Paediatric Non-Alcoholic Fatty Pancreas Disease and Aortic Intimal Medial Thickness: A study identifying potential fatty infiltration of the pancreas and its association with aortic IMT in children exposed to teratogens during pregnancy

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Declaration

I, Amy Thomas, declare that:

(a) The research reported in this dissertation, except where stated otherwise, is my original work;

(b) This dissertation has not been submitted for any degree or examination at any other university;

(c) This dissertation does not entail other people's work unless specifically attributed as such, in which case their words have been rephrased and referenced. However, where their exact words have been used, their writings have been placed in quotation marks and referenced.

Signed:  Date: 9th December 2020

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Abstract

The incidence of non-communicable diseases is increasing worldwide, with South Africa being no exception. Non-communicable diseases are classified as non-infectious and are often referred to as lifestyle diseases as they are caused by common, modifiable risk factors such as unhealthy diet, obesity, tobacco use and lack of physical activity. Due to the increasing prevalence of childhood obesity diseases such as fatty pancreas and fatty liver are becoming more common. Cardiovascular disease, and more specifically atherosclerosis is the underlying cause in most adult deaths. Disease pathogenesis starts in childhood and can be detected via Aortic intima-media thickness (IMT). The developmental origins of health and disease hypothesis (DOHaD) proposes that exposures in-utero may result in persistent adaptations including alterations in metabolism. The overall aim of this study is to investigate the effect of intrauterine exposure to nicotine and alcohol on fatty accumulation in the pancreas, its association with aortic intima media thickness and adiposity in 5 year old children using ultrasonography and anthropometry.

The participants selected came from a database used in a previous study, in which maternal data was collected during antenatal clinic visits in Belhar and at Bishop Lavis Midwife Obstetric unit. Children who fell into the inclusion criteria underwent anthropometric measurements, as well as ultrasound measurements on the pancreas and abdominal aorta. Pancreatic measurements included head, body and tail size as well as pancreatic volume as an indication of possible fatty infiltration. Clinical measurements conducted included blood pressure, mean arterial pressure and heart rate. Collected data was analysed using SPSS version 26 (Statistical Package for the Social Sciences) and thought to be significant when $p<0.05$. Quantitative data was described as the means along with standard deviation, minimum and maximum values while one-way analyses of variance (ANOVA) was used to evaluate the differences in child anthropometric and adiposity variables

among *in utero* exposure groups. Pearson correlation as well as partial correlations were used to identify possible linear relationships between anthropometric and adiposity variables with aIMT. In order to ascertain if variables were independent of each other, Chi-Square analysis was done. Children exposed to alcohol *in utero* were found to have the largest aIMT, however, increased aIMT was not found to be dependent on teratogen exposure but rather BMI. Participants exposed to alcohol were found to have the lowest pancreatic volume, suggesting potential beta-cell apoptosis due to ethanol exposure *in utero*. Visceral fat was significantly associated with pancreatic tail size, which could indicate the start of possible fatty infiltration. Anthropometry would not be a viable indicator of possible pancreatic fat infiltration as pancreatic volume was not significantly associated with basic anthropometric measurements. However, aIMT could be used as an indicator of pancreatic size as pancreatic size had shown a highly significant association to aIMT even after controlling for BMI.

Keywords: Teratogen, Aortic Intime-media thickness, Pancreatic volume, Pancreatic fatty infiltration, Adiposity, Anthropometry.

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Abbreviations

CVD	Cardiovascular Disease
DOHaD	Developmental Origins of Health and Disease
aIMT	Aortic intima-media thickness
MetS	Metabolic Syndrome
NAFPD	Non-Alcoholic Fatty Pancreas Disease
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
NCD	Non-Communicable Disease
LMIC	Low-Middle Income Country
BMI	Body Mass Index
VAT	Visceral Adiposity
MRI	Magnetic Resonance Imaging
WC	Waist Circumference
CT	Computed Tomography
FASD	Foetal Alcohol Spectrum Disorder
SubSFT	Subscapular Skinfold Thickness
TriSFT	Tricep Skinfold Thickness
Phead	Pancreatic Head
Pbody	Pancreatic Body
Ptail	Pancreatic Tail
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
MAP	Mean Arterial Pressure
HR	Heart Rate
PFF	Pancreatic Fat Fraction

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Chapter 1: Introduction

1.1 Background of the study

Globally, non-communicable diseases account for over two-thirds of deaths and diseases which fall under this umbrella include heart disease, Diabetes and cancer (Beaglehole et al., 2011). Non-Alcoholic Fatty Pancreas Disease refers to the infiltration of fat in the pancreas and is strongly associated with Metabolic Syndrome, and because of this it could be a predictor of cardiovascular disease as well as diabetes. The exact prevalence of fatty pancreas is unknown due to lack of a clear definition and diagnostic criteria. Due to the increase in obesity worldwide, it is thought that over time the prevalence of fatty pancreas would be increasing as well (Shah et al.,2019). Another predictor of CVD is Aortic Intima-Media thickness which, if increased, is indicative of sub-clinical atherosclerosis (Rohani et al., 2005). Microscopic lesions in the aorta, which later become the sites of plaque formation, can be seen in childhood and begins in-utero (Napoli et al., 1997). The developmental origins of health and disease hypothesis (DOHaD) proposes that exposures *in utero* may result in persistent adaptations including alterations in metabolism which have lifelong effects on offspring (Mandy & Nyirenda, 2018). These exposures, can be referred to as teratogens, can be chemical or biological (Alwan & Chambers, 2015). In this study the effects of intrauterine exposure to nicotine and alcohol on the pancreas and aIMT in offspring are investigated to ascertain possible increased risk for heart disease and diabetes in adult life.

1.2 Statement of the research problem

The burden of non-communicable diseases is estimated to surpass that of communicable diseases by 2020 (Borthwick & Horton, 2010). Cardiovascular disease (CVD), more specifically atherosclerosis is thought to be a large contributor to adult death and it is the underlying cause in

most myocardial infarctions (Lusis et al., 2010). Effects of Atherosclerosis are only experienced in adulthood, however, disease pathophysiology begins *in utero* and increased intima-media thickness (aIMT) has been found to indicate subclinical atherosclerosis in children and adolescents (Skilton et al., 2019). Since fatty pancreas shares several risk factors with CVD, and given its close association to Metabolic Syndrome (MetS) it could bear an association to increased aIMT, however literature is limited (Catanzaro et al., 2016; Kul et al., 2019). Whether or not children who are exposed to teratogens *in utero* are more susceptible to fatty accumulation in the pancreas is currently unknown, and whether or not this fatty accumulation is associated with an increased aIMT has not been explored. Adequate prevention strategies cannot be implemented without sufficient research about causation and a possible association between fatty pancreas and aIMT offers clinicians a simple alternative to investigating possible subclinical atherosclerosis in a patient. In response to this problem, this study aims to not just identify the prevalence of fatty pancreas, but also aIMT in association with foetal exposure to nicotine and alcohol.

1.3 Research Aims/Objectives

The overall aim of this study is to investigate the effect of intrauterine exposure to nicotine and alcohol on fatty accumulation in the pancreas and its association with aortic intima media thickness in five year old children using ultrasonography and anthropometry.

The specific objectives of the study are:

- Identifying the prevalence of pancreatic fatty accumulation in all four groups, namely both alcohol and nicotine exposed, nicotine exposed, alcohol exposed and non-exposed children.
- Identifying the effect of alcohol and nicotine exposure on aIMT, pancreatic fat accumulation and adiposity (i.e. visceral and subcutaneous fat).
- Ascertaining aortic intima media thickness in the above mentioned groups.

- Establishing possible associations between pancreatic fat accumulation and aortic intima media thickness.
- Determine the value of anthropometry as a predictive tool for NAFPD and aortic IMT.

1.4 Hypothesis

Children exposed to nicotine and alcohol *in utero* will have an increased aIMT in comparison to the control. When looking at fat accumulation in the pancreas, these children will exhibit signs of fatty infiltration, indicating a possible association between aIMT and fatty pancreas. Anthropometry will be useful in acting as a tool to identify children who are more likely to be at risk of fatty pancreas and sub-clinical atherosclerosis.

1.5 Significance of study

The investigation into the effect of teratogens on the risk of cardiovascular disease in offspring in this study allows for insight into the life-long effects of an unfavourable environment *in utero*. Ascertaining associations between nicotine and alcohol exposure to disease risk will enable more efficient future prevention strategies, as they can be focussed on patient groups most at risk. When looking at aIMT and its association to the accumulation of fat in the pancreas, the literature is limited. Based on this study's findings, recommendations can be made to physicians on whether one condition could be indicative of the other, due to shared risk factors. Independently, aIMT and fatty pancreas are both signs of more serious health conditions in the future, such as Atherosclerosis (thickening aIMT is a sign of subclinical atherosclerosis) and Type 2 Diabetes Mellitus (fat accumulation in the pancreas alters pancreatic function and adds to the risk of developing Type 2 Diabetes Mellitus), both of which can experience a slowed disease progression and even reversal if detected early and followed with the implementation of lifestyle changes. This

study acts as an early screening for these conditions among high-risk individuals in this sample group.

1.6 Overview of study

The study is divided into five chapters, with chapter one being an introduction into the research being conducted, chapter two focuses on the literature around aIMT, pancreatic fatty infiltration and the effects of teratogens on offspring and chapter three examines the methodology used. Chapters four and five focus on the analysis of results and the discussion thereof, after which conclusions are drawn and recommendations for future research are made.

Chapter 2: Literature Review

2.1 Background

Non-communicable diseases (NCDs) are classified as non-infectious diseases and are the leading cause of death globally (Sanders, n.d.). According to the World Health Organisation, it is estimated that the worldwide disease burden will increase by 17% in the next ten years, and in the African region by 27%. These diseases include heart disease, cancer, stroke and diabetes which accumulatively accounts for more than two-thirds of the global mortality rate (Beaglehole et al., 2011). These illnesses are often referred to as diseases of lifestyle as they are caused by common, modifiable risk factors such as unhealthy diet, obesity, tobacco use and lack of physical activity. The burden of disease brought by NCDs now tower above those of infectious disease, and the transition is thought to be brought on by a number of factors caused by economic development, especially in low and middle income countries (LMIC), such as individuals moving from traditional foods to processed foods which are high in fat, salt and sugar, as well as a decrease in physical activity (Hancock, Kingo, & Raynaud, 2011).

The Burden of Disease Research Unit at the South African Medical Research Council has analysed mortality levels and trends for NCDs over a 14-year period and found that in 2010, thirty-nine percent of deaths in South Africa were due to NCDs, and more than a third of these deaths occurred before the age of 60 (Nojilana et al., 2016). It was found that the overall NCD mortality rate decreased over time, however there were both increasing and decreasing trends depending on the year. This highlights the changing lifestyle and risk factor profiles of individuals living in South Africa. The increase in mortality from diabetes mellitus and nutritional and endocrine blood disorders speak to more individuals falling into the obese category while the effects of the tobacco control interventions was seen in the decrease in deaths caused by obstructive pulmonary disorder

and Ischemic heart disease (Nojilana et al, 2016). NCDs are believed to be a major challenge to the developmental progression of a low to middle income country in the 21st century (UN, 2012). The relationship between social disadvantage and health status is complex, especially in South Africa where one's socioeconomic status has most times been shaped by historic political, social and economic factors. The poor are more vulnerable to NCDs for numerous reasons, including psychosocial stress, higher levels of risk behaviour, unhealthy living conditions, limited access to high-quality health care and reduced opportunity to prevent complication (Williams, Allen, & Roberts, 2018). It is vital that intervention strategies are implemented in these areas in order to reduce the burden of disease and to improve the quality of life as a whole. Often these individuals do not have access to adequate health screening systems to diagnose the early onset of disease followed by lifestyle intervention.

Prevention methods include primary intervention, which refers to the modification of risk factors, and primordial prevention which is aimed at the prevention of risk factor development (Mhatre V. Ho, Ji-Ann Lee, 2012). Early detection of the development of atherosclerosis helps in deciding which interventions should take place. This chapter looks at two potential predictors of cardiovascular disease, namely Non-Alcoholic Fatty Pancreas Disease (NAFPD) and Aortic Intima-Media Thickness (aortic IMT).

2.2 Non - Alcoholic Fatty Pancreas Disease (NAFPD)

Non-Alcoholic Fatty Pancreas Disease (NAFPD) is defined as fat accumulation in the pancreas, in the absence of significant alcohol consumption (Smits & van Geenen, 2011). NAFPD refers to a wide spectrum of diseases, which include pancreatic fat accumulation, inflammation and pancreatic fibrosis (Romana, Chela, Dailey, Nassir, & Tahan, 2018). A common cause of chronic liver disease – Non-Alcoholic Fatty Liver Disease (NAFLD) – is often thought of as the hepatic

manifestation of Metabolic syndrome (MetS) and it is believed that NAFPD could be the pancreatic equivalent (Pinte, Balaban, Băicuş, & Jinga, 2019). Studies have found that fatty infiltration in the pancreas may represent a manifestation of MetS as it correlates with the metabolic risk factors (Wu & Wang, 2013). In contrast to NAFLD however, both the local and systemic consequences of pancreatic fat accumulation have not been well investigated (Lesmana, Pakasi, Inggriani, Aidawati, & Lesmana, 2015).

NAFPD was first described in 1933, yet due to the differences in terminology and the lack of clear diagnostic criteria, the exact epidemiology of pancreatic fatty accumulation is not well defined and is usually an accidental finding when a patient is having abdominal imaging done for reasons unrelated to the pancreas (Ogilvie, 1933; Pinte et al., 2019). Due to the drastic increase in obesity worldwide, it is believed that the incidence of both liver and pancreatic fatty accumulation would increase (Shah et al., 2019). A study conducted among routine check-up adult patients at a hospital in Indonesia found the prevalence of NAFPD to be 35% and found that the condition is strongly correlated with other metabolic conditions such as NAFLD (Lesmana et al., 2015). This result is closely related to the average adult prevalence of 27% in the United States (Sepe et al., 2011). While there is very little data available on the presence of pancreatic fat accumulation in children, a study done in both obese and 'slim' children (BMI 5th-85th age- and sex-specific percentiles) found that 58% of obese children had fatty pancreas and these children are more susceptible to insulin resistance and metabolic syndrome (Elhady et al., 2019). These results are similar to those found in a study conducted among adolescents, in which it was found that obese participants accumulate fat in the pancreas (Maggio et al., 2012).

2.2.1 Pathophysiology of NAPLD

When looking at the pathophysiology of pancreatic steatosis, the literature indicates that a clear distinction needs to be made between steatosis caused by fat accumulation which is associated with MetS, and steatosis which is caused by the death of acinar cells which are then replaced by adipose tissue (Pinte et al., 2019). Fatty replacement caused by cellular apoptosis is often caused by congenital diseases, viral infections or alcohol abuse and as such it does not fit the case definition of NAFPD.

In contrast to these previously mentioned conditions, with NAFPD, obesity is thought to be the main cause of fatty infiltration (Smits & van Geenen, 2011). The exact pathway of how obesity leads to ectopic fat accumulation is unclear, however some patients may be more susceptible to ectopic fat accumulation due to impaired subcutaneous fat storage capacity (Catanzaro et al., 2016). Organs such as the liver and pancreas experience lipid deposits once the adipose tissue expansion limit has been exceeded(Virtue & Vidal-Puig, 2008). The pathogenesis of NAFPD is often compared to NAFLD, however, unlike fatty liver where fat accumulates in the hepatocytes, fat accumulation in the pancreas takes place in its adipocytes, found within the pancreatic tissue. These ectopic adipocytes infiltrate the tissue resulting in the dysfunction of pancreatic β-cells, increasing one's risk of developing Type 2 Diabetes Mellitus (T2DM) (Pinnick et al., 2008; Weng et al., 2018).

2.2.2 NAFPD and Clinical consequences

Metabolic Syndrome

While there is ongoing debate about whether or not fatty pancreas develops as a result of MetS or if it is a condition leading to MetS, there is consensus that MetS is in close correlation with NAPFD. These findings were supported by a review looking at three publications with a total

of 49,329 subjects (Bi, Wang, Li, Zhou, & Sun, 2019). MetS consists of five components, of which the patient needs to have three or more to be successfully diagnosed. The World Health Organisation defines MetS as the presence of insulin resistance (impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes mellitus) in addition to two of the following risk factors: obesity (waist–hip ratio or body mass index), hyperlipidaemia (hypertriglyceridemia, low high-density lipoprotein [HDL] cholesterol), hypertension, or microalbuminuria (Grundy, Hansen, Smith, Cleeman, & Kahn, 2004). NAFPD is strongly associated with obesity and the risk of T2DM.

Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus refers to a condition in which the body's response to insulin has decreased and eventually, becomes non-existent, leading to insulin resistance. Whether or not fatty pancreas aids in the development of T2DM is controversial, however, there are three main proposed ideas with regards to this association with the first focusing on increased amounts of triglycerides in pancreatic β-cells which can lead to their dysfunction, the second looking at intrapancreatic

adipocytes and their effect on β-cells, and lastly, the hypothesis that NAFPD and T2DM are simply consequences of obesity (Catanzaro et al., 2016; Yu & Wang, 2017).

Cardiovascular Disease Risk

Due to fatty pancreas having such a close association to MetS, it is thought to be associated with an increased risk of cardiovascular disease (Catanzaro et al., 2016). This mentioned association is however, focused on obese individuals. A study conducted by Kim *et al.* found that fatty accumulation in the pancreas could be an independent risk factor for the development of atherosclerosis in non-obese patients with T2DM, indicating that this could be used as a predictor

of CVD in individuals with a normal BMI (Kim et al., 2014). While there is not much data looking at the actual increase in risk for the development of CVD that fatty pancreas causes, studies have grouped shared risk factors together, indicating that one may be at risk of developing both conditions. One of the most important risk factors found to be associated with fatty pancreas and the development of Atherosclerosis is aortic intima-media thickness (aortic IMT) (Kul et al., 2019).

2.3 Aortic Intima-Media Thickness (IMT)

The accumulation of substances such as cholesterol, calcium and fats in the arterial wall resulting in the development of plaques and arterial wall thickening forms the foundation of the development of Atherosclerosis, which is believed to be the underlying cause in about 50% of all deaths (Lusis et al., 2010). This process is the underlying cause of most myocardial infarctions, and although symptoms are only felt during adulthood, the disease process begins in childhood (Skilton et al., 2019).

Intima-media thickness has been found to indicate subclinical atherosclerosis (Rohani et al., 2005). The formation of fatty streaks resulting from lipid deposits later form lesions in the intima of systemic arteries (McGill, McMahan, Malcom, Oalmann, & Strong, 1997). The IMT of the abdominal aorta has been found to be more susceptible to the early development of lesions and fatty streaks when compared to internal or common carotid arteries and it is because of this that abdominal aortic IMT is believed to be a better indication of preclinical atherosclerosis in children who are at high-risk of CVD (Järvisalo et al., 2001). The body undergoes adaptive intimal thickening as a response to changes in blood flow and wall tension, and it is at these sites that one is most likely to develop fatty lesions, when looking at the abdominal aorta, these sites are along the dorsal wall (Stary et al., 1992). The earliest lesions can be seen in early childhood and even in

the foetus. These microscopic lesions are a build-up of macrophage foam cells and also appear on the dorsal wall of the abdominal aorta (Napoli et al., 1997). A study conducted by Järvisalo *et al.* found that aortic IMT is more affected by preclinical atherosclerosis that carotid IMT in young children. The study also found that because atherosclerosis begins to develop first in the aortic intima, aIMT could be used as a marker for preclinical atherosclerosis (Järvisalo et al., 2001).

2.3.1 Association of aIMT with CVD risk factors

The lesions in the aorta progress with time, and accumulation increases as the individual gets older, however, the severity of these lesions are associated with cardiovascular risk factors (Skilton et al., 2019). These risk factors include hypertension, obesity and lipoprotein levels (Perrone, Hollander, De Roos, & Berenson, 1998). The above mentioned risk factors affect lesions in adolescents and young adults, however since the lesions are ubiquitous in the foetus, these lesions are associated directly with maternal cholesterol levels and inversely with birth weight (Napoli et al., 1999). In a study conducted by Dawson *et al.* among 635 adolescents and young adults found that in individuals aged between 11 to 17 years, aIMT was associated with systolic and diastolic blood pressure, body mass index, waist/hip ratio after adjustments were made for age, sex and height. The risk factors were shared in young adults, with the addition of high density lipoprotein cholesterol (Dawson, Sonka, Blecha, Lin, & Davis, 2009a). Early detection of Atherosclerosis is thus important as many of the above mentioned risk factors are modifiable and can reduce the severity of fat accumulation.

2.4 Association between fatty pancreas and aortic IMT

While the association between fat accumulation in the pancreas and fatty lesions in the aorta has not been well investigated, a study conducted by Kul *et al.* looked at this association in adults. It is stated that according to the researcher's knowledge this study was the first of its kind at the time

published (2019), when searching literature for this review, it was found that although there have been more publications, literature is still limited. The study looked at 54 patients with NAFPD and 49 patients without. It was found that aIMT was significantly higher in patients with NAFPD compared to those without. It is important to note that there were no significant differences in BMI, family history of coronary artery disease, diabetes mellitus or history of hypertension between the two groups. This study indicates that NAFPD may indicate the presence of subclinical atherosclerosis (Kul et al., 2019). The above mentioned study was conducted on adults with a mean age of 52 for NAFPD patients and 49 for those without NAFPD, whether the association is already present in children and adolescents is currently unknown.

2.5 Risk factors associated with Fatty Pancreas and Aorta IMT

Although the pathophysiology's of fat accumulation in these two conditions are different, there are shared risk factors such as obesity, socioeconomic status and adiposity which play a role in the development of cardiovascular disease.

2.5.1 Socio-economic status

Worldwide, inequalities in socio-economic status have led to inequalities in health, with the poor facing many predisposing factors which are often social determinants of illness (WHO, 2008). Infectious diseases such as HIV are often referred to as diseases of poverty and prevalence is highly concentrated among the poor, however when looking at NCDs, results are more varied. Diabetes is shown to be more prevalent in high socioeconomic groups and hypertension in lower socioeconomic groups (Ataguba, Akazili, & McIntyre, 2011). Overall, the NCD burden is shifting toward poorer individuals in developing countries due to numerous reasons, such as an increase in high risk behaviour and the inaccessibility to appropriate inpatient and outpatient treatment (Engelgau, Rosenhouse, El-Saharty, & Mahal, 2011). A review conducted by WHO found that the

poor in South Africa have 15% greater exposure to environmental tobacco smoke in the home as well as 50% more exposure than individuals with more than 12 years of education (WHO, 2006). Other risk factors such as excessive alcohol consumption is also more common in low socioeconomic communities and contributes to high levels of hypertension among the poor as well as psychosocial changes and physical injury (Bradshaw & Steyn, 2001). Lastly, obesity is also thought to be as a result of food choices made due to income and food insecurity, with individuals choosing foods high in carbohydrates and sugar to avoid hunger (Bennett, Probst, & Pumkam, 2011). In order for the above mentioned differences in risk behaviour and disease prevalence among socioeconomic groups to become smaller, intervention plans will need to be focused on the disadvantaged, to promote prevention and treatment through education and lifestyle intervention (Howe et al., 2011).

2.5.2 Adiposity

Abdominal obesity is associated with diabetes and cardiovascular disease predisposition while visceral adiposity (VAT) is associated with insulin resistance and systemic inflammation (Boyko, Fujimoto, Leonetti, & Newell-Morris, 2000; Després & Lemieux, 2006).

In a study done by Demerath *et al*, it was found that VAT mass is a strong predictor for MeS even after adjusting for factors such as BMI and physical activity status and this is consistent with recent progress which suggests that visceral adiposity is more influential than body mass in predicting fatty liver disease (Demerath et al., 2008; Paula et al., 2013). Damaso et al. found that the group of adolescents with NAFLD presented significantly higher values of insulin, BMI, visceral and subcutaneous fat in both genders when compared to non-NAFLD patients (Paula et al., 2013). This is significant because data has shown a statistically significant correlation between the percentage of fat in the pancreas and the liver (Singh et al., 2017). Visceral adiposity (VAT) has

been evaluated by Magnetic Resonance Imaging (MRI) in children and it has been related to glucose metabolism, lipids abnormalities and hypertension (Brambilla et al., 2006). Due to cost and technical difficulties, direct methods such as MRI cannot be used for field studies. Several anthropometric indexes have now been suggested as indexes of VAT (Brambilla et al., 2006). In adults, waist circumference (WC) is commonly used as an indication of central fat distribution, but in children it may be influenced by growth and puberty, reducing its accuracy in estimating VAT (Goran, 1999). Currently suprailiac skinfold thickness is used as a measure of subcutaneous adiposity in both males and females (Ayonrinde et al., 2015).

Increased central or abdominal adiposity is often measured due to its previously mentioned association with an increased risk of cardio-metabolic disorders, however associations between disease incidence and individuals with a 'healthy' BMI but high subcutaneous fat levels are still to be established. In a study conducted by Ozbulbul *et al*, it was found that when looking at pancreatic fatty infiltration, visceral fat was found to have a stronger correlation to fatty infiltration than BMI (Isiksalan Ozbulbul, Yurdakul, & Tola, 2010).

2.6 Diagnostic tools

Fatty Pancreas

Currently there is no general consensus on the best way to diagnose pancreatic steatosis, however imaging techniques such as transabdominal ultrasonography and endoscopic ultrasonography have been used (Dite, Blaho, Bojkova, Jabandziev, & Kunovsky, 2020). While the transabdominal ultrasound is non-invasive and widely used, it is not without its limitations as the transabdominal ultrasound of the pancreas is not always possible in obese patients. Endoscopic ultrasonography offers a better visualisation of the pancreas, however it is an invasive method, and is once again operator dependent. It is still not recommended to make a diagnosis solely on this method and

alternatives like computed tomography (CT) or magnetic resonance imaging (MRI) should be used (Dite et al., 2020; Shah et al., 2019). It is important to note that although CT and MRI are preferred methods, they too have limitations such as higher costs and exclusions of patients who suffer from certain conditions such as kidney disease. MRI is currently the best method for the diagnosis of fatty pancreas due to it's high sensitivity and safety, its results have been found to be comparable with those retrieved from histological examination (Hannukainen et al., 2011).

Pancreatic volume is thought to be a holistic measurement relating to the size of the pancreas, however variations in volume are often indicative of a number of conditions, such as T2DM and obesity (DeSouza et al., 2018). It has been suggested that age and adiposity also have an effect on pancreatic volume but literature is limited (Saisho et al., 2007). Another significant variable which impacts pancreatic volume is Body Mass Index, as individuals who were obese reported higher pancreatic volumes in comparison to lean individuals, while T2DM causes a decrease in pancreatic volume (DeSouza et al., 2018). Due to BMI having a significant impact on pancreatic volume, in this study the associations between pancreatic volume and visceral fat will be done while controlling for BMI, as to indicate possible fatty infiltration in the pancreas.

When looking at the pancreas, it is crucial to remember that even if a fatty pancreas is visualized using one of the imaging techniques mentioned above, it does not mean that the patient has NAFPD. In order for the patient to be diagnosed with NAFPD, it needs to fit the case definition of it being a disease associated with MetS and that the fat infiltration in the pancreas happened in the absence of other conditions which lead to fatty replacement (Grundy, n.d.; Pinte et al., 2019).

Aortic IMT

Similarly, to pancreatic steatosis, aIMT is visualized using sonography. It is safe, non-invasive, inexpensive compared to its counter parts and can be easily performed. B-mode ultrasonography

is the most widely accepted method for the detection of subclinical atherosclerosis (Schäberle, Leyerer, Schierling, & Pfister, 2015). The use of sonography to measure aIMT has not only enabled the early detection of atherosclerosis but also the study of cardiovascular risk factors on aIMT in both children and adolescents (Skilton et al., 2019). Due to NAFPD and Atherosclerosis being lifestyle diseases, indications around malnutrition and obesity are useful. Since aIMT has been found to be associated with hip to waist ratio and BMI, anthropometry could be used as an indicator of disease.

Anthropometry

Anthropometry has been a tool for clinical practices since the 19th century and manifested in the measurements of weight, height, circumference and skinfold thickness which were used to identify influences that affected child growth (Utkualp & Ercan, 2015). When looking at NCDs anthropometry is used to screen individuals who would most likely be affected by the disease for referral to various imaging techniques to confer diagnosis (Maffeis et al., 2011).

Anthropometric measurements used in screening include body mass index (BMI), weight, height, skinfold thickness, hip to waist ratio and mid-upper arm circumference (WHO, 1995). In a study conducted by Brambilla *et al*, anthropometry was found to be a good predictor of abdominal adiposity in comparison to MRI as waist circumference can predict VAT and BMI can predict SAT explaining 64% and 90% of its variance respectively (Brambilla et al., 2006). Studies have also found that maternal anthropometry can be useful for predicting low birth weight babies using certain cut-offs in measurements (Pengoriaet al., 2019). Both of these are valuable as adiposity as well as low birth weight were previously identified as NCD risk factors.

According to WHO, when conducting anthropometric measurements on children, indices are derived by comparing the weight and height of a child to pre-established reference curves and

these serve as an indication of conditions such as malnutrition (WHO, 1995). These indices include height for age and weight for age. When looking specifically at aIMT, a study conducted by Gomez-Roig *et al.* found that foetuses who were growth restricted (with a fetal weight lower than the 10th percentile) had a thicker aIMT compared to those who were appropriate for gestational age, indicating possible preclinical atherosclerosis (Gomez-Roig et al., 2015).

2.7 Intrauterine exposure

The developmental origins of health and disease hypothesis (DOHaD) proposes that exposures in-utero may result in persistent adaptations including alterations in metabolism (Harris, Willett, Michels, & Institutet, 2014). It has been suggested that a maternal obesogenic environment contributes to obese offspring as well as MetS (Whitaker, 2004). A study looking at the role of LDL oxidation in the accumulation of monocytes in the fetal aorta has found that these lesions, indicating preclinical atherosclerosis, are significantly enhanced by maternal hypercholesteremia, once again highlighting the effect conditions in-utero have on the health of offspring (Napoli et al., 1997). When the growth or structure of an embryo is altered by exposure to a certain agent, these agents are referred to as human teratogens (Alwan & Chambers, 2015). These agents range from maternal illnesses such as Rubella, which cause heart malformations and deafness, to ingested substances such as Thalidomide which caused birth defects (Gregg, 1991; Somers, 1962). The effects of more common teratogens, nicotine and alcohol, will be explored below with special focus on their role in CVD pathogenesis.

2.7.1 Nicotine

Maternal smoking during pregnancy remains a great public health concern and nicotine-exposed children are more likely to have numerous health issues, not just at birth but throughout their lives, affecting the respiratory, neurologic and cardiovascular systems (Holbrook, 2016). The foetus is

protected from environmental toxins including the chemicals found in tobacco smoke through not only maternal and placental metabolism, but by the liver of the foetus itself (O'Shaughnessy, Monteiro, Bhattacharya, & Fowler, 2011). However, the liver starts to form in the fourth week of gestation and receives 70% of its blood supply directly from the placenta via the umbilical vein which results in the foetal liver being exposed to high concentrations of maternally derived xenochemicals (Krauer & Dayer, 1991; O'Shaughnessy et al., 2011).

A study conducted by Gunes *et al.* aimed to look at the effect of nicotine on aIMT in neonates, and it was found that neonates whose mothers smoked have significantly increased aIMT, indicating an increased risk of atherosclerosis in adult life (Gunes et al., 2007). In the same study it was found that birth weight was negatively associated with aortic IMT, indicating thicker aIMT in low birth weight babies. Irrespective of diet, studies have found a higher prevalence of obesity in children of mothers who have smoked during pregnancy as infants are born with a lower birth weight, increasing the risk of obesity in adult life (Kries, Toschke, Koletzko, & Slikker, 2002). An increased risk of obesity isn't the only potential lifelong consequence of maternal smoking as a study done by Karin *et al.* found that smoking during pregnancy is strongly associated with aortic narrowing in adolescence (Anna-Karin, JohanBengtsson, Nagy, De Keyzer, & Norman, 2008).

When looking at the effect of nicotine on the fetal pancreas with regard to fetal growth restriction, a study conducted among mothers who smoked more than 10 cigarettes per day while pregnant found that endocrine pancreatic dysfunction was not a major contributing factor to nicotine-associated fetal growth restriction (Lockhart et al., 2017). This, however, is not to suggest that nicotine has no significant effects on the pancreas. A study conducted by Somm *et al.* on rat pups found that prenatal exposure to nicotine resulted in a decrease in islet size, resulting in a decrease in gene expression of certain factors and hormones such as insulin and glucagon (Somm et al.,

2008). In a different study done on Wistar rats, structural abnormalities in the mitochondria were observed beginning at 3 weeks and progressing with age, usually preceding glucose intolerance in nicotine exposed rats (Bruin et al., 2008). Lastly, maternal smoking is also associated with a lack of appetite in expecting mothers, resulting in poor nutrition and energy intake, resulting in offspring being small for gestational age (Slotkin, 1998).

2.7.2 Alcohol

With regards to maternal alcohol consumption, most of the research is focused on the structural and biochemical effects on the fetal brain, which are consequently believed to affect cognitive ability and behaviour in the long term (Jacobson & Jacobson, 1994). Studies have found that Ethanol causes apoptosis of neural crest cells, which are cells which differentiate into several cell types such as neurons and mesenchymal cells, which play a role in tissue repair and regeneration (Delfino-Machín, Chipperfield, Rodrigues, & Kelsh, 2007; Srinivasan, Fults, Supronowicz, Esquivel, & Zamilpa, 2019). Ethanol is a teratogen which often causes these effects and it is collected under the umbrella of foetal alcohol spectrum disorder (FASD) (Burd, Blair, & Dropps, 2012). Intrauterine exposure to alcohol affects other areas of the human body as well, with both animal and human studies suggesting that alcohol affects the development of the immune system and offspring are at an increased risk of infection and disease in comparison to their non-alcohol exposed counterparts (Gauthier, 2015). Numerous studies have been done to support the notion that a high alcohol intake during pregnancy is detrimental to fetal organs, especially in the cardiovascular system restricting the growth of vital organs, however a study investigated the effects on a relatively low alcohol intake during pregnancy in rats and found that although the damage caused by the teratogen was not seen immediately as fetal cardiac gene expression had stayed the same, but after 8 months there was a significant increase in left ventricular wall

thickness during diastole in ethanol-exposed pups as well as a significant increase in fibrosis(Nguyen et al., 2014). This indicates that low amounts of ethanol can still be detrimental to long-term cardiac health.

While pre-natal ethanol exposure is thought to affect organs such as the brain, liver and heart more severely, a study conducted among guinea pig offspring found that exposure to ethanol had resulted in numerous insults, including increased adipocyte infiltration into the pancreas in adulthood and had decreased insulin-like immunoreactivity (Dobson et al., 2012). An increase in fatty infiltration in the pancreas could inhibit proper pancreatic function, increasing the risk of developing T2DM. While there are several animal models investigating the adverse effects of maternal drinking on the foetus, more research is needed looking at the effects on fatty infiltration in the pancreas in humans.

2.8 Treatment and prevention

NAFPD has recently been added as a new entity to MetS and because of this, research with regards to the most effective treatment is limited but because the main cause of pancreatic fatty infiltration is obesity, it is thought that weight loss could be the simplest way to reduce fat content in the pancreas (Pinte et al., 2019). Reducing weight by consuming a low fat diet and daily exercise are thought to work as a treatment for fatty pancreas by increasing the number of functionally competent insulin receptors (McCarty, 1998). Pharmacological therapies include the use of oral hypoglycaemic agents such as *Metaformin* and *Troglitazone. Troglitazone* acts by suppressing the inflammatory changes in the pancreas and has been found to reverse fibrosis and inflammatory cell infiltration, however the first derivative of the drug was withdrawn in 2000 due to its negative effects on the liver (Jia, Fukumitsu, Tabaru, Akiyama, & Otsuki, 2001).

Conditions which fall under cardiovascular disease often share the same risk factors and because of this, treatment should be aimed at reducing these risk factors in order to decrease the severity of the illness or even reverse disease progression. These risk factors include smoking, diabetes mellitus, presence of plaque and low density lipoprotein (Martinsson et al., 2014). Changes made to one's lifestyle to include a balanced diet, exercise and a reduction in smoking and alcohol consumption is the simplest way to reduce the risk of CVD. Due to the first signs of preclinical atherosclerosis appearing *in utero*, it is vital that the approach toward CVD prevention is a lifelong one, with efforts beginning in childhood (Hayman et al., 2007).

This study aims to not only ascertain if there is an association between intrauterine alcohol and nicotine exposure on the development of fatty pancreas and aortic IMT in five year old children, but also to highlight the consequences of participating in a high-risk lifestyle in relation to disease incidence while acting as a screening for children who are at risk of developing NCDs. Early detection enables treatment and a slower disease progression.

Chapter 3: Research Design and Methodology

3.1 Ethical approval

The Biomedical Research Ethics Committee (BMREC) at the University of the Western Cape and the health and Research Ethics Committee (HREC) at Stellenbosch University provided ethical clearance prior to the study taking place. Clearance was granted based on the research proposal submitted. Participation in the study is completely voluntary and individuals had the right to withdraw at any time. Prior to ultrasonography and anthropometry, participants as well as their guardians were given an information leaflet providing insight into the study as well as the relevant contact information should they require more information. As the participants are too young to legally consent to participation, informed assent was ascertained from the children prior to testing.

3.2 Study Design and Selection of participants

The study was conducted at The University of Stellenbosch in the Department of Obstetrics and Gynaecology at Tygerberg Hospital, Cape Town over an 18 month period. The participants selected came from a database used in a previous study, in which maternal data was collected during antenatal clinic visits in Belhar and at Bishop Lavis Midwife Obstetric unit. Women who were booking for antenatal care were invited to participate in the study. The Safe Passage Study aimed to investigate the role of maternal drinking and smoking on Sudden Infant Death Syndrome. Apart from age and gender, the inclusion criteria set includes individuals who are free of physical or mental abnormalities and those who are not on medication or any treatment for cardiovascular disease or diabetes.

3.3 Overview of Paediatric Assessments

3.3.1 Anthropometry

Anthropometric measurements taken included weight, height, body mass index (BMI), triceps and subscapular skinfold thickness, and waist to hip ratio (WHR). These measurements served as an indication of obesity and nutritional status. The protocol which was followed during the anthropometric data collection is as stated in the *National Health and Nutrition Examination Survey (Nhanes): Anthropometry Manual*.

Body weight was measured with a digital scale in kilograms and participants were asked to remove shoes during the weighing process. Height was measured using a mechanical stadiometer. In order to measure skinfold thickness, a Holtain calliper was used to take subscapular and triceps skinfold measurements. The triceps skinfold was measured at the upper arm mid-point mark on the posterior surface of the right upper arm while the subscapular skinfold was measured on the left side of the body, 20mm below the tip of the scapula at an angle of 45°, to the nearest 0.1mm while the fingers continued to hold the skinfold. Measurement readings were taken 3 seconds after the calliper was released.

The anthropometry protocol calls for two circumference measures as participants are older than 2 months, such as arm and abdominal, or waist circumference. The arm circumference is measured on the right arm at the level of the upper arm mid-point mark using a measuring tape, while the waist circumference is measured at midpoint between the last rib and the iliac crest using a tape measure. Participants were measured 3 times and mean values were calculated from these measurements. All measurements, except skinfolds, were taken to the nearest tenth of a centimetre or 1.0 millimetre. Skinfold measurements were taken to the nearest 0.1 millimetre.

3.3.2 Blood Pressure

Patients were asked to remain still for a minute before readings took place. A validated CAS 740 MAXNIBP automated digital sphygmomanometer was used to record systolic diastolic blood pressure and mean arterial pressure while patients sat in an upright position. Readings were taken using a size-appropriate blood pressure cuff fitted on the right upper arm. Heart rate was recorded as well and each reading was taken 3 times.

3.3.3 Ultrasonography

Pancreas

Abdominal ultrasounds were performed on each child by a qualified sonographer using a Voluson E8 ultrasound machine (GE Healthcare). Measurements were taken with the child in the supine position and fasting approximately 4 hours prior to the examination to prevent lack of visibility due to gas. Some children were asked to turn into an oblique or prone position in cases with poor visualization of the pancreas. Imaging of the pancreas was attained on held inspiration or in some cases with the abdomen extended. The head, body and tail of the pancreas were measured with the transducer 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 mediolaterally and anteroposteriorly whereas the body and tail were measured anteroposteriorly only.

aIMT

Measurements of Aortic intima-media thickness were also taken using the Voluson E8 ultrasound machine, according to the standardized IMT protocol as listed in the updated Mannheim Carotid Intima-Media Thickness and Plaque Consensus (Touboul et al, 2012). Similarly, to the pancreas,

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, followed by placement in the midline, inferior to the xiphoid, for superior visualization of the abdominal aorta intima-media thickness (aIMT).

Adiposity

Visceral adipose tissue was measured using peritoneal maximum (Pmax) as an indication thereof, it was measured from 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) and minimum thickness of subcutaneous fat layer (S) and refers to the abdominal wall thickness.

3.4 Maternal assessments

Mothers were asked to complete a questionnaire in order to gather information on pregnancy history, alcohol and tobacco use and nutrition. Anthropometric measurements collected on the mothers of participating children included Body Mass Index (BMI) and Mid upper arm circumference (MUAC).

3.5 Statistical analysis

The statistical package used was SPSS (Statistical Package for the Social Sciences, version 26). Quantitative data was described as the means along with SD, minimum and maximum values with 95 % confidence intervals (CI). Pancreatic volume and size were used as a possible indication of fatty infiltration in the pancreas. Pancreatic fat remains proportional to pancreas parenchyma volume until fifty years of age (Yoshifumi Saisho, 2016). Pancreatic volume is also useful as it

can be indicative of other disease states such as Diabetes in which a reduced pancreatic volume will be seen, or obesity which causes an increased pancreatic volume (DeSouza et al., 2018).

For certain analyses the exposure groups were collapsed into dual and alcohol exposure, as well as dual and nicotine exposure in order to identify the effects of nicotine and alcohol respectively. Independent t-tests were done to compare pancreatic volumes, aIMT and visceral adiposity among these groups. One-way analyses of variance (ANOVA) was used to evaluate the differences in child anthropometric and adiposity variables among *in utero* exposure groups. Post hoc Tukey/LSD comparisons was used to identify group differences. Pearson correlation was used to identify possible linear relationships between anthropometric and adiposity variables with aIMT. It was also used to identify a relationship between aIMT and pancreatic variables. Due to the effect of BMI on pancreatic size, partial correlations were done controlling for BMI. Chi square analysis was conducted for categorical variables to explore the likelihood of children with pre-/hypertension, high aIMT or high pancreatic volumes to fall in the exposure groups. Although literature around standardized pancreatic cut-offs are limited, dimensions of the pancreas within the 5^{th} to 95^{th} percentile are considered normal (Raut, Raje, Dandge, & Singh, 2018). Increased or abnormal aIMT was classified as an aIMT above the 95^{th} percentile (Skilton et al., 2019).Pre-hypertension was classified between the 90^{th} to less than 95^{th} percentile, and stage 1 hypertension as $>95^{th}$ percentile (Falkner & Daniels, 2004). BMI was classified into three categories being normal, overweight, and obese based on the gender and age specific cut-offs provided by the World Health Organisation (WHO). Data was analysed per exposure group. The exposure groups consisted of four groups according to intrauterine teratogen exposure, namely, alcohol only, nicotine only, dual exposure (being exposed to both alcohol and nicotine) and lastly, the control. Statistical significance was set at $p < 0.05$.

Chapter 4: Results

Table 1: Paediatric anthropometric characteristics at age 5 years according to the four exposure groups.

	Controls	Dual exposed	Alcohol exposed	Smoking exposed	p value	F	dF
	N=147	N=154	N=33	N=167			
Weight	18.69±3.58	18.16±2.71	17.82±2.74	17.70±2.3	0.21	3.29	3
BMI	15.35±2.07	15.21±1.47	15.09±1.75	15.07±1.37	0.48	0.82	3
WC	51.93±5.20	51.43±3.93	51.22±3.70	50.66±3.53	0.08	2.27	3
SubSFT	7.66±4.24	7.03±1.89	7.61±3.85	6.92±2.33	0.12	1.97	3
TriSFT	10.14±3.77	9.38±2.65	9.64±4.38	9.34±2.71	0.11	2.03	3

BMI – Body Mass Index, WC – waist circumference, SubSFT - subscapular skinfold thickness, TriSFT – Triceps skinfold thickness. Significant values ($p<0.05$) are indicated in bold.

Table 1 shows the study cohort divided into exposure groups to assess mean differences between paediatric anthropometric measurements among the groups. Differences among these characteristics are not significant at this time.

Table 2: Paediatric sonographic characteristics of the aorta and pancreas at age 5 years according to the four exposure groups

	Controls	Dual exposed	Alcohol exposed	Smoking exposed	p value	F	dF
	N=141	N=151	N=33	N=164			
Pancreatic Measurements							
Phead	18.26±2.58	17.85±2.55	18.44±2.75	17.58±2.5	0.077	2.29	3
Pbody	7.16±1.58	7.04±1.60	7.24±1.69	6.69±1.5	**0.037***	2.86	3
	N=27	N=31	N=8	N=34			
Pancreatic volume	13.33±5.21	14.64±5.68	12.75±3.97	13.84±4.82	0.715	0.46	3
aIMT Measurements							
	N=146	N=153	N=33	N=164			

aIMT	0.54±0.08	0.54±0.09	0.55±0.10	0.52±0.09	**0.049***	2.64	3

Phead – Pancreas head, Pbody – Pancreas body, aIMT – aortic intima-media thickness,
Significant values ($p<0.05$) are indicated in bold.

In Table 2 pancreatic and aortic mean measurements are compared among exposure groups. Pancreatic measurements consist of the size of three regions of the pancreas, namely, the head, body and tail. Aortic measurements consist of aortic intima-media thickness. There was a significant difference (p=0,037) in pancreatic body (Pbody) size among the groups, with participants exposed to nicotine in-utero having the smallest pancreatic body size (6,69) and alcohol exposed participants the largest (7,24).

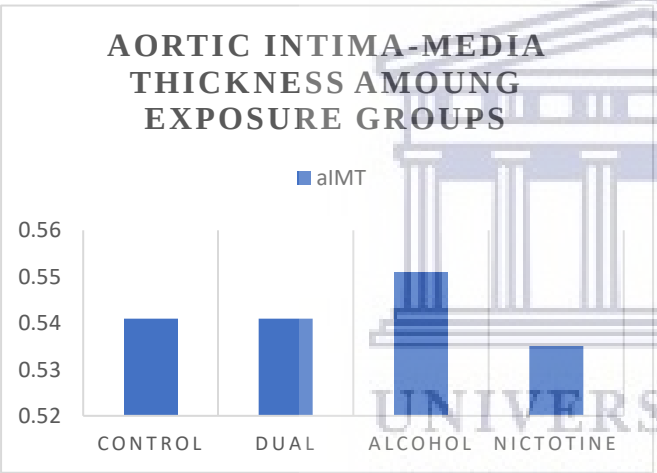

Figure 1. Mean values for aortic intima-media thickness (mm) according to the four exposure groups.

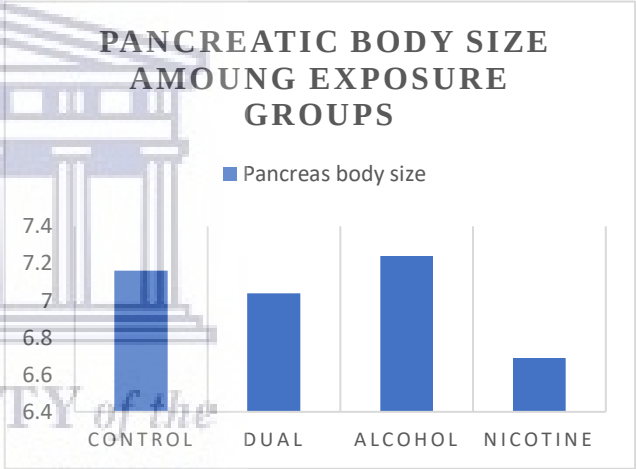

Figure 2. Mean values for pancreatic body size (mm) according to the four exposure groups.

Table 3: Paediatric clinical measurements at age 5 years according to the four exposure groups

	Controls	Dual exposed	Alcohol exposed	Smoking exposed	p value	F	dF
	N=146	N=154	N=33	N=167			
Clinical Measurements							
SBP	106.49±10.66	107.15±10.02	105.76±11.72	104.27±9.33	0.07	2.41	3
DBP	65.25±9.07	65.75±9.05	65.64±11.41	63.88±9.01	0.29	1.24	3
MAP	78.84±9.48	79.45±9.28	79.28±11.33	77.29±9.30	0.20	1.55	3
HR	91.72±12.43	92.47±14.42	91.79±8.68	91.35±13.27	0.90	0.20	3

HR-heart rate, BMI - body mass index, SBP - systolic blood pressure, DBP- diastolic blood pressure, MAP - mean arterial pressure

Table 3 indicates the differences in means among clinical measurements such as systolic and diastolic blood pressure mean arterial pressure and heart rate across exposure groups. There are no significant differences between the exposure groups at this age.

	Controls	Dual exposed	Alcohol exposed	Smoking exposed	p value	F	dF
	N=146	N=153	N=33	N=166			
Adiposity measurements							
Pmax	3.89±1.47	3.48±0.11	1.29±0.23	3.57±0.10	**0.04***	2.84	3
P/S Ratio	0.48±0.41	0.54±0.44	0.57±0.10	0.55±0.48	0.54	0.73	3

Table 4: Paediatric adiposity characteristics at age 5 years according to the four exposure groups

Pmax – subcutaneous fat, P/S Ratio – visceral fat

Mean values for subcutaneous and visceral fat are compared among exposure groups in Table 4, with a significant difference found (p=0.04) between groups when looking at subcutaneous fat. The control group has the largest mean subcutaneous fat measurement (3.89) while participants exposed to alcohol *in utero* having the lowest (1.29). A graphical representation of this can be seen in Figure 3 and Figure 4 below.

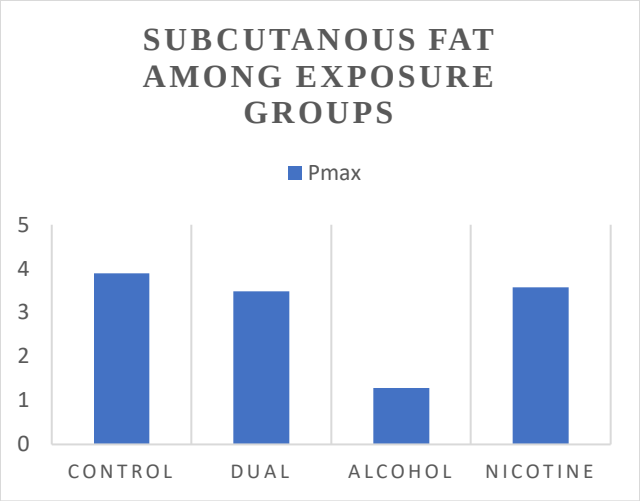

Figure 3. Mean values for subcutaneous fat (Pmax) (mm) according to the four exposure groups.

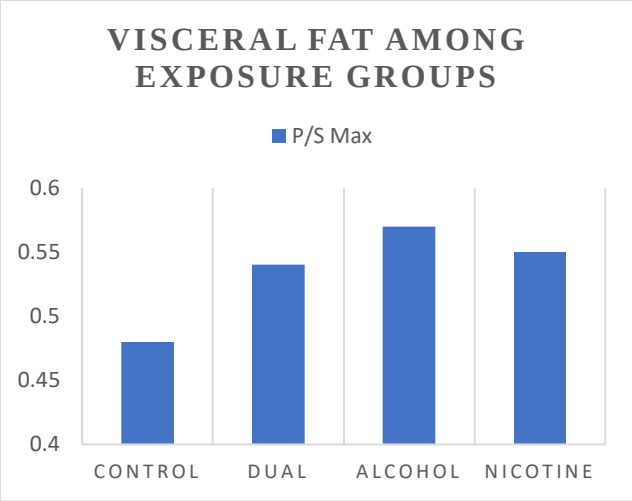

Figure 4. Mean values for Visceral fat (P/S Max) (mm) according to the four exposure groups.

Table 5: Associations between child aortic intima-media thickness and child adiposity anthropometric measurements expressed as Pearson's correlation coefficients (r).

	aIMT		
	r	p	n
Weight	0.21	**p<0.01***	496
BMI	0.21	**p<0.01***	496
TriSFT	0.71	0.12	497
SubSFT	0.15	**P<0.01***	496
WC	0.23	**p<0.01***	466

In Table 5 Pearson correlation is used to identify associations between participant aortic IMT and child anthropometric measurements. Significant associations ($p<0.01$) were seen between participant weight, BMI and waist circumference with aortic IMT. A significant positive association was also seen between subscapular skinfold thickness and aortic IMT.

Table 6: Associations between child aortic intima-media thickness and child clinical measurements expressed as Pearson's correlation coefficients (r).

	aIMT		
	r	p	n
SBP	0.42	0.35	495
DBP	0.04	0.33	495
MAP	0.03	0.51	495
HR	0.05	0.31	495

Table 6 looks at possible associations using Pearson correlation between systolic, diastolic and mean arterial pressure as well as heart rate to aortic IMT. While there are positive correlations seen between these variables and aortic IMT, none of the associations are significant.

Table 7: Associations between child aortic intima-media thickness and child pancreatic measurements expressed as Pearson's correlation coefficients (r).

	aIMT		
	r	p	n
Phead	0.22	**p<0.01***	490
Pbody	0.25	**p<0.01***	490
Ptail	0.19	**p<0.01***	490
Pancreatic volume (cm^3)	-0.08	0.44	100

When looking at associations between pancreatic variables and aortic IMT, Table 7 indicates significant ($p<0.01$) positive associations between pancreatic head, body and tail size with aortic IMT. Although currently weak and not significant, a negative association was seen between pancreatic volume and aortic IMT ($r=-0.08$).

Table 8: Associations between child aortic intima-media thickness and child adiposity measurements expressed as Pearson's correlation coefficients (r).

	aIMT		
	r	p	n
Pmax	0.19	**p<0.01***	498
P/S Ratio	0.19	0.697	498

Table 8 indicates possible associations between subcutaneous and visceral fat with aortic IMT, using Pearson correlations. A significant ($p<0.01$), although weak correlation ($r=0.19$) was seen between subcutaneous fat (Pmax) and aortic IMT.

Table 9: Associations between child adiposity and child anthropometric measurements expressed as Pearson's correlation coefficients (r).

	Pmax			**P/S Ratio**		
	r	p	n	r	p	n
Weight	0.41	**p<0.01***	498	0.44	**p<0.01***	498
BMI	0.42	**p<0.01***	498	0.53	**p<0.01***	498
TriSFT	0.30	**p<0.01***	499	0.54	**p<0.01***	499
SubSFT	0.36	**p<0.01***	498	0.58	**p<0.01***	498
WC	0.44	**p<0.01***	467	0.51	**p<0.01***	467

Correlations between participant anthropometric measurements and adiposity (subcutaneous and visceral fat) are seen in Table 9. While all anthropometric measurements are significantly ($p<0.01$) associated with subcutaneous and visceral fat, some associations are stronger than others. BMI, tricep and subscapular skinfold thickness have a moderate association ($r>0.5$) to visceral fat, while subcutaneous fat had a weaker association ($r<0.5$) to these variables.

Table 10: Associations between pancreatic measurements and child anthropometric measurements expressed as Pearson's correlation coefficients (r).

	Phead			**Pbody**			**Ptail**			**Pancreas Volume**		
	r	p	n	r	p	n	r	p	n	r	p	n
Weight	0.24	**P<0.01***	489	0.13	**.005***	489	0.18	**p<0.01***	489	-0.09	0.39	100
BMI	0.26	**p<0.01***	489	0.13	**.004***	489	0.17	**p<0.01***	489	-0.13	0.21	100
TriSFT	0.18	**p<0.01***	490	0.16	**.001***	459	0.09	**.048***	490	-0.12	0.24	100
SubSFT	0.15	**.001***	489	0.11	**.015***	489	0.07	.102	489	-0.11	0.27	100
WC	0.29	**p<0.01***	459	0.16	**.001***	459	0.22	**p<0.01***	459	-0.09	0.37	96

Table 10 shows possible associations using Pearson correlation between pancreatic variables and anthropometric measurements. Pancreatic measurements consist of pancreatic head, body and tail measurements as well as pancreatic volume. Anthropometric measurements were all significantly associated with pancreatic head and body size, while only subscapular skinfold thickness was not significantly associated with pancreatic tail size. It is important to note however that these are weak positive associations ($r<0.4$). Although not currently significant, a negative association exists between anthropometric measurements and pancreatic volume.

Table 11: Associations between child pancreas size and child pancreatic volume expressed as Pearson's correlation coefficients (r).

	Pancreatic Volume		
	r	p	n
Phead	0.20	**0.04***	100
Pbody	0.29	**0.004***	100
Ptail	0.41	**p<0.01***	100

In Table 11, associations between pancreatic size and pancreatic volume are shown using Pearson correlation. Although weak, pancreatic tail size shows the largest significant correlation (r=0.41, p<0.01) to pancreatic volume. Both pancreatic head and body size are significantly associated with pancreatic volume.

Table 12: Associations between child pancreatic variables (size and volume) and child adiposity measurements expressed as Pearson's correlation coefficients (r).

	Pmax			**P/S Ratio**		
	r	p	n	r	p	n
Phead	0.09	**0.04***	491	0.64	0.16	491
Pbody	0.07	0.124	491	-0.91	0.91	491
Ptail	0.20	0.654	491	0.09	**0.05***	491
Pancreatic Volume	-0.05	0.61	100	-0.16	0.12	100

Possible associations between pancreatic measurements and volume with adiposity (subcutaneous and visceral fat) are seen in Table 12. Pancreatic head size was found to have a weak significant (r=0.09 p=0.04) association with subcutaneous fat while pancreatic tail size was significantly associated with visceral fat (p=0.05).

Table 13: Associations between child adiposity and child clinical measurements expressed as Pearson's correlation coefficients (r).

	Pmax			**P/S Ratio**		
	r	p	n	r	p	n
SBP	0.17	**p<0.01***	497	0.09	**0.04***	497
DBP	0.21	**p<0.01***	497	0.11	**0.01***	497
MAP	0.21	**p<0.01***	497	0.08	**0.03***	497
HR	0.08	0.07	497	0.10	**0.03***	497

Table 13 explores associations between clinical measurements and adiposity. Systolic, diastolic and mean arterial pressure are significantly ($p<0.01$) associated with subcutaneous fat while heart rate is not. Visceral fat has a weak but significant association to all of the above mentioned clinical measurements.

Table 14: Partial correlation between child pancreas size and child pancreatic volume expressed as Pearson's correlation coefficients (r) while controlling for BMI.

	Pancreatic Volume		
	r	p	df
Phead	0.25	**0.01***	97
Pbody	0.35	**p<0.01***	97
Ptail	0.46	**p<0.01***	97

In order to investigate whether associations previously seen were due to participant size, Table 14 looks at associations between pancreatic size and volume while controlling for BMI. Pancreatic head, body and tail were still significantly associated with pancreatic volume, with pancreatic tail size having the strongest association to volume (r=0.46).

Table 15: Partial correlation between child pancreas size and child adiposity expressed as Pearson's correlation coefficients (r) while controlling for BMI.

	Pmax			**P/S Ratio**		
	r	p	df	r	p	df
Phead	0.10	0.317	97	-0.16	0.112	97
Pbody	0.16	0.114	97	-0.28	**0.004***	97
Ptail	0.03	0.802	97	0.05	0.633	97
Pancreatic Volume	0.02	0.88	97	-.012	0.296	97

Table 15 indicates possible associations between subcutaneous and visceral fat with pancreatic size and volume while controlling for BMI, using partial correlations. A negative correlation between visceral fat and pancreatic volume was still present, however not significant. Visceral fat was found to have a significant negative correlation (r=-0.28 p=0.004) with pancreatic body size.

Table 16: Partial correlation between child pancreas size and child aortic intima-media thickness expressed as Pearson's correlation coefficients (r) while controlling for BMI.

	aIMT		
	r	p	df
Phead	0.17	**p<0.01***	485
Pbody	0.23	**p<0.01***	485
Ptail	0.16	**p<0.01***	485

Pancreatic size are still significantly (p<0.01) associated with aortic IMT, after controlling for BMI in Table 16. Associations are weak (r<0.4), with pancreatic body size having the largest association to aortic IMT (r=0.23).

Table 17: Partial correlation between pancreatic measurements and child anthropometric measurements expressed as Pearson's correlation coefficients (r).

	Phead			**Pbody**			**Ptail**		
	r	p	df	r	p	df	r	p	df
Weight	.081	0.83	456	0.04	0.42	456	0.07	0.135	456
TriSFT	-0.01	0.82	456	-0.03	0.49	456	-0.03	0.47	456
SubSFT	-0.03	0.49	456	0.02	0.67	456	-0.06	0.21	456
WC	0.13	**0.005***	456	0.08	0.104	456	0.140	**0.003***	456

Table 17 looks at possible associations between anthropometric measurements and pancreatic size while controlling for BMI, using partial correlations. While not significant, tricep and subscapular skinfold thickness were found to have a negative association to pancreatic head, body and tail size, while waist circumference had a weak but significant (r=0.13 p=0.005) association to pancreatic head and tail size (r=0.140 p=0.003).

Table 18: Comparison of means among exposure groups and child anthropometric, adiposity and ultrasound measurements using the independent T-test (t)

	Exposure	**N**	**Mean**	**SD**	**t**	**p**
Anthropometric Measurements						
BW	Control	147	3.06	0.52	1.106	0.269
	Alcohol ±Dual	187	2.99	0.57		
	Control	151	3.06	0.52	1.443	0.150
	Smoking ± Dual	316	2.98	0.58		
BMI	Control	147	15.35	2.07	0.782	0.435
	Alcohol ±Dual	187	15.19	1.52		

	Control	151	15.32	2.05	0.948	0.344
	Smoking ± Dual	317	15.15	1.42		
Adiposity						
Pmax	Control	146	3.89	1.47	0.233	**0.007**
	Alcohol ±Dual	186	3.47	1.33		
	Control	150	3.90	1.48	2.667	**0.008**
	Smoking ± Dual	315	3.52	1.28		
P/S Ratio	Control	146	0.48	0.41	-1.327	0.185
	Alcohol ±Dual	186	0.55	0.47		
	Control	150	0.48	0.41	-1.559	0.120
	Smoking ± Dual	315	0.55	0.47		
Ultrasound measurements						
aIMT	Control	146	0.54	0.08	-0.251	0.802
	Alcohol ±Dual	186	0.54	0.87		
	Control	150	0.54	0.79	1.783	0.075
	Smoking ± Dual	313	0.53	0.09		
Pancreatic						
Volume	Control	27	13.33	5.22	-0.692	0.491
	Alcohol ±Dual	39	14.25	5.38		
	Control	27	13.33	5.22	-0.744	0.459
	Smoking ± Dual	65	14.22	5.22		

Independent T tests were used in Table 18 to investigate possible significant differences between means in different exposure groups. Exposure groups were collapsed and Alcohol and Dual exposure was grouped together as well as Nicotine and Dual exposure in order to isolate which teratogen, alcohol or nicotine had a larger effect on anthropometric measurements, adiposity and ultrasound measurements.

Table 19: Chi-Square analysis conducted between aortic intima-media thickness and exposure groups.

	Control		**Alcohol+ Dual Exposure**		**Chi- Square**	**dF**	**Sig.**
Normal aIMT	Count	138	Count	171	0.848	1	0.357
	Expected	135.9	Expected	173.1			
Increased aIMT	Count	8	Count	15			
	Expected	10.1	Expected	12.9			
	Control		**Smoke+ Dual Exposure**		**Chi- Square**	**dF**	**Sig.**
Normal aIMT	Count	140	Count	296	0.282	1	0.596
	Expected	141.3	Expected	294.7			
Increased aIMT	Count	10	Count	17			
	Expected	8.7	Expected	18.3			

Table 19 explores possible dependence/ association between teratogen exposure and increased aortic IMT using Chi-square analysis. Increased aortic IMT was found to be independent of teratogen exposure.

Table 20: Fisher's exact test (2 cells have a count <5) conducted between pancreatic volume and exposure groups.

	Control		**Alcohol+ Dual Exposure**			**Sig.**
Normal pancreatic volume	Count	26	Count	36		0.639
	Expected	25.4	Expected	36.6		
Increased pancreatic volume	Count	1	Count	3		
	Expected	1.6	Expected	2.4		
	Control		**Smoke+ Dual Exposure**		**dF**	**Sig.**
Normal pancreatic volume	Count	26	Count	61	1	1.000
	Expected	25.5	Expected	61.5		
Increased pancreatic volume	Count	1	Count	4		
	Expected	1.5	Expected	3.5		

Due to not meeting requirements for Chi-Square analysis, Fisher's exact test was used in Table 20 to ascertain if there is an association between teratogen exposure and increased pancreatic volume. At this stage, there was no association found between increased pancreatic volume and intrauterine exposure to alcohol and nicotine.

Table 21: Chi-Square analysis conducted between BMI and exposure groups.

	Control		**Alcohol+ Dual Exposure**		**Chi-Square**	**dF**	**Sig.**
Normal BMI	Count	128	Count	168	0.624	1	0.430
	Expected	130.3	Expected	165.7			
Increased BMI	Count	19	Count	19			
	Expected	16.7	Expected	21.3			
	Control		**Smoke+ Dual Exposure**		**Chi-Square**	**dF**	**Sig.**
Normal BMI	Count	132	Count	284	0.634	1	0.426
	Expected	134.5	Expected	281.5			
Increased BMI	Count	19	Count	32			
	Expected	16.5	Expected	34.5			

Table 21 investigates possible dependence between increased BMI and teratogen exposure using the Chi-Square analysis. No association was found between intrauterine exposure to alcohol and nicotine and increased BMI.

Table 22: Chi-Square analysis conducted between BMI and Aortic IMT.

	Normal BMI		**High BMI**		**Chi-Square**	**dF**	**Sig.**
Normal aIMT	Count	420	Count	45	15.968	1	**P<0.001***
	Expected	413.3	Expected	51.7			
Increased aIMT	Count	20	Count	10			
	Expected	26.7	Expected	3.3			

Increased BMI was found to be significantly ($p<0.01$) associated with increased aortic IMT in Table 22, using Chi-Square analysis.

Table 23: Chi-Square analysis conducted between child blood pressure categories (normal and prehypertensive) and exposure groups.

	Control		**Alcohol+ Dual Exposure**		**Chi- Square**	**dF**	**Sig.**
Normal BP (child)	Count	130	Count	164	0.060	1	0.806
	Expected	129.5	Expected	164.5			
Pre-hypertension	Count	7	Count	10			
	Expected	7.5	Expected	9.5			
	Control		**Smoke+ Dual Exposure**		**Chi- Square**	**dF**	**Sig.**
Normal BP	Count	134	Count	287	0.088	1	0.766
	Expected	134.6	Expected	286.4			
Pre-hypertension	Count	7	Count	13			
	Expected	6.4	Expected	13.6			

Chi-Square analysis was used in Table 23 to investigate if blood pressure states (normal or pre-hypertensive) are associated with teratogen exposure. Pre-hypertensive states were not associated with alcohol and nicotine intrauterine exposure at this time.

Table 24: Chi-Square analysis conducted between child blood pressure categories (normal and hypertensive) and exposure groups.

	Control		**Alcohol+ Dual Exposure**		**Chi- Square**	**dF**	**Sig.**
Normal BP (child)	Count	130	Count	164	0.091	1	0.763
	Expected	129.5	Expected	164.7			

Hypertensive	Count	9	Count	22			
	Expected	9.7	Expected	22.0			
	Control		**Smoke+ Dual Exposure**		**Chi- Square**	**dF**	**Sig.**
Normal BP	Count	134	Count	286	0.083	1	0.774
	Expected	134.7	Expected	285.3			
Hypertensive	Count	9	Count	26			
	Expected	8.3	Expected	26.0			

Chi-Square analysis was used in Table 24 to investigate if blood pressure states (normal or hypertensive) are associated with teratogen exposure. Hypertensive states were not associated with alcohol and nicotine intrauterine exposure at this time

Table 25: Fisher's exact test (1 cell has a count <5) conducted between child blood pressure categories (normal and prehypertensive) and maternal hypertension.

	Normal maternal BP		**Hypertensive mother**		**Sig.**
Normal BP (child)	Count	399	Count	17	0.275
	Expected	1397.3	Expected	18.7	
Pre-hypertension	Count	48	Count	4	
	Expected	49.7	Expected	2.3	

Table 25 investigates whether child blood pressure states (normal or pre-hypertensive) is associated with maternal blood pressure states (normal or hypertensive) using Fisher's exact test. Offspring blood pressure states were found not to be associated with maternal hypertension.

Table 26: Fisher's exact test (1 cell has a count <5) conducted between child blood pressure categories (normal and hypertensive) and maternal hypertension.

	Control		Smoke+ Dual Exposure		Sig.
Normal BP (child)	Count	398	Count	26	0.756
	Expected	399.0	Expected	25.0	
Hypertensive	Count	48	Count	2	
	Expected	47.0	Expected	3.0	

Table 26 investigates whether child blood pressure states (normal or hypertensive) is associated with maternal blood pressure states (normal or hypertensive) using Fisher's exact test. Offspring blood pressure states were found not to be associated with maternal hypertension

Chapter 5: Discussion and Conclusion

This chapter discusses the results with respect to the research objectives of the study and from this conclusions are drawn. Implications of the findings as well as recommendations for future research are included.

5.1 Discussion

It was hypothesized at the beginning of the study that children exposed to nicotine and alcohol will have an increased aIMT compared to the control, the results derived from the data show that those children who have been exposed to alcohol *in utero* have a larger aortic intima-media thickness in comparison to the other three groups (Table 2). While other studies looking specifically at the effect of *in utero* exposure to alcohol on foetal aIMT is limited, a study conducted by Nguyen *et al* investigating the effects of low to moderate maternal alcohol consumption on foetal cardiac structure in rats, found that at 8 months during diastole there were significant increases in the left ventricular anterior and posterior wall thickness in ethanol-exposed offspring. This is indicative of ventricular hypertrophy (Nguyen et al., 2014). Carotid intima-media thickness, which is indicative of atherosclerosis, was also found to be affected by alcohol consumption in a study conducted among men aged between thirty and seventy (Qu et al., 2015).

When looking at aortic intima-media thickness among the offspring exposed only to nicotine, it was the lowest among the groups (Figure 1). This is contrary to what has been seen in animal studies, as rat pups exposed to nicotine showed an increase in aIMT compared to the control (Gunes et al., 2011). However, this increase was seen to be dose dependent and the pups were forty-five days old, which indicates that the pups were in mid-adolescent stage, this could potentially explain the disparity in the animal model to the current study as the current population is younger, and this effect could possibly be seen later in life. Seeing that the offspring exposed to

both nicotine and alcohol have a greater aIMT than the nicotine only (Table 2), it could be suggested that intrauterine exposure to alcohol and nicotine has more of an effect on aIMT than nicotine alone, however offspring exposed to alcohol *in utero* still had the largest aIMT.

While there is no current, significant difference at five years old, to the weight of participants among the groups (Table 1), there is a significant difference in subcutaneous fat (Table 4). The control group had the largest peritoneal fat reading, indicating that possible external factors such as diet and inactivity could play an equally, if not more important role than the teratogens in question in the current study. In a similar study conducted among 35 participants aged 9-15 years, it was found that preperitoneal fat (Pmax) could be the most sensitive way to predict insulin resistance-associated metabolic derangements in children (Tamura et al.,2000).

In the current study, aIMT was found to be strongly associated with subcutaneous fat (Table 8) as well as pancreatic head size (Table 12). Using Chi-Square analysis, aIMT was also found to be significantly associated with BMI, indicating that increased aIMT was dependent on increased BMI (Table 22).

When looking at the indicator of visceral fat in the current study (Table 4), children born to mothers in the three exposure groups all have higher measurements in comparison to the control, with the highest being participants who were exposed to alcohol *in utero*. This finding is consistent with an animal study conducted on zebrafish, which found that adults who had undergone embryonic alcohol exposure had an increased visceral fat measurement (Weeks et al., 2020). Although the differences are not currently significant in this study population, it is suggested that a follow-up study be done when the children are older to see if the difference in visceral fat among exposure groups has persisted. A review on the literature around intra-abdominal adipose tissue, or visceral fat, and disease risk found that visceral fat has a positive correlation to insulin resistance in obese

pre-pubertal children and adolescents (Goran & Gower, 1999). In a study conducted by Staaf *et al* in 2017 among 116 adolescents, it was found that visceral fat is also associated with pancreatic fat fraction. The current study did not use MRI to investigate pancreatic fat fraction (PFF), instead it looked at pancreas size and volume using ultrasound, and there is still a significant correlation between pancreas tail size and visceral fat in this population (Table 12) (Staaf et al., 2017). Interestingly, another finding investigating pancreatic fat fraction in the above-mentioned study was that PFF was not associated with BMI. In contrast, using partial correlations and controlling for BMI, and, although not significant, visceral fat is negatively associated with pancreatic body size and pancreatic volume in the current population (Table 15). This finding is supported by a study investigating the relationship between pancreatic volume and regional body fat distribution among patients with T2DM, there was a significant inverse correlation between pancreatic volume and VAT (Oz et al., 2017). Patients with T2DM were reported to have a decreased pancreas volume as well possibly due to beta cell apoptosis (Cho et al.,2011).

When investigating possible associations between pancreas size and aIMT, in the current study population, there was a highly significant positive association between pancreas head, body and tail size with aIMT (Table 7). In order to ensure that the association seen was not due to participant body size, partial correlations were done controlling for BMI, and aIMT still had a strongly significant association to pancreatic size (Table 16). Pancreatic size, regardless of region (head, body or tail) is also associated with pancreatic volume, even when controlling for BMI (Table 14). However, aIMT was not found to be significantly associated with pancreatic volume, even given its association to size. Sample size could be a possible explanation to this as pancreatic volume was measured on a random subset of 100 participants while pancreatic size and aIMT measurements were taken on a sample of 500 participants. In future studies it would be

recommended to increase the number of participants undergoing pancreatic volume measurements in order to improve the accuracy around possible associations to aIMT. While the research being conducted and variables measured in this study around pancreatic fatty infiltration and aIMT is novel, a similar study conducted by Kul *et al* found that NAFPD is associated with increased aIMT (Kul et al., 2019).

When investigating possible pancreatic fatty infiltration, adiposity measurements (visceral and subcutaneous fat) were correlated with pancreatic size and volume while controlling for BMI. This was done to ensure that organ size was not due to larger patient size as pancreas volume was found to have a positive correlation with BMI, in a different study among a Japanese population (Kou *et al.,* 2014). No significant associations were seen between these adiposity measurements and pancreatic volume at this age in the current study population (Table 15). In order to identify if increased pancreatic volume and aIMT was dependent on teratogen exposure, Chi-square analysis was conducted (Table 19 and 20). Although the results were not significant, it is important to note that when collapsing data groups into common groups (i.e. grouping exposures) reduces the statistical power. In future it would be advised to increase the sample size as previously mentioned with pancreatic volume. When looking at pancreatic volume, mean comparisons among exposure groups (Table 18), no significant differences were seen at the present age of the children. However, it is interesting to note that both groups exposed to teratogens (alcohol and dual exposure as well as smoking/nicotine and dual exposure) have higher pancreatic volumes in comparison to the control. While literature is limited when examining the effects of nicotine and alcohol on pancreas volume, no significant associations were also seen in a different study exploring a possible relationship between alcohol consumption, nicotine and pancreatic volume in a different population prone to suffer from pancreatitis. In that population, age seemed to be the only variable

associated with a significantly reduced pancreatic volume (Stuart et al., 2020). Although not significant at this time, when looking at individual mean differences in pancreatic volume (Table 2), participants exposed to alcohol only *in utero* have the lowest pancreatic volume. It is in the researcher's view that this should be further investigated once the participants are older as a study done to investigate the effect of ethanol on pancreatic beta-cell death found that ethanol along with fatty acids generate oxidative stress, causing beta-cell death (Dembele *et a*l.,2009). Reduced beta-cell mass results in a decreased pancreatic volume.

Lastly, the study aimed to investigate possible associations between anthropometry, aIMT and pancreatic measurements. AIMT was positively associated with all the basic anthropometic measurements, namely weight, BMI, waist circumference (WC) and subscapular skinfold thickness except tricep skinfold thickness (Table 5). Also, it was not significantly associated with clinical measurements, systolic and diastolic blood pressure or heart rate. This is contrary to what was found in a study conducted by Dawson *et al*, in which aIMT was positively associated not just with BMI but with systolic and diastolic pressure as well (Dawson *et al.*, 2009). It is possible that with age, the association between aIMT and blood pressure could become more apparent, as the above-mentioned study was conducted among adolescents and young adults, while the current study cohort is five years old.

Pancreatic size, regardless of region, was positively associated with weight, BMI and WC (Table 10). However, only pancreas head and tail size were associated with tricep skinfold thickness. After controlling for BMI, WC was still seen to have a significant association with pancreatic head and tail size (Table 17). These associations between anthropometry and pancreatic measurements prior to controlling for BMI were consistent to research done by Hema Latha Gowraiah et al. in which BMI, weight and body height were associated with pancreas weight during adult autopsies,

Furthermore, an association between pancreas volume and BMI was seen as well, which was not found in the current study (Gowraiah *et al.*, 2019). Pancreatic volume was not found to be significantly associated with any of the anthropometric variables. However, it is interesting to note that all Pearson correlation coefficients (r) when looking at pancreatic volume and anthropometry were negative, indicating an inverse correlation even though pancreatic size had been positively associated to anthropometry. Given the highly significant association between pancreatic size and volume, it is recommended that the measurements around anthropometry and pancreatic volume be redone once the children are older, to investigate whether this association is still present and if at all significant.

5.2 Conclusion

In conclusion, children exposed to alcohol *in utero* have the largest aIMT among the four groups, However, increased aIMT was not found to be dependent on teratogen exposure but rather BMI at this time. Although not currently significant, visceral fat was found to be increased among alcohol, nicotine and dual exposure groups with intrauterine alcohol exposure being the highest. When looking at the effect of teratogens on the pancreas, increased pancreatic volume was not dependent on intrauterine nicotine and alcohol exposures while. In fact, participants exposed to alcohol *in utero* had the lowest pancreatic volume. This could suggest that exposure to alcohol influences beta-cell atrophy, thus causing a decrease in pancreatic volume. Pancreatic size was found to have a highly significant association to aIMT after controlling for BMI. While pancreatic size and volume are associated with each other, no significant association was seen between aIMT and pancreatic volume, which could be due to having a smaller cohort undergo pancreatic volume measurements in comparison to aIMT. When looking at adiposity associations to the pancreas, after controlling for BMI, visceral fat was still significantly associated with pancreatic tail size, which could indicate possible fatty infiltration starting from the tail region. Anthropometry would not be a viable indicator of possible pancreatic fat infiltration as pancreatic volume was not significantly associated with basic anthropometric measurements. However, pancreatic size as well as aIMT were significantly associated with BMI, weight and WC. Recommendations for future studies include conducting a follow-up on participants once they are adolescents to assess significant associations to teratogen exposure, as both aIMT and pancreatic volume change over time, as well as increasing the number of participants who had undergone pancreatic volume measurements.

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