children at age five years. Findings by studies such as Prentice and Moore et al., 2005 suggest the *in utero* period and early life insults result in risk of developing cardiometabolic diseases in later life [41]. Although the exposed children were smaller with less abdominal adiposity at age five years, they had higher visceral abdominal adiposity which might

more detrimental. Also, they might be at higher risk of developing obesity should nutritional transition occur. Furthermore, low income countries suffer greatest from *in utero* insults such as alcohol and nicotine exposure, thus we expect to see an increase prevalence of obesity in these children. Although children from our study did not display high prevalence of overweight and obesity as an increased BMI at age five years, they had increased adiposity according to WHO reference values[42]. According to Rerksuppaphol et al., 2014, WC measurement is a more sensitive tool than BMI to detect cardiometabolic risk in paediatric populations[43]. And, according to Yajnick et al. 2003 who described the thin-fat Indian baby[44], there is an association between the body composition and impaired insulin sensitivity later in childhood. As the children from the Pune study, our children might also be described as "thin-fat" and we speculate they might develop obesity with impaired insulin sensitivity in the adolescence or early adulthood [16, 44, 45]. In summary, confirmed by factorial analysis (figure 7.1), the influence of the current weight of the child has the strongest association with all other variables (Chapter 3). Thus, illustrating the importance of tracking weight gain and the development of obesity in these children, especially females.

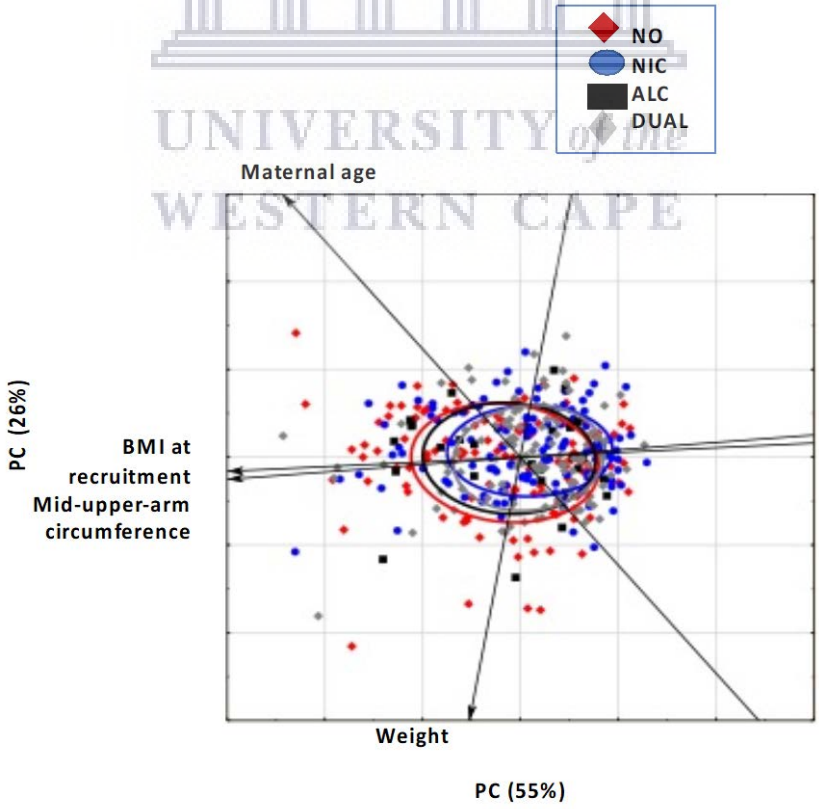


Figure 7.1 Factorial analysis: maternal and child adiposity variables influencing all other outcome variables

**Maternal anthropometry and nutritional status**

In this study population, we found maternal anthropometry significantly predicts the anthropometric measures of their children at age five years. Maternal BMI and MUAC at recruitment correlated significantly and positively with the SFT and WC of their children at age five years (Chapter 3). In particular, the maternal parameters of adiposity correlated significantly and positively with skinfold thickness, waist circumference, IMT and visceral adiposity of the children age five years. In addition, maternal age at recruitment correlated with SBP and triceps SFT. Furthermore, literature suggests maternal nutrient-restricted intake is associated with low nephron numbers, altered kidney function and HPT later in life [46 -48]. In addition, mothers who continue to smoke during pregnancy tend to have reduced appetite leading to nutritional deficiency impacting on the development of the foetal organs [49, 50]. Furthermore, according to Barouki et al. (2012), maternal high carbohydrate diet, especially during the first trimester of pregnancy, is associated with methylation of the CpG gene which in turn is associated with adiposity of the child at 6 or 9 years of age[51]. Typically found in low-income settings is a diet high in carbohydrates and saturated fats which impact negatively on the cardiometabolic risk of the children. Another important factor related to the socioeconomic status, the age of the mother, might explain antenatal and post-natal food choices [4, 7, 19, 52]. Maternal age is associated with the educational level of the mother which influences food choices which in turn is associated with programming of increased childhood adiposity[7, 13, 19, 35]. Other contributing factors of increased adiposity includes gestational weight gain, maternal pre-pregnancy body mass index (BMI), parity[53], as well as maternal and paternal genetics.

The following diagram (figure 7.2) illustrates the influence of nutritional transition from poverty to affluence on the development of obesity explained by the transgenerational and intergenerational inheritance in this low-income study population [41].

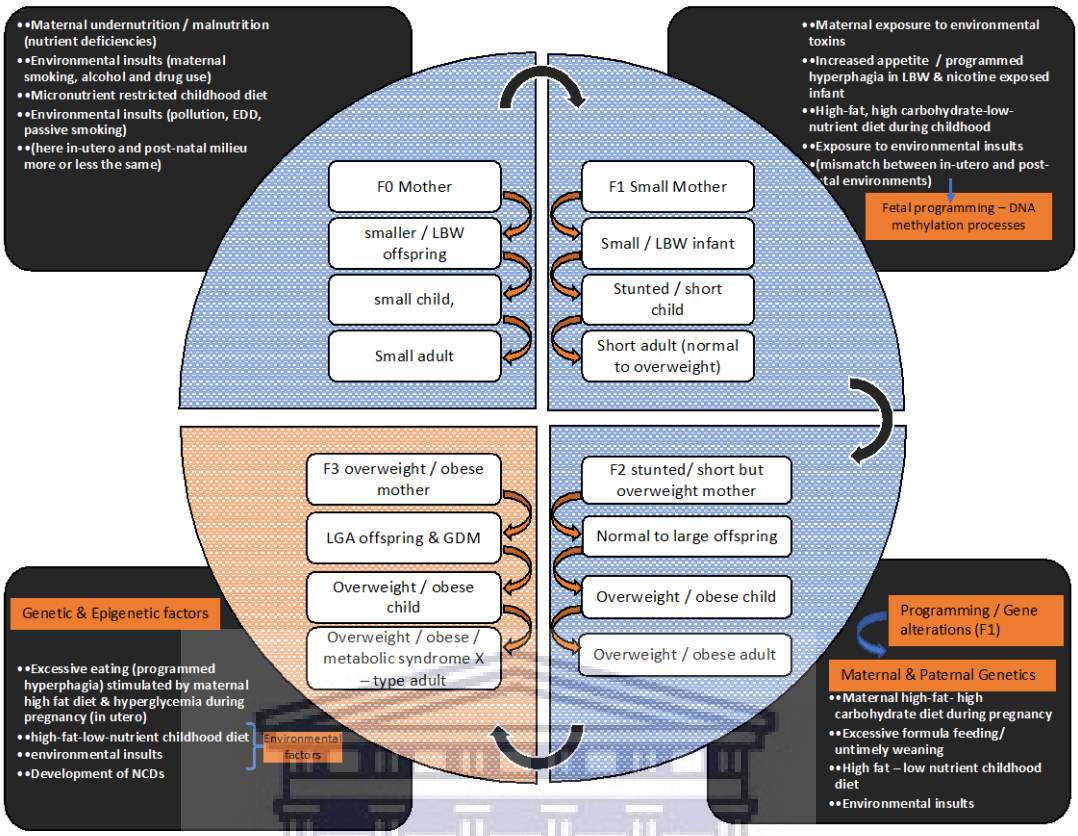


Figure 7.2 Obesity programming due to transgenerational and intergenerational inheritance from mother to child

Although the $F_1$ mothers (grandmothers of present study population) were not part of the present study, literature suggests they were generally shorter with normal weight, stunted children (thin-fat babies) [41]. The offspring formed the $F_2$ generation and represent the mothers of the present study population, who are generally short and normal to overweight with increase adipose tissue as indicated by a higher MUAC. They gave birth to normal BW children, the $F_3$ generation, study subjects of current study. The $F_3$ mothers (females of the present study) are generally more likely to become overweight with larger offspring, $F_4$ generation. Another important reason to conduct follow-up studies to track the evolution of cardiometabolic risk in this low-income setting.

**Maternal smoking, alcohol consumption and vascular dysfunction**

*Aorta and carotid IMT*

One of the main objectives for this thesis was to demonstrate the harmful effect of maternal exposure to alcohol and nicotine during pregnancy on vascular dysfunction. In this study, dual *in utero* exposure to alcohol and nicotine was associated with

higher carotid IMT values observed in the males at age five years. We utilized higher intima media thickness values as an indicator for subclinical atherosclerosis. Supported by the findings of Chen et al., (2011) and Jones et al., (2011) who used animal models to illustrate the enhanced vascular dysfunction in the male offspring[54, 55]. In their studies, the enhanced vascular dysfunction was caused by maternal nutrient restriction and placental insufficiency. They also described the vascular dysfunction as a possible cause for the development of hypertension (HPT) in males in their later life[54, 55]. Liu et al., 2017 was the first to demonstrate an association of higher carotid IMT measurements in children from low-income settings during mid-childhood[56]. According to a review done by Park et al., 2015, adiposity positively correlated with cIMT in adolescents, but not in younger children. Studies in this review were conducted mostly in Western Europe and the United States[57]. However, in our study population, the higher cIMT values were detected in early childhood and in a low-income setting. Findings from our study also confirmed the positive correlations between IMT values and adiposity at age five years. These findings demonstrate the detrimental effect of *in utero* exposure to alcohol and nicotine during pregnancy on the development of atherosclerosis. In addition, the programming effect of vascular dysfunction caused by *in utero* exposure to alcohol and nicotine might affect males and females differently [17].



*Blood pressure*

In our study population, females had significantly higher SBP and MAP compared to the males. In contrast with other literature, Grigore et al., 2008, Ojeda et al., 2008, Jones et al., 2011, male offspring normally remained hypertensive due to *in utero* programming of HPT and foetal growth restriction[48, 55, 58]. Chen et al., 2011 also confirmed this sex specific foetal programming of HPT and vascular dysfunction only in male offspring[54]. In addition, we found strong positive correlations between blood pressure measurements and measures of adiposity. SBP, DBP and MAP measurements correlated significantly and positively with all anthropometric and ultrasound measurements of adiposity. These findings are supported by multiple previous studies (birth to twenty cohort of Soweto, South Africa) [16], Generation R study of Rotterdam, Netherlands[10, 59]. Blood pressure measurements at age five years fell between the 75$^{th}$ and the 90$^{th}$ percentiles for age and body length. More than 40 % of the children in this study fell in the category of pre-hypertension (according to

WHO reference values). There was no significant association between the blood pressure measurements and intima media thickness (cIMT or aIMT) at age five years[60, 61]. These findings are supported by findings of Edstedt Bonamy et al., 2008 and Meenakshisundaram et al., 2011. Furthermore, maternal HPT during pregnancy was not associated with blood pressure measurements of their children. According to findings by Benschop et al., 2018, an association between maternal hypertension and hypertension in offspring exists[59]. Gillman et al., 2005 described the reduced activity of the placental enzyme 11β -hydroxysteroid dehydrogenase type 2 associated with hypertension and hyperglycemia in the offspring. In summary, these authors describe some of the known factors associated with the development of premature hypertension as *in utero* exposure to nicotine, maternal hypertension as well as a maternal high carbohydrate diet during pregnancy. Foetal programming of hypertension takes place during the embryonic phase of development according to Jones et al., 2011. The programming effects of HPT were observed in both sexes but only males remain hypertensive in adulthood. We did not find an association between BP and cIMT, aIMT nor kidney size. Other researchers also did not find an association between BP and cIMT[59, 61, 62] nor BP and kidney size[63]. However, these associations might become evident when children from our study population enter adolescence and young adulthood[64]. According to Gillman et al., 2005, increased in BP might be present in the second or third decade of life.



**Maternal smoking, alcohol consumption and organ size**

*Kidneys*

Another main objective of this thesis was to investigate the effects of maternal smoking and alcohol consumption during pregnancy on organ size five years after birth. Results from our study support the hypothesis of foetal growth and kidney size programming described by Oken et al., 2008, Gigore et al., 2008, Koleganova et al., 2012 and Luyckx et al., 2013. Lower mean kidney length, width, height and volume values for both right and left kidneys were observed in LBW children at age five years. These values were between the 5$^{th}$ and 50$^{th}$ percentile for the child's height and age according to reference values described by Konus et al., 1998. Both BMI and WC of the child at age five years correlated significantly with all the kidney measurements, indicating kidney size might be proportional to body size[65 - 67]. The relationship between reduced kidney size and lower WC persisted after controlling for

the BMI of the child. These findings suggest the use of WC, as a proxy for smaller visceral organs[44]. Although the majority of the infants in this study population had NBWs, body weight might be masked by increased adiposity and lower lean muscle mass described by the thin-fat hypothesis[34, 44]. According to Kooijman et al. 2013, smaller third trimester foetal kidney size was associated with lower kidney function in school-aged children[63]. Even though kidney function was not measured in our study, we observed a high percentage of children with pre-hypertension at age five years. The pre-hypertension was not associated with reduced kidney size and volume at age five years. Multiple other studies also support our findings of no association between kidney volume and BP at age five years[63, 68, 69]. Yet, other studies, found an association between reduced kidney volumes and higher BP[46, 55, 58, 70]. Animal studies confirming the *in utero* programming hypothesis and altered kidney function state the underlying mechanism to be due to maternal nutrient restriction, placental insufficiency and hypoxia [55, 71, 72]. Moreover, increased risk of kidney disease and higher BP in adulthood are illustrated by the *in utero* programming hypothesis where induced bilateral uterine artery ligation affects foetal growth [71]. In addition, post mortem studies confirmed lower nephron number and or smaller kidney size in adults with primary hypertension in those whom had LBW[48, 63]. Moreover, at this age, waist circumference was independently and strongest associated with kidney size[44]. Controlling for BMI as covariate, this relationship persisted, thus confirming the use of WC as a proxy for smaller organ size described by Yajnick et al. (2003).



*Pancreas*

The *in utero* exposed children had lower pancreas head and body measurements compared to the controls at age five years. However, these differences did not reach statistical significance in our study sample of 500 children (Chapter 5). Pancreas measurements show great variability within individuals. From all the pancreas measurements, the pancreas body is most likely the easiest to measure and also the most reliable of all measurements performed in a transvers section [73]. Furthermore, the sex of the child as well as the WC of the child at age five years were significantly associated with a reduced pancreas head size. Waist circumference was independently and the strongest from all other variables, associated with lower pancreas measurements, thus supporting the hypothesis of WC as an indication of smaller visceral organs proposed by Yajnick et al. (2003). Moreover, WC is also an easy

measuring tool for detection of cardiometabolic risk factors in pre-pubertal children[43, 74]. Other findings included, significantly lower values for the pancreas body were observed in the nicotine only exposed group compared to the control group at age five years. Research done by Jovanovic et al., 2013 confirmed the association of *in utero* exposure to teratogens and the association with reduced organ size, growth and development[75]. The effect of *in utero* exposure to nicotine on the development of organs was also described by Anblagan et al., 2013. Normally vital organs such as the brain and heart are spared to the expense of non-vital visceral organs[72]. In addition, according to findings by Saisho et al., 2010 and Altobelli et al., 1998 ß cell mass decreases in type 1 and 2 diabetes due to ß cell apoptosis but, increases with obesity [76, 77]. Obesity is often associated with fatty infiltration in various organs, including the pancreas. This can be described as non-alcoholic fatty pancreas disease[78, 79]. Pancreas size and volume may increase due to fatty infiltration [78] thus masking the teratogenic effect of *in utero* exposure. These measurements fell within normal ranges i.e. Furthermore, the pancreas body values, recorded from our study population, are at the lower end of normal range and the tail and head measurements were significant lower when compared to reference values for pancreas size reported by Siegal et al., 1987, 1999. On the one hand these findings suggest a reduced pancreatic size associated with lower ß cell numbers due to either foetal programming or apoptosis. On the other hand, an increased pancreas size due to fatty infiltration both these conditions may be induced by the *in utero* nicotine exposure[80, 81].



Both maternal smoking and alcohol consumption during pregnancy are documented teratogens involved in foetal programming of organ size, growth and development [13, 19, 75]. One aim of this thesis was to demonstrate the additive effect of *in utero* exposure to smoking and alcohol. In this study population, a compounding effect of *in utero* teratogen exposure was demonstrated on the vascular system (Chapter 4). In addition, individual negative effects of *in utero* nicotine exposure were seen in kidney and pancreas size (Chapter 5) but also on the anthropometry of male offspring (Chapter 3, 4 and 5).

**Future research**

Future longitudinal assessments are needed to map the trajectory of metabolic risk factors in low-income settings. Given the statistics of this setting, we expect these

children to be predisposed to secondary as well as smoking themselves at a younger age, thus the perpetuation cycle of smoking among childbearing aged women continues. A useful addition to our study would have been to look at body composition and lean body mass index instead of BMI and body weight alone. Also, gestational weight gain, paternal lifestyle, dietary intake and individual SES assessments are recommended for studies of this nature. Furthermore, prospective follow-up studies to evaluate pancreas and kidney function in association with ultrasound measurements of organ size are recommended.

**Conclusions**

These studies described in this thesis highlight the importance of not only the lifestyle of the mother (prenatal) but also that of her child (post-natal). This can be described in two ways. First, an expecting mother should abstain from alcohol consumption as well as cessation of cigarette smoking. In the public health sector, new, effective strategies and interventions are needed to decrease the smoking rates among pregnant women. Also, to study the relationship between modifiable factors or epigenetic factors such as diet, active and passive smoking exposure and exercise and those non-modifiable factors such as genetics and male sex as determinants of cardiometabolic diseases. In addition, extension of the study to include children at the primary school level could provide useful insight into the evolution of cardiometabolic risk over time in the South African environment. In conclusion, our study supports the value WC measurements, vascular and abdominal ultrasound techniques for early detection and timely management of cardiometabolic risk in children exposed to *in utero* alcohol and nicotine. Also, these may be use as reference values for future research studies.

Finally, *in utero* exposure to alcohol and nicotine has individual negative effects on the anthropometric, blood pressure and ultrasound measures but the additive effect, is sex specific, affecting male vascular structures and female adipose tissue. As described in this thesis, the etiological *in utero* effects of alcohol and nicotine may not have the same effects on all the organs, the size or function.

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