THE ASSOCIATION BETWEEN LEVELS OF FISH CONSUMPTION EARLY IN PREGNANCY AND BIRTH OUTCOMES OF PREGNANT WOMEN IN JOHANNESBURG, SOUTH AFRICA

by

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I declare that the above dissertation is my own work and that all the sources used or quoted have been indicated and acknowledged by means of complete references.

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DEDICATED

This dissertation is dedicated to the LORD, GOD ALMIGHTY- The author and the finisher of my faith.

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ABSTRACT

Background: Neonates born with low birth weight or preterm are at an increased risk of long-term adverse health outcomes. Research studies on the association of fish consumption during pregnancy and birth outcomes, have led to inconsistent conclusions. Maternal dietary intakes during pregnancy have a significant impact on foetal development and growth. The aim of this project is to determine levels of maternal fish intake at <18 weeks during pregnancy and to determine the association with birth outcomes in pregnant women from Johannesburg, South Africa.

Methods: This Master's study is nested in a larger study with a longitudinal observational research design was conducted on 250 pregnant women in Johannesburg, South Africa. For this Master's study, data from the first 102 participants were used. Data for this study were collected early in pregnancy (<18 week's gestation) and at birth. The birth data were collected by the study mid-wife. Maternal fish consumption during early pregnancy was measured using a Quantitative Food Frequency Questionnaire (QFFQ). Correlation analysis was used to examine the association between maternal fish consumption during early pregnancy and neonatal anthropometry (birth weight, crown heel length and head circumference) and gestational age at birth.

Results: Majority (88.1%) of the mothers were black South Africans between the ages of 18 and 39 with a mean age of 28 ± 5 years. At enrolment, the mean BMI of the women was 27.8±5.8kg/m^2 having a mean height of 158.8±6.7cm and a mean weight of 70.4±15.2kg. Most of them were unmarried (45.4%), living in households of 2 – 5 members (86.3%), wage-earning (44.6%) and had Grade 11 or 12 schooling (58.4%). Most (76.5%) of the pregnant women consumed fish rarely (once a month) and the overall median fish intake was 4.8g/day (0; 25). In the study sample 12.5% of new-borns had a low birth weight (<2500g), the percentages of preterm births were 1.0% - extremely preterm (<28 weeks), 2.0% - very preterm (28 – <32 weeks) and 10.0% - moderate to late preterm (32 – 37 weeks). The mean birth weight was 2999.2±624.4g with boys having a mean birth weight of 3157.3±571g and girls at 2819±671g. The new-borns' mean gestational age at birth was 38.8±2.4weeks (271.6days). The percentage of new-born head circumference ≤ 31.49cm was 9.2% and the mean head circumference was 34.3±3.6cm with the boys having a mean head circumference of 34.5±2.4cm and the girls 34.1±4.3cm. In this study sample, 3.7% of new-borns were born with crown heel length of 31 – 40cm and the mean crown heel length mean was 49.5±4.6cm with the boys having a mean crown heel length of 49.8±4.9cm and the girls having mean crown heel length of 49.3±4.3cm. In this study, there were no statistically significant

associations between fish consumption at early pregnancy and birth outcomes such as gestational age at birth (r=0.051; p=0.625), birth weight (r=-0.043; p=0.695) and crown heel length (r=0.008; p=0.943). There was a positive association between maternal fish consumption in early pregnancy and head circumference of the new-born which tended towards statistical significance (r=0.193; p=0.079).

Conclusions: In this study of pregnant women living in Johannesburg, a few women consumed fish at early pregnancy compared with women who did not consume fish during pregnancy. We found no statistically significant association in this study between fish consumption at early pregnancy and birth outcomes.

Key words: Maternal, Nutrition, Fish Consumption, Pregnancy, New-Born, Birth Weight, Crown Heel Length, Head Circumference, Gestational Age at Birth and Quantitative Food Frequency Questionnaire (QFFQ).

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACOG	American College of Obstetricians and Gynaecologists
ALA	Alpha-linoleic acid
ANC	Antenatal clinic
CS	Caesarean section
DGLA	Dihomogamma-linolenic acids
DHA	Docosahexaenoic acid
DOHaD	Developmental Origins of Health and Disease
DRI	Dietary Reference Intake
EER	Estimated Energy Requirement
ELBW	Extremely-low-birth-weight
EPA	Eicosapentaenoic acid
GDM	Gestational diabetes mellitus
GLA	Gamma-linoleic acid
GOED	Global Recommendations for EPA and DHA intake
ICF	Informed consent form
IOM	Institute of Medicine
IUGR	Intrauterine growth restriction
LA	Linolenic acid
LBW	Low birth weight
LCPUFA	Long chain polyunsaturated fatty acids
LSM	Living Standards Measure
MUFA	Monounsaturated fatty acids
NASEM	National Academies of Sciences Engineering and Medicine
NCCIH	National Centre for Complementary and Integrative Health
NCDs	Non-Communicable Diseases
NuPED	Nutrition during Pregnancy and Early Development study

NVD	Normal vaginal delivery
NWU	North-West University
PCBs	Polychlorinated biphenyls
PUFA	Polyunsaturated fatty acids
QFFQ	Quantitative food frequency questionnaire
RDA	Recommended daily allowance
RMMCH	Rahima Moosa Mother and Child Hospital
RSA	Republic of South Africa
SAARF	South African Audience Reference Foundation
SADHS	South Africa Demographic and Health Survey
SANHANES-1	South African National Health and Nutrition Examination Survey
SPSS	Statistical Package for Social Sciences
T2DM	Type 2 Diabetes Mellitus
THUSA	Transition and Health during Urbanisation of South Africans
UNICEF	United Nations International Children's Emergency Fund
UNISA	University of South Africa
VLBW	Very-low-birth-weight
WHO	Word Health Organization

DEFINITIONS LIST

Preterm birth: preterm birth can be defined as babies born alive before 36 weeks' pregnancy are completed (i.e. <37 weeks gestation). This is divided into three groups based on gestational age at birth, namely extremely preterm (<28 weeks), very preterm (28 to 32 weeks) and moderate to late preterm (32 to 37 weeks) (WHO, 2015).

Low birth weight: low birth weight can be defined as birth weight less than 2500g. It is one of the main predictors of adverse perinatal outcomes and death (Chen *et al*, 2013). There are categories of LBW such as very low birth weight (VLBW) and extremely low birth weight (ELBW) (Fayed, 2016 & Ballot *et al*, 2012).

Very low birth weight: VLBW can be defined as birth weight less than 1500g (Koller-Smith *et al*, 2017; Hornik *et al*, 2012)

Extremely low birth weight: ELBW can be defined as birth weight less than 1000g (Cutland *et al*, 2017; Natarajan *et al*, 2012).

Gestational age: the American Academy of Pediatrics (2013) defines gestational age as "the time elapsed between the first day of the last normal menstrual period and the day of delivery". Gestational age is used to describe foetal age at birth (Boyle *et al*, 2012; Srinivasjois *et al*, 2015; Oken *et al*, 2003).

Early term pregnancy: Early term pregnancy can be defined as the new-born delivered between 37 weeks 0 days and 38 weeks, six days (ACOG, 2013).

Full term pregnancy: Full term pregnancy can be defined as the new-born delivered between 39 weeks and 40 weeks, six days (ACOG, 2013).

Late term pregnancy: Late term pregnancy can be defined as the new-born delivered between 41 weeks and 41 weeks, six days (ACOG, 2013).

Post term pregnancy: Post term pregnancy can be defined as the new-born delivered between 42 weeks and beyond (ACOG, 2013).

CHAPTER 1

INTRODUCTION

1.1 Introduction

Maternal nutrition concerns the nutritional needs and intake of women during the time they are pregnant and breastfeeding and also may refer to nutrition prior to pregnancy (Huffman *et al,* 2001). Many major foetal body structures are formed during early pregnancy. Maternal nutrition is important during this time because the maternal body stores the nutrients needed and provides the environment for the growth and development of a new human being (Rolfes *et al*, 2012). Furthermore, foetal nutritional exposures have shown to have long term health consequences. Epidemiological studies of new-born and adult mortality have supported this and the Developmental Origins of Health and Disease originated (Wadhwa *et al*, 2009). The hypothesis explains the relationship between adult death rates and nutrient variations in early life. Foetal nutritional exposure may affect the growth pattern of the foetus at different stages of development (Barker *et al*, 1993). This may result in low birth weight and risk of diseases as an adult (Bloomfield *et al*, 2006; Barker *et al*, 1993). Longitudinal studies of men and women around the world confirmed an association between low birth weight and coronary heart disease (Barker, 2007).

Maternal nutrition has been a global challenge as both developed and developing countries are faced with under and over-nutrition which calls for urgent attention [United Nations International Children's Emergency Fund (UNICEF) (2009)]. Unbalanced or inadequate maternal nutrition may lead to various complications and poor birth outcomes such as low birth weight, premature birth, neural tube defects, macrosomia, foetal facial and heart abnormalities (Leddy *et al*, 2008), mental retardation, impaired mental and physical development (Kapil, 2007), anaemia (Ladipo, 2000), foetal alcohol syndrome (Ornoy & Ergaz, 2010) and development of diabetes later in life (Guelinckx *et al,* 2008).

Globally, an estimated 15 million babies are born preterm every year with the prevalence ranging from 5% to 18% of live births across 184 countries [Word Health Organization (WHO) (2018); Ferre *et al*, 2016)]. Preterm birth is the leading cause of death in children below the age of five. According to WHO (2018) “more than 60% of preterm births occur in Africa and South Asia”. Risk factors for preterm birth are disease, poor maternal nutrition, disaster, divorce, loss of employment (Scorgie *et al*, 2015) teenage and unplanned pregnancy (Chigona & Chetty, 2008).

1.2 Problem statement and Motivation

Birth outcomes are the category of measures that describe health at birth of which birth weight is most often used. Additional newborn assessments used to assess birth outcomes are gestational age, Apgar score, size for gestational age and live/still birth (Hayatbakhsh *et al*, 2011). Birth weight has been identified as an essential predictor of health at population level specifically. Low birth weight, preterm birth, and intrauterine growth restriction (IUGR) have all been recognised as components of major adverse birth outcomes (Abu-Saad & Fraser, 2010). These adverse birth outcomes represent the principal causes of neonatal death among children born without hereditary abnormalities (Bhutta *et al*, 2005; Scholl & Johnson, 2000); likewise, increased health care costs, quality of life and development of the new-born may be attributed to the adverse birth outcomes (Abu-Saad & Fraser, 2010).

Low birth weight (LBW) (<2500g at birth) specifically is known as a risk factor for adverse outcomes such as higher incidences of mortality and chronic disease; increased hospital costs and impaired growth (Muthayya, 2009). According to Slyker *et al*, (2014) preterm birth is as an important predictor of health outcomes for the infant. Twenty eight percent of global neonatal deaths have been accounted to preterm birth through direct and indirect mortality (Slyker *et al*, 2014). Later in life, preterm birth is associated with both short and long term adverse health outcomes such as increased risk of chronic diseases such as diabetes and cardiovascular disease (Derraik *et al,* 2016; Ota *et al*, 2014).

Optimal maternal nutritional status during pregnancy is essential because of the high nutrient demands and the critical role of the nutrients for both the developing foetus and the mother because maternal nutritional status during pregnancy has been regarded as an important determinant for foetal growth (Godfrey & Barker, 2007). Likewise, nutrients found in foods perform different functions in the growth of the developing foetus. Some nutrients are obtained from limited food sources, for example long chain polyunsaturated fatty acids (LCPUFA) from fatty fish, crustaceans, mollusks, meat, egg, milk, flax seed, soybean and canola oil (Abedi & Sahari, 2014; Gogus & Smith, 2010) and may be lacking in monotonous diets.

Similarly, there are indications that positive foetal neuro-developmental outcomes are associated with fish intake during pregnancy which was supported by seven of eight articles reviewed by Starling *et al* (2015) and Daniels *et al* (2004). South African data on maternal fish consumption is scarce.

Therefore, this research study will provide information on maternal fish intake during early pregnancy; and the association between different levels of maternal fish consumption and birth outcomes of pregnant women attending antenatal care in Johannesburg, South Africa.

1.3 Aim and objectives

1.3.1 Aim of the study

The aim of this study is to determine levels of maternal fish intake during early pregnancy and to determine any association with selected birth outcomes in pregnant women from Johannesburg, South Africa.

1.3.2 Objectives of the study

The objectives of this project are:

- To assess types and levels of maternal fish consumption during early pregnancy.
- To examine the association between maternal fish consumption levels during early pregnancy and neonatal anthropometry, specifically birth weight and head circumference.
- To examine the association between maternal fish consumption levels during early pregnancy and gestational age at birth.

1.4 Significance of the study

This research study provided information on the association between dietary intake, specifically fish intake during early pregnancy, and birth outcomes such gestational age at birth, birth weight, head circumference and crown heel length of the new-born. It will further supply insight on the association between dietary intake during early pregnancy and major adverse birth outcomes such as low birth weight, preterm birth, and intrauterine growth restriction (IUGR) which are the major causes of neonatal death among children born without hereditary abnormalities.

Likewise, this research study will provide information on dietary intake, specifically fish consumption, of South African pregnant women of which there is a paucity of information. Furthermore, limited information is available regarding the association of dietary practices in pregnancy women and the birth outcomes.

1.5 Limitations of the study

This research study is limited to the city of Johannesburg. Therefore, the results of this study cannot be applied to all pregnant women in South Africa but could allow for comparison in similar urban settings.

Dietary recall methods (Quantitative food frequency questionnaires) are dependent on the participants' memory and cannot estimate deliberate under- or over reporting (Moghames *et al*, 2016; Shim and Kim, 2014).

1.6 Background of the study

This Master's research project is a sub-study of a larger project, entitled: *Nutrition during Pregnancy and Early Development, the NuPED study.* The larger project involves team members from the University of Witwatersrand, University of South Africa (UNISA) and North-West University (NWU). The aim of the larger study is to assess dietary intake and nutritional status of urban pregnant women in Johannesburg, South Africa; to determine associations with birth outcomes, measures of maternal health, as well as measures of offspring health and development. Participants in the larger study (n=250) are assessed at <18, 22 and 36 weeks' gestation as well as at birth.

1.7 Structure of Dissertation

Chapter 1 supplies the background of the study. Chapter 2 focuses on the literature available relevant to the topic. Chapter 3 presents the research methods. The results of the study are reported and discussed in Chapter 4. Chapter 5 supplies conclusions drawn from the study as well as relevant recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Pregnancy is an exceptional period in a woman's life in which a woman carries a growing foetus in her uterus for approximately 40 weeks (NIH, 2017). The role of nutrition when a woman becomes pregnant and during pregnancy has a significant influence on foetal health, but also on infant and maternal health outcomes (WHO, 2013).

Maternal nutrition during pregnancy is essential for pregnant women to provide adequate nutrients and oxygen for foetal health and its survival because inadequate foetal nutrient supply may lead to poor foetal growth and development (Rocco *et al*, 2005; Westenberg *et al*, 2002).

Poor nutrition status during pregnancy may increase the risk of prenatal complications such as low birth weight (LBW), preterm birth, preeclampsia, gestational diabetes mellitus (GDM), IUGR, prenatal and infant mortality and morbidity (Daba *et al*, 2013; Widen & Siega-riz, 2010; Rocco *et al*, 2005) placing foetus and mother at risk. Type 2 diabetes mellitus (T2DM) and obesity complications during pregnancy may contribute to the offspring developing these conditions later in life (Barger, 2010; Blumfield *et al*., 2012).

Foetal adaption and response to insufficient nutrition during pregnancy may permanently alter the function and structure of the body (Daba *et al*, 2013). For example, comprehensive studies done on malnutrition and foetal programming, show that maternal nutrition is specifically important in the metabolic pathways during the pre- and postnatal development and maternal diet lacking vital nutrients may be responsible for permanent brain alterations (Castrogiovanni & Imbesi, 2017).

Nutrition during pregnancy has been shown to predict birth outcomes (Abu-Saad and Fraser, 2010; Bang and Lee, 2009). "Birth outcomes are a category of measures that describe health at birth" (Hatchell et *al*, 2016). The birth outcomes, such as birth weight, gestational age, Apgar score and size for gestational age, may predict a child's current and future morbidity (Gavin *et al*, 2012; Hayatbakhsh *et al*, 2011).

Maternal dietary patterns high in vegetables, fruit, pulses, fish and dairy products are considered to be high in protein and several key vitamins and minerals, which are associated with healthy birth outcomes (Tanha *et al,* 2013). The following sections will discuss the role of nutrition during pregnancy on the health of the growing foetus.

2.2 Dietary requirements during pregnancy

Optimal nutrient supply during pregnancy is vital for suitable foetal growth and development (Grieger & Clifton, 2014). The dietary requirements during pregnancy increase because of the high nutrient demands of both the mother and the growing foetus (Sharlin & Edelstein, 2011). This research study limits itself to the literature available on the nutrients that are available in fish and which are needed in higher quantities during pregnancy than in non-pregnant women: carbohydrates, protein, energy, folate, vitamin B_{12}, vitamin A, vitamin D, iron, zinc, calcium, phosphorus, magnesium and essential fatty acids.

2.2.1 Carbohydrates

The fast growth rate of the developing foetus requires sufficient amounts of energy, mainly in the form of glucose (Sharlin & Edelstein 2011). Insufficient carbohydrate intake during pregnancy may lead to poor foetal growth. The recommended daily allowance (RDA) for carbohydrate during pregnancy is 175g/day while 130g/day is recommended for non-pregnant women (Rolfes *et al*, 2012; Sharlin & Edelstein 2011). Dietary sources high in carbohydrates include whole grains, vegetables, fruits, milk, and milk products (Meyer *et al,* 2000).

However, if a mother develops diabetes while she is pregnant or enters pregnancy with preexisting diabetes, a mild restriction of dietary carbohydrate may be recommended, especially for refined carbohydrates (Magon & Seshiah, 2011; Sharlin & Edelstein 2011). In addition, it is essential for her to work closely with a health-care team in order to provide adequate but not too much glucose to ensure optimal growth of her baby (Magon & Seshiah, 2011; Sharlin & Edelstein 2011).

2.2.2 Protein

Sufficient protein is needed during pregnancy for foetal development, enzyme formation, and muscle and collagen development. The framework of skin, bones, blood vessels and other body tissue requires collagen (Sharlin & Edelstein 2011; Picciano, 2003). Protein is also essential for maternal physical changes to carry the foetus. Inadequate protein intake during pregnancy may lead to foetal growth retardation and LBW (Borazjani *et al,* 2013; Liberato *et al*, 2013). The Dietary Reference Intake (DRI) of protein for pregnant women is 1.1g/kg/day or an additional 25g/day while 0.8g/kg/day (46g/day) is the reference for non-pregnant women (Rolfes *et al*, 2012 p. 443; Sharlin & Edelstein 2011). Dietary sources high in protein are fish, meat, eggs, poultry and dairy products (Sharlin & Edelstein 2011).

In special populations, such as women experiencing severe nausea and vomiting, vegetarians, vegans and low-income women experiencing food insecurity, protein intake should be cautiously monitored for protein quality and sufficiency during pregnancy. If the pregnant woman does not consume high-quality sources of protein which contain all essential

amino acids, such as those obtained from animal sources, she should be encouraged to eat a variety of plant-based foods to ensure that all essential amino acids are available to the foetus (Sharlin & Edelstein 2011; Dwyer, 1991).

2.2.3 Fats and Lipids

Adequate fat is required in the maternal diet for more than only a source of energy. Certain fats, such as long chain polyunsaturated fatty acids (LCPUFA), cannot be synthesized in the human body and are therefore essential. Thus, the type of fatty acids consumed is important to the various functions in different physiological systems.

Fat is also a source of concentrated calories and may be helpful to women at risk of energy malnutrition during pregnancy. Excess dietary fat is not recommended during pregnancy because it may lead to unwanted weight gain above the recommendations for pregnant women, since 68% of the South African women are overweight or obese (SADHS, 2016; Shisana *et al*, 2013; Borazjani *et al,* 2013; Vorster *et al*, 2013). Likewise, it may lead to cardiovascular disease and risk of diabetes especially if intake is high in saturated fat (NASEM, 2017). The Acceptable Macronutrient Distribution Range (AMDR) for fat intake for all people (including pregnant women) is 20% to 30% of the total calories intake (Rolfes *et al*, 2012; Sharlin & Edelstein 2011). The South Africa food based dietary guidelines also recommends that total fat intake must provide 20-30% daily energy intake for two years and above for ideal health (Vorster *et al*, 2013). However, there are controversies over the dietary fat recommendations because of their different roles in human health. According to Aranceta and Pérez-Rodrigo (2012) “recommendations vary between countries regarding the levels of fat intake advised, the process followed to set the recommendations” while many recommendations do not include a recommendation for the cholesterol intake which shows that there is a gap in the available evidence (Aranceta & Pérez-Rodrigo 2012; German & Dillard 2004). However, fat consumption during pregnancy should emphasize sources that supply the essential fatty acids and choline, a component of phospholipids essential for healthy brain function (Sharlin & Edelstein 2011).

2.2.3.1 The essential fatty acids

Even though many different fatty acids are needed for good health in humans, mammalian cells cannot synthesize all of them (Grosso *et al*, 2014; Monroig *et al*, 2013). The essential fatty acids which should be obtained from the diet include linoleic acid (LA, omega-6) and alpha-linolenic acid (ALA, omega-3) (Almaas *et al*, 2015; Ros, 2010). Human cells are able to desaturate and elongate LA and ALA (18-carbon chain fatty acids) to form the long chain polyunsaturated fatty acids (LCPUFA) arachidonic acid (AA) and EPA, respectively (20-carbon chain fatty acids) and ultimately DHA (22-carbon chain) (see figure 2.1). Thus, if LA and ALA are deficient in the diet, the LCPUFA may also be deficient. However, the efficiency

by which the conversion is taking place in human metabolism is still uncertain (Amjad Khan *et al*, 2017; Alhazzaa et al, 2013). Therefore, the recommendations to obtain the specifically LCPUFA from the diet and not only the shorter chain precursors. There are limited dietary sources of LCPUFA such as fatty fish, crustaceans, mollusks, and limited amounts in meat, eggs, milk, flax seed, soybean and canola oil (Abedi & Sahari, 2014; Gogus & Smith, 2010).

LCPUFA such as DHA and arachidonic acid (AA) are essential for the foetal central nervous system (Almaas *et al*, 2015; Coletta *et al,* 2010), cell membrane formation, hormone formation and for development of brain and eye tissue (Sharlin & Edelstein 2011; Coletta *et al,* 2010). The foetal brain is largely made-up of lipid material, and therefore essential fatty acids are required for its growth, function and structure (Rolfes *et al*, 2012).

Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) differ structurally in their number of carbon-carbon double bonds. The PUFA contain two or more double bonds (therefore not saturated with hydrogen atoms) within the molecule, while the MUFA only one double bond. The PUFA can be classified by their chemical structure in omega-3 and omega-6 fatty acids. The omega-3s refers to a group of PUFA in which the first double bond is 3 carbons from the methyl end (the omega carbon atom of the molecule); while the omega-6 double bond is at the 6^{th} carbon from the methyl end (Grosso *et al*, 2014).

The recommended intake of essential fatty acids is higher for pregnant women than for non-pregnant women because of its critical role in the building of the foetal brain, retina and nerve tissues (Daniels *et al*, 2004). Between 400 and 550mg of omega-3 PUFAs (EPA and DHA) is recommended per day for pregnant women (Swanson *et al*, 2012; Greenberg *et al*, 2008) while 250mg of omega-3 PUFAs (EPA and DHA) is recommended per day for non-pregnant women (GOED, 2014).

In general, essential fatty acids are associated with lower blood pressure and decreased risk of sudden death, heart attack, abnormal heart rhythms and stroke (Mozaffarian & Wu, 2011; Minihane, 2005; Kris-Etherton *et al*, 2002). The intakes of these fatty acids are associated with a lower risk of diabetes, dementia and Alzheimer's disease (Rylander *et al*, 2014). In pregnancy, increased intake of omega-3 fatty acids may reduce the risk of depressive symptoms in the postpartum period because omega-3 fatty acids may decrease proinflammatory cytokine production, which may be elevated in depressed patients, since the pregnant women supply omega-3 fatty acids to the foetus during pregnancy which may lead to the reduction of maternal stores of omega-3 fatty acids during pregnancy (Markhus *et al*, 2013; Coletta *et al*, 2010; Jensen, 2006; Freeman, 2006). The ratio of omega-3 to omega-6 fatty acids intake has been shown to be important (Simopoulos, 2016).

The modern diet has an imbalance of omega-3 to omega-6 fatty acids, with a higher omega-6 intake. This has been associated with preterm birth (McGregor *et al*, 2001). The combination and quantity of essential fatty acids are therefore important for healthy birth outcomes and health in later years.

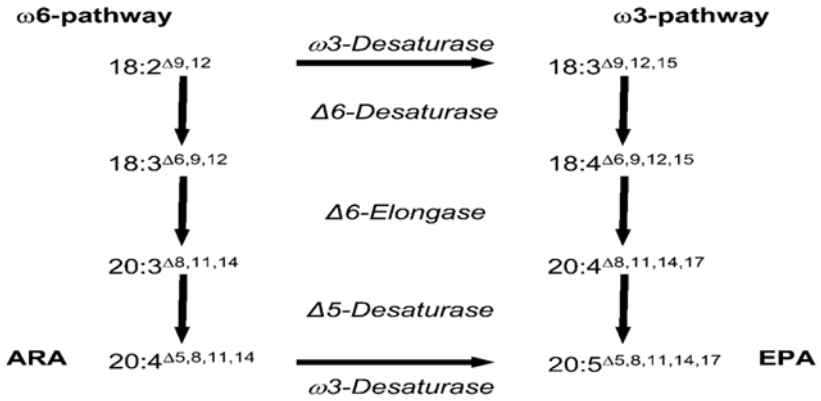

Figure 2.1: The biosynthetic pathways of long-chain polyunsaturated fatty acids (Abbadi *et al*, 2004).

2.2.4 Total energy

During pregnancy, intake of nutrient-dense foods should be increased and prioritised while empty-energy foods should be minimized because they provide extra energy without the required micronutrients (Sharlin & Edelstein 2011; Picciano, 2003). The total energy DRI for pregnant women is higher than for non-pregnant women The DRI supplies total energy references for each trimester. No additional energy is required for the first trimester; an additional 1428kJ is required for the second trimester and 1898.4kJ for the third trimester (Sharlin & Edelstein 2011; Butte *et al,* 2004; Trumbo *et al*, 2002).

2.2.5 Fluid

Fluid intake during pregnancy needs to be increased in order to provide for maternal fluid needs and increasing amniotic fluid; increased blood volume, to maintain body temperature, to transport nutrients and waste products, to moisten of the digestive tract and tissues and for cushioning and protection of the developing foetus (Wright *et al*, 2010; Sharlin & Edelstein 2011; Story & Hermanson, 2000). Water is also a source of fluoride, which assists in the bone formation of the foetus. Pregnant women must avoid water that contains heavy metals, such as lead, because it may contribute to spontaneous abortion, gestational hypertension, reduced stature and deficient neurodevelopment (ACOG, 2013; Brown & Margolis 2012; Montgomery, 2002).

2.2.6 Folate

Folic acid is the synthetic form of folate. The production of neurotransmitters in the manufacturing of DNA cells in early pregnancy requires folate. (Hisam *et al*, 2014; Sharlin & Edelstein 2011; Almeida & Cardoso 2010). The RDA of folate for pregnant women is 600µg/day while 400µg/day is recommended for non-pregnant women of childbearing age

(Rolfes *et al*, 2012; Sharlin & Edelstein 2011). Good food sources include dried beans, avocados, peas, bananas, green leafy vegetables, orange juice, asparagus and fortified dry cereals (Sharlin & Edelstein 2011).

Importantly, supplementation of 400µg/day of folic acid in preconception and early pregnancy seems to be sufficient in preventing neural tube defects such as spina bifida and anencephaly (Grieger & Clifton, 2014; Sharlin & Edelstein 2011). Spinabifida happens when there is only a partial closure of the spinal column and spinal cord whereas severe underdevelopment of the brain is referred to as anencephaly. Folic acid supplementation is recommended for women of child-bearing age (capable of becoming pregnant) because neural tube defects occur during the first 28 days of pregnancy; at this time most women do not know they are pregnant (Rolfes *et al*, 2012; Cavalli, 2008; Blom *et al*, 2006). The South African Department of Health, through the food fortification programme has included folic acid among the nutrients to be added to maize meal and bread flour (staple foods) with the aim to improve the micronutrient status of the South African population; the food fortification programme shows a positive result because there was a decline on the prevalence of neural tube defect in South Africa (Hoddinott, 2018; Sayed *et al*, 2008) and Sayed *et al*, 2008 state that "30.5% was observed, from 1.41 to 0.98 per 1,000 births". Sufficient intake of folic acid during pregnancy prevents maternal deficiency and reduces the risk of birth defects (Hisam *et al*, 2014; Sharlin & Edelstein 2011; Almeida& Cardoso 2010).

2.2.7 Vitamin A

Vitamin A is required during pregnancy for foetal development because of its critical contribution to growth, cell differentiation and protein synthesis. Inadequate intake of vitamin A during pregnancy may result to night blindness for pregnant women (Christian *et al*, 2000). Vitamin A deficiency during pregnancy may lead to an increased risk of maternal mortality and it is also associated with LBW, premature birth, antepartum haemorrhage and IUGR (NASEM, 2017; Ladipo, 2000). Vitamin A may be obtained from a variety of foods in the form of either vitamin A or beta-carotene (provitamin A carotenoids). Animal foods are the only source of vitamin A, while plant foods are the source of beta-carotene (NASEM, 2017). The RDA for vitamin A for pregnant women is 770µg/day while 700µg/day is for non-pregnant women. Dietary sources of vitamin A include fish, meat and milk while dietary sources of beta-carotene include darkly coloured fruits and vegetables (orange, mango, carrot and spinach), as well as oily fruits and red palm oil (NASEM, 2017; Tang *et al*, 2009). Since vitamin A is a fat-soluble compound, dietary fat is required for its absorption (Rolfes *et al*, 2012).

2.2.8 Vitamin D

An adequate level of vitamin D is very important during pregnancy for foetal development because it helps to build and maintain strong bones and teeth (NASEM, 2017; Grieger &

Clifton, 2014; Sharlin & Edelstein 2011). There is increasing evidence that vitamin D also plays a vital role in preventing cancer, autoimmune diseases, influenza, type 1 diabetes, heart disease osteoporosis and depression (Mozos & Marginean 2015; Nair & Maseeh 2012; Sharlin & Edelstein 2011). Inadequate levels of vitamin D during pregnancy may lead to foetal rickets, neonatal tetany and abnormal teeth development (NASEM, 2017; Ladipo, 2000). The human body, through the action of sunlight, can synthesize vitamin D from 7-dehydrocholesterol. The metabolism of vitamin D is influenced by the colour of the skin (Mostafa & Hegazy, 2015). However, vitamin D can be obtained from diets and supplements (NASEM, 2017). The RDA for both pregnant and non-pregnant women is 15µg/day (Grieger & Clifton, 2014; Rolfes *et al*, 2012; Sharlin & Edelstein 2011). Fatty fish (sardine, salmon, and mackerel), fish liver oil, fortified milk, liver and egg yolks are all good dietary sources of vitamin D (NASEM, 2017; Grieger & Clifton, 2014; Sharlin & Edelstein 2011).

2.2.9 Vitamin B_{12}

In pregnancy, vitamin B_{12} is vital for healthy functioning of the nervous system, manufacturing of red blood cells and genetic material (Bonilla*et al*, 2012; Sharlin & Edelstein 2011). Insufficient intake of vitamin B_{12} during pregnancy and lactation may cause neurologic damage in children (Kocaoglu *et al,* 2014; Black, 2008). The RDA for pregnant women is 2.6µg/day and for non-pregnant women 2.4µg/day (Rolfes *et al*, 2012; Sharlin & Edelstein 2011). Dietary sources of vitamin B_{12} include fish (especially oily fish), meat, dairy products, eggs, fortified cereal (high fibre bran flakes) and fortified non-dairy milk (soy milk) (Bonilla *et al*, 2012; Vanderjagt *et al*, 2011).

2.2.10 Iron

Iron, is a component of haemoglobin, which allows red blood cells to carry oxygen and it is an essential nutrient (Grieger & Clifton, 2014; Sharlin & Edelstein 2011). Iron furthermore plays a vital role in oxidation-reduction reactions during metabolism and in pregnant women, it is essential to compensate for the increasing blood volume (Sharlin & Edelstein 2011). Inadequate iron intake during pregnancy may increase the possibility of adverse birth outcomes such as preterm birth and LBW (Grieger & Clifton, 2014). It may cause iron-deficiency anaemia which has been associated with maternal mortality (Ladipo, 2000). The DRI for iron during pregnancy is 27mg/day while the recommendation is 18mg/day for non-pregnant women (Rolfes *et al*, 2012; Sharlin & Edelstein 2011). Good dietary sources of iron include red meat, poultry, seafood, eggs, nuts, legumes, spinach, whole wheat, broccoli, nuts and seeds (Samaniego-Vaesken *et al,* 2017; Stewart, 2006; Hunt, 2003).

2.2.11 Zinc

Adequate zinc intake during pregnancy is important because of its role in the immune system development, especially in the first trimester when foetal organs are formed (Shah & Sachdev,

2006). It is also a structural component of cells making zinc vital for cell growth, development and differentiation (Wang *et al*, 2015; Hirano *et al*, 2008). Insufficient zinc intake during pregnancy may affect the immune response because of its consequences in reductions in T cell development, thymic hormone release, and T cell functions (Ladipo, 2000). Likewise, adverse pregnancy outcomes such as stillbirth, foetal neural tube defects, preterm birth and spontaneous abortion have been associated with zinc deficiency during pregnancy (Wang *et al*, 2015; Graham *et al,* 1994; Scholl *et al*, 1993; Buamah *et al,* 1984). The RDA for zinc during pregnancy is 11mg/day while 8mg/day is for non-pregnant women (Rolfes *et al*, 2012; Sharlin & Edelstein 2011; Grieger & Clifton, 2014). Good dietary sources of zinc include fish, beef, veal, pork, lamb, lentils, beans, fortified maize meal and fortified white and brown bread flour (Tietz, 2006; Ma & Betts, 2000).

2.2.12 Calcium

Adequate intake of calcium during pregnancy is necessary for foetal bone formation and maintains maternal skeletal structure (Grieger & Clifton, 2014; Sharlin & Edelstein 2011). Inadequate intake of calcium during pregnancy may lead to reduced maternal bone density, which may result in maternal osteoporosis later in life (Heringhausen & Montgomery, 2005; Prentice, 2000). The South Africa Department of Health National Guidelines for Maternity Care in South Africa (2015) recommends calcium supplementation during pregnancy as part of the prevention of pre-eclampsia complication (GMCSA, 2015). The RDA/DRI for calcium is 1000mg/day for both pregnant and non-pregnant women (Rolfes *et al*, 2012; Sharlin & Edelstein 2011). Dietary sources of calcium include sardines with bones, milk, cheese, leafy green vegetables and yoghurt (Sharlin & Edelstein 2011).

2.2.13 Magnesium

Over 300 enzymes in the body use magnesium as a cofactor. Insufficient magnesium intake during pregnancy is associated with an increased risk of premature labour, preeclampsia, prolonged pregnancy-induced hypertension and placental dysfunction (Zarean & Tarjan, 2017) and developing of both gestational and type 2 diabetes (Barbagallo & Dominguez 2007). The RDA of magnesium during pregnancy is between 350 and 400mg/day and between 310 and 360mg/day for non-pregnant women (Rolfes *et al*, 2012). A good dietary source of magnesium includes legumes, peanuts, nuts, wheat germ and bran (Sharlin & Edelstein 2011).

In brief, an adequate, varied diet is essential for foetal growth and healthy birth outcomes and must be given special attention during pregnancy in order to prevent possible adverse effects. As indicated in chapter 1, fish consumption of pregnant women is the focus of the next section.

2.3 Fish Consumption

Fish and fishery products produced a much-appreciated source of vital micronutrients and protein for balanced nutrition and sound health. In 2009, global consumption of fish accounted for 16.6% and 6.5% of all worldwide protein intakes (FAO, 2018). Internationally, fish offers almost 3.0 billion people with approximately 20% of their intake of animal protein, and 4.3 billion people with nearly 15% of such protein (FAO, 2018). In South Africa, 20.8g/person/day (7.6kg/capita/year) raw fish products are consumed and 12.5g/capita/day edible portions (cooked product) are consumed (Schonfeldt & Hall, 2013).

Fish is generally low in saturated fats, carbohydrates and cholesterol. It is considered a good source of high quality protein, polyunsaturated omega-3 fatty acids (only in specific fish species), selenium, iodine, vitamin D and B_2 (riboflavin), calcium, phosphorus, potassium, iron, magnesium and zinc (Brantsæter *et al*, 2017; Leventakou *et al,* 2014; FAO, 2012).

Although average per capita fish intake may be little, even negligible quantities of fish can have a positive significant nutritional impact by providing fats, micronutrients and essential amino acids that are rare in plant-based diets. Fish consumption plays a role in health, not specific to pregnancy only. The American Heart Association recommends consumption of fish at least two times per week to lower blood pressure and to reduce the risk of a heart attack or stroke (Kris-Etherton *et al*, 2003) which is mainly attributed to the fact that fish is a source "omega-3 fatty acids known to reduce the likelihood of blood clotting" (Fernandes *et al,* 2012). Dietary intake of fish in women may inhibit cataract development, loss of cognitive function and psychological syndromes such as depression and psychotic symptoms (Fernandes *et al*, 2012). There is sufficient evidence of the beneficial effects of fish consumption in relation to coronary heart disease (Béné *et al,* 2009), stroke, age-related macular degeneration and mental health (Mora *et al*, 2009). In addition, there is convincing evidence of the benefits in terms of growth and development especially in women during pregnancy and in children and infants for optimal brain development (Marangoni *et al*, 2016; Bogard *et al*, 2015; Hiddink *et al,* 2011).

2.3.1 The role of dietary fish intake during pregnancy

Fish and other seafood serve as the main dietary source for elongated omega-3 PUFA including DHA, a vital structural component of the brain (Bloomingdale *et al*, 2010; Oken *et al*, 2008). Sufficient intake of these fatty acids through fish intake may also protect against other adverse perinatal and longer-term outcomes such as preterm birth, low birth weight, stillbirth, neonatal death, gestational diabetes, hypertension, and maternal deaths (Mitao *et al*, 2016; Bloomingdale *et al*, 2010; Clausson *et al*, 2001).

In addition, iron and long chain omega-3 fatty acids (EPA and DHA) through dietary intake of fish during pregnancy may be beneficial to the development and function of the nervous system in a foetus (Elias & Innis, 2001; Kesmodel *et al*, 2002) as well as cognitive development experienced later by the child (Daniels *et al*. 2004; Hibbeln *et al*, 2007; Oken *et al.* 2008).

Equally, maternal homeostasis, foetal neurological development, placental formation and processes associated with normal gestational progress as well as maternal and paediatric health involved intake of essential fatty acids such as omega-3 fatty acids during pregnancy (Genuis, 2008). Increased likelihood of early labour may occur if there is an insufficient omega-3 fatty acids intake during pregnancy. (Genuis, 2008; McGregor *et al*, 2001).

A small case-control study conducted at the Swedish Medical Centre in the United States indicated that preeclampsia maybe developed 7.6 times if pregnant women intakes of omega-3 fatty acids is low during pregnancy (Genuis, 2008; Williams *et al*, 1995).

According to Coletta *et al*, (2010) “deprivation of omega-3 fatty acids during pregnancy is associated with visual and behavioural deficits that cannot be reversed with postnatal supplementation”. From the above reasons, adequate omega-3 fatty acids need to supply to foetus throughout the pregnancy (Jensen, 2006). Balanced diet both in omega-6 and omega-3 fatty acids might be less immunosuppressive and inflammatory (Coletta *et al*, 2010).

There is abundant literature signifying the general health of adequate omega-3 fatty acids consumption. These include a diminished risk of various diseases such as breast cancer (Genuis, 2008; Maillard *et al*, 2002), osteoporosis (Genuis, 2008; Genuis and Schwalfenberg, 2007), heart disease (Bucher *et al*, 2002), arthritic problems (Genuis, 2008; Kremer *et al*, 1990), psychiatric illness (Zboyan *et al*, 2000), and Alzheimer’s disease (Morris *et al*, 2003).

Therefore, the consumption of fish during pregnancy may be beneficial in terms of maternal and foetal health; this was in line with the recommendation for the Norwegian pregnant women (Brantsæter et al, 2012).

2.3.2 Types of fish and its nutritional value

Fish can be classified based on their nutritional value (see Table 2.1). Fish such as pilchards, sardines, tuna, salmon, Green land halibut, mackerel, herring, eel and halibut are classified as fatty fish. Cod, coalfish, tusk, hake, haddock, European perch and European plaice are classified as lean fish (Mohanty *et al*, 2016; Grygus, 2013). The fish listed in Table 2.1 allows for comparison of nutrient content for fish available locally and internationally. Fish commonly consumed in South Africa includes herring, sardines, tuna, salmon, mackerel, and hake (Schonfeldt & Hall, 2013).

Table 2.1: Nutritional value of selected fish per 100g

Fish	**Protein** (g)	**Fat** (g)	**MUFA** (g)	**PUFA**(g)	**Vitamin D** (µg) RDA 15	**Vitamin B_2** (mg) RD 1.4	**Calcium** (mg) RDA 1000-1300	**Iron** (mg) RDA 27	**Mg** (mg) RDA 350 - 360	**Zinc** (mg) RDA 11
Fatty fish										
Pilchard in brine	20.0	5.4	1.09	2.13	8.0	0.47	360	3.6	31	1.60
Pilchard in tomato sauce	18.8	5.4	1.09	2.13	8.0	0.29	300	2.7	39	1.60
Sardines, canned in oil (drained solid)	24.6	11.5	3.87	5.15	7.28	0.23	382	2.9	39	1.31
Sardines, canned in tomato sauce (drained)	16.4	12.0	3.67	4.30	6.79	0.23		2.3	34	1.40
Tuna, canned in oil (drained solid)	29.1	8.2	2.95	2.89	7.40	0.14	13	1.4	31	0.90
Salmon	19.7	10.5	3.21	3.36	8.0	0.14	12	0.4	28	0.4
Greenland halibut	17.6	15.6	7.16	2.55	11.4	0.08	8	0.1	19	0.4
Mackerel	18.5	24.4	9.66	6.52	12.5	0.36	12	0.9	27	0.6
Herring	17.0	19.0	5.59	7.83	11.5	0.30	38	1.0	38	0.5
Eel	17.3	31.5	13.9	5.8	30.0	0.04	35	0.4	15	20
Halibut	16.2	10.4	0.86	1.2	18.0	0.08	6	0.2	16	0.3
Lean fish										
Tuna, canned in water (drained solid)	25.5	0.8	0.16	0.34	0	0.07	11	1.5	27	0.77
Cod	18.1	0.6	0.04	0.25	1.4	0.11	8	0.1	29	0.5
Coalfish	16.5	0.3	0	0.01	0.8	0.20	8	0.1	22	0.7
Tusk	16.1	0.3	0.04	0.05	0	0.15	37	0.1	23	0.4
Haddock	16.6	1.0	0.16	0.38	0.7	0.11	19	0.1	27	0.3
European perch	18.1	0.9	0.31	0.16	0.8	0.07	110	0.6	26	0.8
European plaice	13.4	1.5	0.31	0.57	6.6	0.09	34	0.1	19	0.6

(Fisheries and Aquaculture Industry Research Fund, 2010; Langenhoven *et al*, 1991)
RDA - Recommended Dietary Allowance for pregnant women of reproductive age (18 - 39 years)
Mg - Magnesium
MUFA - Mono-unsaturated fatty acids
PUFA - Poly-unsaturated fatty acid

Fish contain typically 0.1- 31.5g lipids per 100 g of flesh (see table 2.1) (Venugopal & Shahidi, 1996). Lean fish are fish with a low fat content ranging from 0.1 to 2.9% (Murray & Burt 2001; Langenhoven *et al*, 1991) and most of its fat is deposited in the guts (Engeset *et al*, 2015). It contains more iodine and less energy compared to fatty fish. In addition, lean fish, such as cod, are a good source of protein (Rylander *et al*, 2014).

Fatty fish are fish with high fat content and different profiles of fatty acids ranging from 11 to 30% (Langenhoven *et al*, 1991) and its fat is found intramuscularly (Engeset *et al*, 2015; Venugopal and Shahidi, 1996). It is an excellent source of omega-3 fatty acids, specifically EPA and DHA (Kris-Etherton *et al*, 2002).

2.3.3 Essential fatty acids in fish

The primary producers of DHA are the marine microalgae and the concentration of DHA increases in the food chain with these microalgae at the base (Mohanty *et al*, 2016). Globally, fatty fish such as mackerel, tuna, sardines, pilchard and herring are the main dietary sources of EPA and DHA (Abedi and Sahari, 2014; Zivkovic *et al*, 2011; Gogus & Smith, 2010). Table 2.1 indicates which types of fish are high in PUFA.

Fatty fish is therefore considered a healthy food option (Mohanty *et al*, 2013). This is because these oils especially those rich in omega-3 fatty acids, may intervene in prevention and modulation of certain diseases such as heart disease, high blood pressure, diabetes and cancer that are common in many populations (Sahena *et al*, 2009; Mohanty *et al*, 2016).

Fish oils are common dietary supplements (Albert *et al*, 2015). In the United States in 2012, 7.8% (18.8 million) adults used fish oil / omega-3 / DHA, EPA fatty acids as supplement among adults who consumed dietary supplements (NCCIH, 2017). South African literature on fish oils consumption among adults is scarce.

2.3.4 Controversy over fish consumption during pregnancy

Fish may be a recognised means of exposure to pollutants such as dioxins, polychlorinated biphenyls (PCBs), methylmercury, and other heavy metals (Oken *et al* 2013; Turunen *et al*, 2010; Costa & Fattori, 2010).

According to Sidhu (2003) “PCBs and dioxins are lipophilic, so high levels may be found in the adipose tissue of fatty fish”. Thus, fish PCBs content may be reduced by 12 – 40% through removing skin, cooking and trimming fat (Mozaffarian & Rimm, 2006). However, the fat in fish is also rich in the essential fatty acids and fat-soluble micronutrients. Furthermore, fish is an important dietary source of selenium, which may offer some protection against mercury toxicity (Ralston & Raymond 2010) because selenium binds to methylmercury, making it unobtainable to the brain (Ralston, 2008). In addition, mercury and PCBs were poorly associated with birth

weight (Taylor *et al,* 2016; Grandjean *et al,* 2001). Foran *et al*, (2005) “developed a risk ratio relating cancer risk and other diseases with the cumulative exposure to organic contaminants and to the omega-3 contents present in fish” but the risk contaminants was outbalanced by the omega-3 fatty acid benefits to health (Fernandes *et al*, 2012; Grandjean *et al,* 2001). Therefore, fish consumption is still recommended during pregnancy.

2.4 Association between maternal fish consumption and birth outcomes

The birth outcomes of interest to this study include gestational age at birth and newborn anthropometry (specifically birth weight and head circumference).

High intake of fish has been associated with health benefits in the general population. LCPUFA and other numerous nutrients found in fish make it a healthy food option for humans. In a Norwegian study, increasing fish consumption from 0–1 times per month, 2–3 times per month, 1–3 times per week, 4–6 times per week and 1–2 times per day in adults have been associated with incremental lower risk of metabolic syndrome and healthy metabolic profile (Tørris *et al,* 2016). In addition, consuming fish may be protective against certain cancers, lowering the risk of coronary heart disease (due to improved lipid profile) (Chapman *et al*, 2011), death or sudden death in adults (Pieniak *et al*, 2008).

The nutrients found in fish, specifically PUFA, protein, selenium, iodine, and vitamin D, are also considered to be beneficial for foetal growth and development (Starling *et al*, 2015; Thorsdottir *et al*, 2004). Maternal fish consumption during pregnancy has shown to lower the risk of preterm birth and associated increased newborn birth weight (Lauterbach *et al*, 2018; Drouillet *et al*, 2009; Muthayya *et al,* 2009; Grandjean *et al,* 2001) and length (Guldner *et al*, 2007). In contrast, some studies have reported lower foetal growth indices (or small for gestational age) with associations of higher intake of seafood or EPA and DHA during pregnancy (Halldorsson *et al*, 2007; Oken *et al*, 2004). These has been ascribed to possible pollutants consumed with fatty fish. Also in a study of Danish pregnant women, no association was found with growth measures and fish consumption during pregnancy (Heppe *et al*, 2011; Halldorsson *et al*, 2007; Knudsen *et al*, 2006). An observational study in the United States found an inverse association between maternal fish consumption and foetal growth and no association was observed with length of gestation (Halldorsson *et al*, 2007; Oken *et al,* 2004). Likewise, a prospective cohort study from early pregnancy onwards in The Netherlands with a low fish consumption population found no consistent associations of total fish consumption and consumption of different types of fish with foetal growth characteristics (Heppe *et al*, 2011; Halldorsson *et al*, 2007; Knudsen et al, 2006). The literature shows inconsistencies in the results, which have been attributed to different types of fish, such as lean fish, fatty fish and shellfish as well as pollutants accumulating in fatty fish.

The following sections will discuss the current literature on fish consumption and the birth outcomes of interest to this study.

2.4.1 Maternal fish consumption and gestational age at birth

As indicated in chapter 1, gestational age describes foetal age at birth (Boyle *et al*, 2012; Srinivasjois *et al*, 2015; Oken *et al*, 2003) while birth weight is the first weight of the foetus obtained shortly after birth (WHO, 2006; De Bernabé *et al*, 2004). Both gestational age and birth weight as birth outcomes are important determinants of neonatal and infant survival (Gebremedhin *et al*, 2015; Sharma *et al*, 2015; Kemfang Ngowa *et al*, 2014 and Shalini & Vipul, 2010). The below paragraph will discuss the association between intake of fish during pregnancy and gestational age at birth.

LBW is an essential determinant of perinatal survival, infant morbidity, and mortality as well as the risk of developing disabilities and illnesses in later life and LBW can be caused by preterm birth and poor maternal nutrition before and during pregnancy (Gebregzabiherher *et al*, 2017; Sharma *et al*, 2015; Ramakrishnan, 2004).

Consuming fish more than once a week during pregnancy has been associated with a lower risk of preterm birth (Olsen *et al*, 2018; Brantsæter *et al*, 2017; Hack *et al,* 2002). A possible biological mechanism responsible for the reduction in risk of preterm delivery is due to elevated levels of PUFA that prevent the synthesis of dienoic prostaglandins F2α and prostaglandins E2 (Facchinetti *et al,* 2005). According to Hong *et al*, (2016) “prostaglandins (PGs) are considered the universal mediators of parturition” and amniotic fluid is the key source of PGE2 and PGF2α. Elevated levels of LCPUFA “may prolong gestation by inhibiting the production of the prostaglandins that seem to play a part in parturition, cervical ripening, and initiation of labour” (Brantsæter *et al*, 2017; Starling *et al*, 2015; Rogers *et al*, 2004; Allen & Harris, 2001) (see figure 2.2).

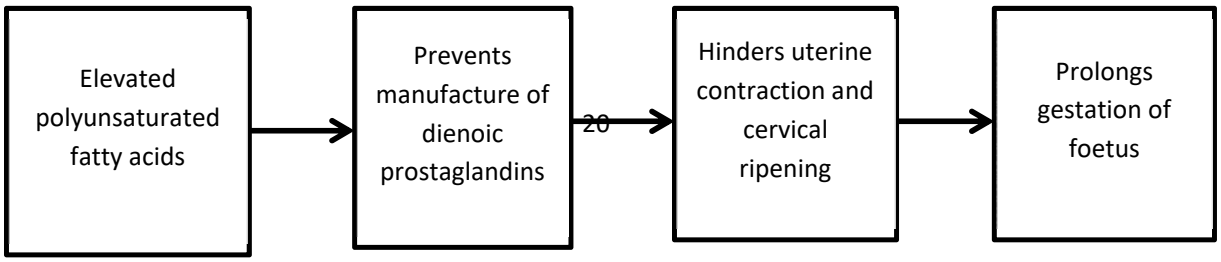

Figure 2.2: Possible biological mechanism by which fish consumption during pregnancy is associated with reduced risk of preterm delivery.

2.4.2 Maternal fish consumption and neonatal anthropometry

Birth weight is the weight of the newborn obtained within the first hour after birth (WHO, 2006). A number of previous studies suggest that high maternal consumption of seafood during pregnancy is associated with increased birth weight (Taylor et al, 2016; Starling *et al*, 2015; Leventakou *et al*, 2014; Brantsaeter *et al*, 2012; Muthayya *et al*, 2009; Allen & Harris 2001). In addition, "besides birth weight, neonatal head circumference has been positively related to maternal fish intake" (Brantsæter *et al*, 2012; Drouillet *et al*, 2009; Thorsdottir *et al*, 2004).

Consuming enough fish or omega-3 fatty acids during pregnancy may prolong gestational age and increase foetal growth rate (Larsen *et al,* 2016). DHA decreases the thromboxane/prostacyclin synthetic ratios through possible biological mechanism for the improved foetal growth rate (Heppe *et al,* 2011; Drouillet *et al*, 2009). Reduced ratios of thromboxane has been link to improved placental blood flow resulting in an increased foetal growth rate (Rogers *et al*, 2004) (see figure 2.3).

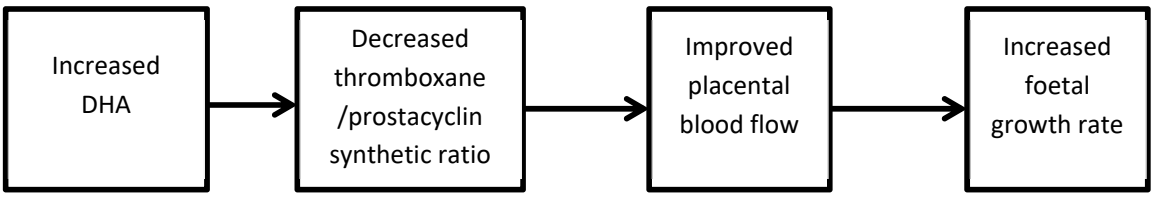

Figure 2.3: Possible Biological Mechanism by which fish intake during pregnancy is associated with increased birth weight

According to Muthayya *et al,* (2009) "fish intake, particularly in the third trimester, is closely associated with birth weight" and pregnant women consuming fish before pregnancy and during pregnancy have been associated to give birth to infants with increased birth weight (Drouillet-Pinard *et al*, 2010; Drouillet *et al*, 2009).

The consumption of fish by France pregnant women for at least five times in a month had significant impact in neonatal birth weight and fish consumption by pregnant women more than nine times in a month increased the neonatal birth weight by 169g (Droulliet *et al,* 2009).

Furthermore, omega-3 fatty acids found in fish increases the neonate's birth weight in pregnant women who smoke. Smoking during pregnancy is known to cause a reduction in birth weight and omega-3 LCPUFA counter the effect of oxidative stress damage on foetal tissues (Zheng *et al*, 2016; Bernstein *et al*, 2005).

Therefore, moderate consumption of fish during pregnancy may lower the risk of preterm birth and may increase newborn birth weight, which may especially benefit developing countries, which account for greater than 50% of global preterm and LBW cases (Ramakrishnan, 2004).

2.5 Conclusion

Although there are some controversies over the recommendations of fish consumption during pregnancy, the nutritional contribution of fish during pregnancy providing essential fatty acids plays an important role in foetal, infant and maternal health. Nutritional health benefits of fish consumption during pregnancy may be of help to the current global challenges on neonatal birth outcomes such as preterm birth, low birth weight, stillbirth, neonatal death and maternal deaths. Preterm birth is the leading cause of death in children below the age of five (WHO, 2015), which may be preventable. In addition, the general population should be educated on the nutritional health benefits of fish consumption (such as EPA and DHA). The health benefits of fish consumption may be of help in reducing the development of diseases associated with the deficiencies of the nutrients found in fish.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Background

This Master's research project is a sub-study of a larger project, entitled: *Nutrition during Pregnancy and Early Development, the NuPED study.* The NuPED study is using a longitudinal observational research design and the aim is to assess dietary intake and

nutritional status of urban pregnant women in Johannesburg, South Africa and to determine associations with birth outcomes, maternal health and offspring health. This larger project involves team members from the University of Witwatersrand, University of South Africa (UNISA) and North-West University (NWU).

The aim of this Master's project (sub-study) is to determine levels of maternal fish intake at early pregnancy and their association with birth outcomes of pregnant women in Johannesburg, South Africa. In this chapter, the research methodology used to collect the necessary data was described.

3.2 Study Design

A longitudinal observational research design was used in order to reach the study aim. However, the dietary intake data obtained once in early pregnancy allowed for cross-sectional analyses as well.

3.3 Study Area

The Republic of South Africa (RSA) has nine provinces which includes Gauteng. The study was conducted in the city of Johannesburg, the provincial capital of Gauteng province (see figure 3.1). It is the largest city in South Africa, also known as Jozi, Jo 'burg, or Egoli. (World Population Review, 2017). In 2017, Johannesburg's population was estimated at 9,823,000 which consist of 76.4% Black African, 12.3% White, 5.6% Coloured, and 4.9% Indian/Asian residents (World Population Review, 2017).

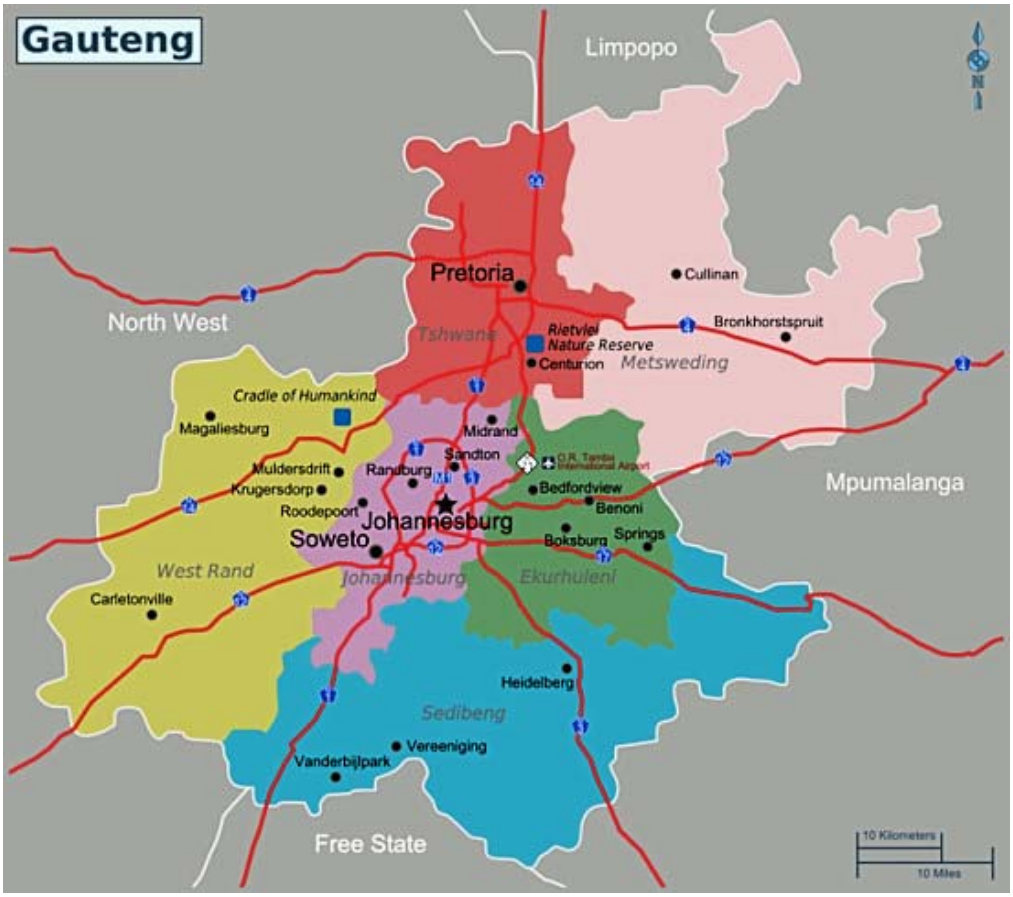

Figure 3.1 Map of Gauteng province (Hypertext, 2014)

3.4 Study Setting

The study participants were recruited from four primary healthcare clinics in regions B and C of the city of Johannesburg (see figure 3.2) as well as the antenatal clinic of a tertiary healthcare facility, namely Rahima Moosa Mother and Child Hospital (RMMCH) (also situated in region B). Data collection took place at the antenatal clinic of RMMCH in Johannesburg.

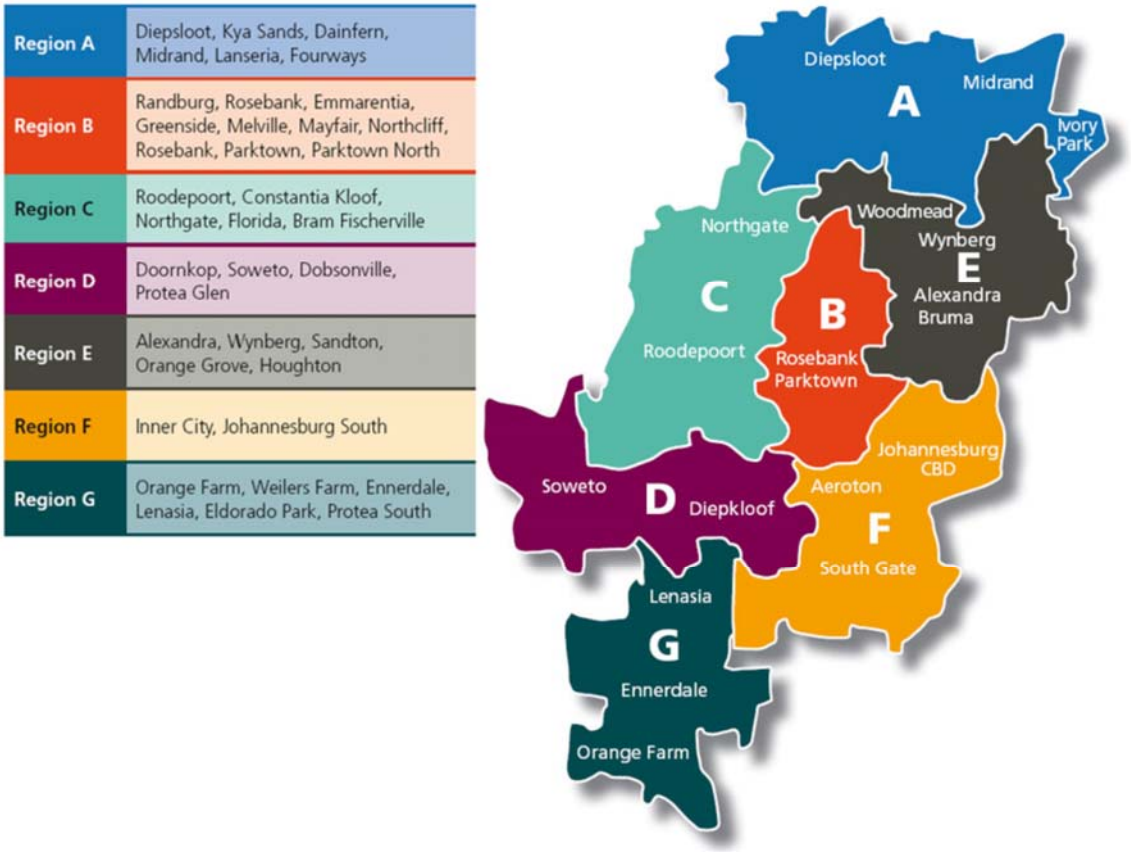

Figure 3.2: The seven regions of the City of Johannesburg (Hypertext, 2014)

3.5 Study Population and Sampling

3.5.1 Study Population

The study population includes all pregnant women attending antenatal care for the current pregnancy at different clinics of regions B and C in the City of Johannesburg. The primary health care clinics where recruitment took place are Zandspruit, Bosmont, Sophiatown and Florida clinics as well as the antenatal clinic of RMMCH.

3.5.2 Sample and Sampling Method

The participants were sampled by means of consecutive sampling, thus, all accessible women at the recruitment site formed part of the sample. The researcher or the nurse, who was also trained and served as part of the field worker explained the study to all pregnant women in the waiting area. Those interested, were screened individually in a private setting according to inclusion and exclusion criteria (see the next paragraph). After screening, those eligible to be included were invited to take part in the study and referred to RMMCH for further antenatal care, signing of written informed consent and data collection on a specific date. Those not

eligible received a thank you note which included guidelines for healthy living during pregnancy.

The participants that were included in the research study met the following criteria: confirmed pregnancy at less than18 weeks' gestation (as confirmed by ultrasound sonography); those who were planning to deliver the baby at RMMCH; those born in South Africa, Lesotho, Swaziland, Zimbabwe, Botswana or Namibia and able to communicate effectively in one of the following languages: English, Afrikaans, Sotho, Zulu or Xhosa. The following women were not included in the research study on the basis of confounding factors for the outcome variables (low-birth weight and premature birth): less than 18 years of age and greater than 39 years of age; multiple pregnancy; women using illicit drugs (self-confessed); smoking (current and/or in past year); known non-communicable diseases (NCDs) namely diabetes, renal disease, high cholesterol and hypertension; known infectious disease such as tuberculosis and hepatitis; and known serious illness namely cancer, lupus or psychosis. HIV status was not an inclusion or exclusion criteria.

3.5.3 Sample Size

The sample size for this project was *N* = 102 pregnant women. The larger study had a sample size of 250 participants based on statistical power determination. However, considering time limitations for a Master's study, the analyses of this study was limited to the first 102 participants enrolled in the study.

3.6 Methods of Data Collection

Larger study sample were assessed four times throughout the pregnancy. The first phase data collection was done in early pregnancy (<18 weeks), the second phase at ±22 weeks gestation and the third phase at ±36 weeks gestation. Each assessment took between 1.5 – 2 hours per person for the first three phases. The fourth and final phase took place at birth. All the study assessments were done at assigned private spaces in the antenatal clinic (ANC) while the assessments performed by the nursing staff were done in consulting rooms. Table 3.1 summarises the assessments relevant for this study. The data collection process is summarised in Figure 3.3. Data collected for the first and final phases were used in this study.

Table 3.1: Schedule of study activities during pregnancy

Visit number (phase)	1	4
Approximate gestational age	<18 weeks	40 weeks (Birth)
Informed consent	**X**	
Medical history*	**X**	**X**
Socio-demographics	**X**	
Ultrasound screen (for confirmation of gestational age)	**X**	
Quantitative food frequency questionnaire	**X**	
New born anthropometrical measurements		**X**
New born assessment		**X**

*: Medical history from Maternity Case Record to determine adverse outcomes or change in health status

Phase 1 data collection
Phase 4 data collection

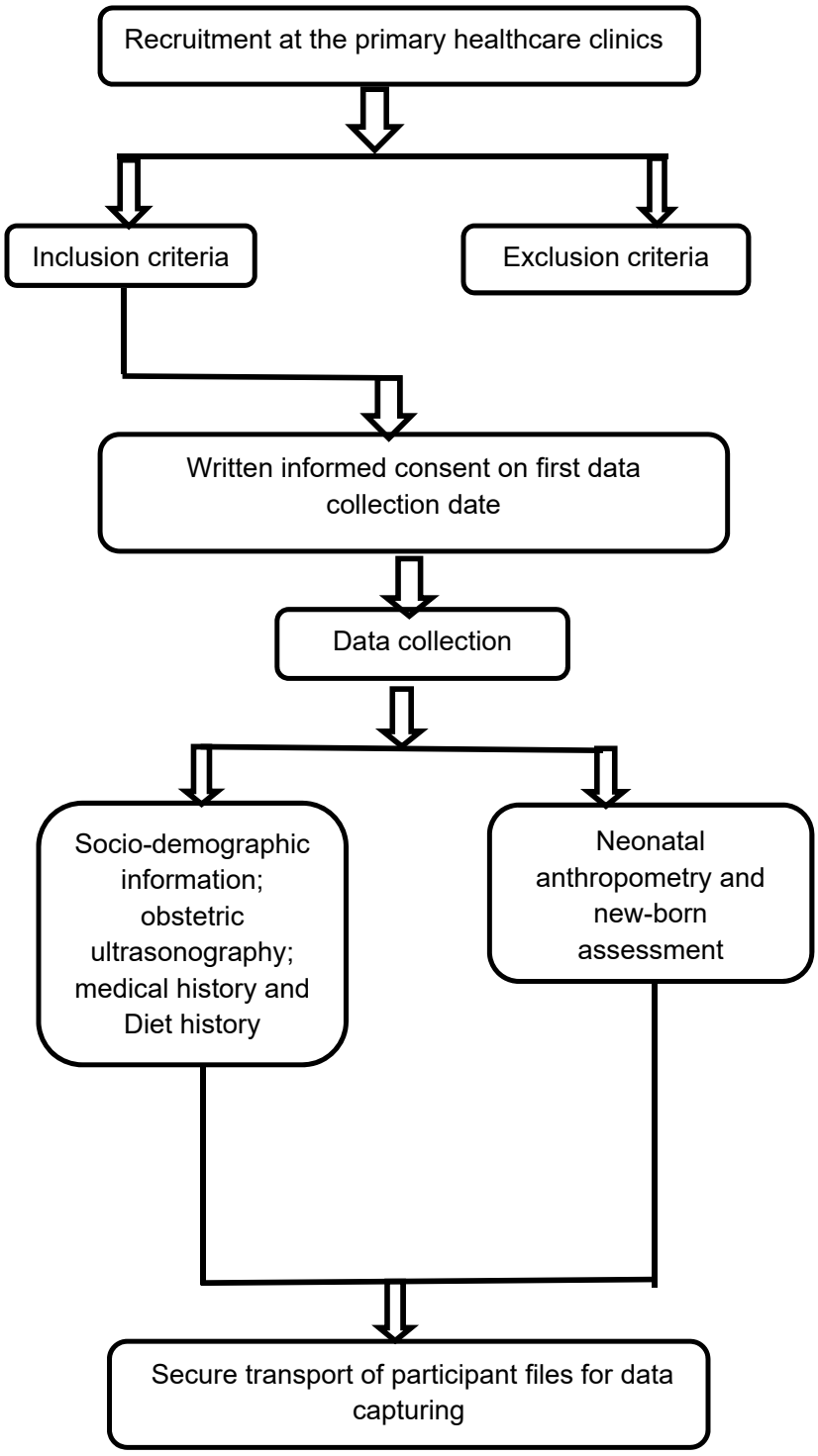

Figure 3.3 Simplistic diagram of the data collection process

In order to reach the research objectives, the type of data collected were socio-demographic data and medical history for descriptive purposes (and determining confounding factors); dietary intake data to determine fish consumption; foetal assessment by means of ultrasonography to determine gestational age; neonatal anthropometry and new-born assessment to determine if the birth was via natural vaginal delivery (NVD) or caesarean section (CS). Seven different types of questionnaires and forms were used in order to obtain the data relevant to this study. These will be described in more detail in the following sections.

3.6.1 Diet History

The pregnant women's usual dietary intake was determined by means of the quantitative food frequency questionnaire (QFFQ) during an interview with trained fieldworkers at <18 weeks. The QFFQ was designed to capture habitual dietary intake, pregnant women fish intake were assessed within 45 – 60 minutes. The QFFQ was validated for the population in the Transition and Health during Urbanisation of South Africans (THUSA) study (MacIntyre *et al*., 2001a) and its reproducibility was proven (MacIntyre *et al*., 2001b; Wentzel-Viljoen *et al*., 2011). The QFFQ questions include intake of 140 food items to determine the overall diet. Fourteen questions were asked about fish and fish products. Participants were requested to recall how often and how much of the listed foods and drinks were consumed in the last four weeks.

This was done as an interview in a quiet room. Dietary kits (see Figure 3.5) with food models, picture books, measuring tools etc. was used to identify food portion sizes. Participants were asked to describe the food, preparation method, time, place and amount of food which was recorded in the QFFQ by the trained fieldworkers (see Appendix 1).

Figure 3.5: Two dietary kits used for data collection in the NuPED study

3.6.2 Socio-Demographic information

The participant's socio-demographic information was obtained by means of a socio-demographic questionnaire. This included the Living Standards Measure (LSM) as developed by the South African Audience Reference Foundation (SAARF) (LSM, 2014; SAARF, 2001). The LSM divides the population into 10 groups; it ranges from 10, as the highest and 1, as the lowest and an online calculator was used to determining the LSM (LSM, 2014). Participants were asked information about their population group, date of birth, home language, country of birth, highest formal education, marital status, employment status and the number of people in household. This was interviewer administered and recorded in the socio-demographic questionnaire by the trained fieldworkers (see Appendix 2).

3.6.3 Obstetric Ultrasonography Information

The larger study obtained ultrasound data at all three phases. The first phase was used to determine the participant's gestational age through ultrasonography examination performed by an obstetrician or sonographer and was captured on the study obstetric ultrasonography form by the obstetrician or one of the researchers during the sonographic examination (see Appendix 3). This study included single, live pregnancies at <18 weeks gestation.

3.6.4 Medical History

The participant's medical history was assessed from the participant's maternity case record at <18 weeks and at birth. This was recorded in the medical history form by the trained fieldworkers (see Appendix 4).

3.6.5 Neonatal Anthropometry Information

Neonatal anthropometry such as birth weight, crown heel length (CHL), mid arm circumference (MAC) head circumference (HC), thoracic circumference (TC) (Symington *et al*, 2018) were measured and recorded by the study mid-wife. If the measurements could not be taken within 24 hours after birth, the hospital records were used to obtain the anthropometrical measures. All scales in the labour ward and theatre were calibrated. The data were recorded in the study's neonatal anthropometry form (see Appendix 5).

3.6.6 New-born Assessment

The new-born was assessed after delivery by the study mid-wife in the delivery room. Data captured included new-born date of birth, time of birth, gender, gestational age, use of resuscitation, total Apgar score, mode of delivery and problems encountered during delivery. The data were recorded in the study's new-born assessment form (see Appendix 6).

3.7 Birth Assessments

The birth assessments were conducted after delivery on the new-born by the study mid-wife in the delivery room and data were recorded in the study's neonatal anthropometry form (see Appendix 6). The new-borns were assessed at birth using World Health Organisation (2006) standard on neonatal birth weight, crown heel length, head circumference, mid-upper arm circumference and thoracic circumference. All the measurements were taken twice and the averages were used for the analyses.

3.8 Statistical Data Analysis

All data apart from the dietary data were captured in Statistical Package for Social Sciences (SPSS) IBM version 23 (2015). The dietary data were captured in a Microsoft Excel (2010) spread sheet for further analyses by the South African Medical Research Council. However, for the purposes of this study, the required data were extracted from Excel for statistical analyses. Descriptive statistical analysis was done on socio-demographic data (population group, home language, country of birth, formal education level, marital status, employment status, number of people living in household and living standards measure). Results are presented as frequencies and percentages in tables.

Association between maternal fish consumption during early pregnancy and the following birth outcomes were analyzed using Spearman correlations: Neonatal anthropometry viz: birth weight, crown-heel length, head circumference and ggestational age at birth.

All data was analysed using Statistical Package for Social Sciences (SPSS) with statistical significance set at $p < 0.05$.

3.9 Quality of Data

3.9.1 Validity

Validity can be defined as the degree to which a measurement measures what it purports to measure (Bolarinwa, 2015; Kimberlin & Winterstein, 2008). The questionnaires and instruments used for this project were examined by experts in the field in content, construction, standard and appearance and are validated in relation to the objectives of the research study and it answers the following:

- The questionnaire measure what it supposed to measure
- It represents the content
- It was suitable for the population
- The questionnaire meet the purpose and goals of the research study

3.9.2 Reliability

Reliability can be referred to the degree to which the results obtained by a measurement and procedure can be stable and consistent (Bolarinwa, 2015; Kimberlin & Winterstein, 2008). The QFFQ has been tested and used in previous studies in similar populations (Richter, 2010). To ensure reliability, measuring instruments should internally validate for internal consistency, test and retest before use, interrater reliability and generalisability coefficient (Sullivan, 2011).

3.10 Research Ethics

The research study required ethical consideration in order to exercise care that the rights of individuals and institutions are protected.

The informed consent form (ICF) integrates the core ethical principles of autonomy, beneficence, non-maleficence and justice that are required. The ICF (see Appendix 7) was given to the pregnant women who were interested in the study and eligible for inclusion at the recruiting clinics. They were therefore able to take the ICF home to read and discuss with family members. The pregnant women brought the ICF with to their first visit for data collection at RMMCH. The trained field-workers explained the ICF and made sure they understood the content of the form (the ICF was available in English, Afrikaans, Sotho, Zulu or Xhosa) (see

Appendix 9 for the English version only). Participants were given time to ask any questions before signing consent. Data was collected after the ICFs were signed.

Ethical clearance has been obtained for the larger NuPED study from University of Witwatersrand (M150968) and North-West University (NWU-00186-15-S1) having the principal investigator (Prof Marius Smuts) and other investigators positioned in these institutions (see Appendix 8, 9 and 10). Similarly, permission was obtained from the Gauteng Department of Health, City of Johannesburg Health District along with permission from the Clinical Manager of RMMCH (see Appendix 11, 12 and 13). Likewise, ethical approval for the Master's study was granted by the College of Agriculture and Environmental Sciences Ethics Committee (UNISA) (2017/CAES/059) (see Appendix 14).

Women were reimbursed for travelling and received an airtime voucher at each visit. They also received refreshments. Furthermore, a token of appreciation was given to participating women at the phase 3 visit of the larger study.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The aim of this project was to determine levels of maternal fish intake at early (<18 weeks gestation) pregnancy and their association with birth outcomes of pregnant women in Johannesburg, South Africa. The participants were sampled by means of consecutive sampling according to the study's inclusion and exclusion criteria as explained in Chapter 3.

This chapter reports the results of the data analyses as tabulated, interpreted and evaluated, having the sampling size as *n* = 102 pregnant women. A total of 102 women were included in this study. There were missing data in some instances as indicated in the tables. Data were missing because of a combination of the following reasons: some women did not give birth at RMMCH; approved amendments to questionnaires (to obtain country of birth and years living in South Africa); women lost-to-follow-up; or data not captured during data collection.

4.2 Study sample characteristics

The women included in this study were between the ages of 18 and 39 (Mean 28 ± 5 years). Most of the women (43.1%) were in the age category of 25 – 31 years. The mean height at <18 weeks gestation was 158.8±6.7cm and mean weight was 70.4±15.62kg. The participants' mean BMI at enrolment was 27.8±5.8kg/m^2. Even though we do not have access to their pre-pregnancy BMI, the BMI at this stage of pregnancy is usually close to the pre-pregnancy BMI and the majority of women were in categories above normal weight, i.e. overweight (35.3%) and obese (29.4%). The South Africa Demographic and Health Survey (SADHS) conducted in 2016 and the South African National Health and Nutritional Examination Survey (SADHS, 2016; Shisana *et al*, 2013) by the Department of Health show that (68% and 40.1%) South African women were overweight or obese which was of high prevalence. In this study one in five women (29.4%) had a BMI ≥ 35.0kg/m^2, placing them in the severely obese category. In this study, only two (2.0%) women were underweight. The mean gestation was 13.5±3 weeks at enrolment. Majority (77.5%) of the women were multigravida while this index pregnancy was the first for the remaining 22.5%. The study sample characteristics are summarized in Table 4.1.

Table 4.1: Study sample characteristics at study entry

Weight (kg), mean (SD) (n=102)	70.4 (15.6)	
Height (cm), mean (SD) (102)	158.8 (6.7)	
Gestational age at enrollment (weeks), mean (SD) (n=102)	13.5 (3.0)	
Maternal age (years) (n=102), frequency (%)		**Mean (SD)**
18 – 24	27 (26.5%)	28.1±5.1
25 – 31	44 (43.1%)	
32 – 38	31 (30.4%)	
Body Mass Index (kg/m^2) (n=102), frequency (%)		**Mean (SD)**
Underweight <18.5	2 (2.0%)	27.8±5.8
Normal weight 18.5 – 24.9	34 (33.3%)	
Overweight 25 – 29.9	36 (35.3%)	
Obese ≥30	30 (29.4%)	
Gravida (n=102), frequency (%)		
Primigravida	23 (22.5%)	
Multigravida	79 (77.5%)	

4.3 Socio-economic and demographic status

The results presented in the table below describe the study sample in terms of socio-economic and –demographic status.

Table 4.2: Socio-economic and -demographic status of the participants

Population group (n=101), frequency (%)	
Black African	89 (88.1%)
Colored	9 (8.9%)
White	2 (2.0%)
Other	1 (1.0%)
Home language (n=101), frequency (%)	
English	11 (10.9%)
Xhosa	7 (6.9%)
Zulu	32 (31.7%)
Sotho	22 (21.8%)
Other	29 (28.7%)
Country of birth (n=92), frequency (%)	
South Africa	66 (71.7%)
Zimbabwe	22 (23.9%)
Lesotho	1 (1.1%)
Swaziland	3 3.3%)
Formal education level (n=101), frequency (%)	
Primary school	2 (2.0%)
Grade 8-10	14 (13.9%)
Grade11-12	59 (58.4%)
Tertiary education	26 (25.7%)
Marital status (n=101), frequency (%)	
Unmarried	45 (44.6%)
Married	27 (26.7%)
Living together	22 (21.8%)
Traditional marriage	7 (6.9%)
Employment status (n=101), frequency (%)	
Unemployed	47 (46.5%)
Self-Employed	5 (5.0%)
Wage-Earner	48 (47.5%)
Self-employed Professional	1 (1.0%)
Number of people living in household (n=99), frequency (%)	
Living alone	6 (6.1%)
2 – 5 members	85 (85.9%)
> 5 members	8 (8.1%)
Living standards measure (LSM) (n=102), frequency (%)	
1 – 3	0 (0%)
4 – 6	40 (39.2%)
7 – 8	38 (37.3%)
9 – 10	24 (23.5%)

The study participants were mainly black African (88.1%), Zulu-speaking (31.7%) women born in South Africa (71.7%). Most of them were unmarried (44.6%), living in households of 2 – 5 members (85.9%), wage-earning (47.5%) and had Grade 11 or 12 schooling (58.4%). None of the participants had living standards between levels 1 – 3, but most had living conditions at levels 4 – 6 (39.2%).

4.4 Dietary intake

The results of the analyses from the QFFQ were supplied and discussed in the sections below.

4.4.1 Total energy, protein, fat and carbohydrate consumption per day

The results presented in Table 4.3 describe the participants' consumption in terms of total energy, protein, fat and carbohydrate.

Table 4.3: Total energy, protein, fat and carbohydrate consumption per day

Total energy intake (kJ) (n=97), frequency (%)	
< 10000	28 (28.9%)
10001 – 13000	20 (20.6%)
>13000	49 (50.5%)
Protein intake (g) (n=97), frequency (%)	
< 70	21 (21.6%)
70 – 80	11 (11.3%)
>80	65 (67.0%)
Fat intake (g) (n=97), frequency (%)	
< 50	9 (9.3%)
51 – 100	40 (41.2%)
101 – 150	30 (30.9%)
>150	18 (18.6%)
Carbohydrate intake (g) (n=97), frequency (%)	
< 175	4 (4.1%)
175 – 340	26 (26.8%)
>340	67 (69.1%)

Most participants had a total energy intake >13000kJ (50.5%). The estimated energy requirement (EER) for women in their second trimester is 11521kJ (IOM, 2005). Many women consumed more than the EER and may therefore explained the high overweight and obesity prevalence in this group. Most women had a total carbohydrate intake >340g (69.1%). The Recommended Dietary Allowance (RDA) for carbohydrate intake of pregnant women per day is 175g/day (IOM, 2005). These results show that most women consumed more than the RDA (almost double the RDA) which might contribute to overweight and obesity among the group. At a 50% carbohydrate intake of a recommended total energy of 11500kJ, carbohydrate consumption can be up to 340g per day, however, most of the women consumed more than this. Majority of the participants' fat intake were between 51 – 100g (41.2%) and most women's protein intake were >80g (67.0%). The RDA for protein intake for pregnant women in the second trimester per day is 71g/day (IOM, 2005). At a 15% protein intake of a recommended total energy of 11500kJ, protein consumption can be up to 100g per day. Many women consumed more than the RDA for all the macronutrients, which might contribute to the high overweight and obesity among the participants.

4.4.2 Fish consumption

The results presented in Table 4.4 below indicated the participants' fish consumption per day. According to the Food-Based Dietary Guidelines for South Africa (FBDG-SA) (Schonfeldt *et*

al, 2013) it is recommended to consume 2 – 3 servings of fish per week (80-90g per portion). However, in order to compare our results with the works of others, the fish consumption categories are based on those presented by (Brantsaeter *et al*, 2012). These categories correspond to; rarely; (<5 g/day); <1 serving/week (5–20 g/day), 1–2 servings/week (20-40 g/day), 2–3 servings/week (40-60 g/day) and 3 or more servings/week (>60 g/day). When seafood was eaten as bread spread the serving size was estimated as 20–25g.

In this study, 3.1% of the women consumed fish 2-3 times per week while the majority consumed fish rarely (76.5%). Similarly, 1.0% consumed fatty fish 2-3 times per week and 1.0% lean fish. The average (median) fish intake was 4.8 g/d (0; 25) which falls in the "rarely consume fish" category.

Table 4.4: Fish consumption per day

Total fish				**Fattyfish (g/d) (n=98)**			**Leanfish (g/d) (n=98)**		
(g/d) (n=97)	**Frequency**	**%**	**Median (25^{th}; 75^{th})**	**Frequency**	**%**	**Median (25^{th}; 75^{th})**	**Frequency**	**%**	**Median (25^{th}; 75^{th})**
<5	75	76.5	4.8 (0; 25)	80	81.6	0.3 (0; 9.3)	83	84.7	0 (0; 8.4)
5 – 20	13	13.3		13	13.3		12	12.2	
20 – 40	0	0.0		0	0.0		0	0.0	
40 – 60	3	3.1		1	1.0		1	1.0	
>60	7	7.1		4	3.9		2	2.0	

This study sample therefore had a very low fish intake. The reasons for the low fish consumption can be speculated as fish being expensive and the fact the inland consumers typically consume less fish than those residing at the coast.

4.5 Birth Results of the study sample

The study birth outcomes included three miscarriages or intra-uterine foetal deaths (IUFD) for which, therefore, there were no birth data. There was also one neonatal death after early delivery, however, the new born data was available and one maternal death after delivery.

The results presented in Table 4.5 contained the information about the gestational age at birth, neonatal birth weight, head circumference and crown heel length.

Table 4.5: The anthropometric birth results of the study sample

Gender (n=92), frequency (%)				
Boys	50 (54.3%)			
Girls	42 (45.7%)			
Gestational age at birth (weeks) (n=100), frequency (%)				
< 28 (very early preterm)	1 (1.0%)			
28 – 32 (early preterm)	2 (2.0%)			
32 – 37 (preterm)	10 (10.0%)			
>37 (full term)	87 (87.0%)			
Birth weight (g) (n=88), frequency (%)		**Mean**	**Mean for boys (SD)**	**Mean for girls (SD)**
<2500	11 (12.5%)	2999.2	3157.3±571	2819.3±671
2500 – 3999	74 (84.1%)			
>4000	3 (3.4%)			
Head circumference (cm) (n=87), frequency (%)		**Mean**	**Mean for boys (SD)**	**Mean for girls (SD)**
≤ 31.49	8 (9.2%)	34.3	34.5±4.3	34.1±2.4
31.50 – 35.81	61 (70.1%)			
>35.81	18 (20.7%)			
Crown heel length (cm) (n=80), frequency (%)		**Mean**	**Mean for boys (SD)**	**Mean for girls (SD)**
31 – 40	3 (3.6%)	49.4	49.8±4.9	49.3±4.3
41 – 50	45 (54.2%)			
>51	35 (42.2%)			

In this study, 54.3% of the babies born were boys. Most of the infants (87.0%) were born at full term (37 weeks and above), 10.0% were born at moderate to late term (32 - 37 weeks) and 2.0% were born at very preterm (28 to 32 weeks) and 1.0% were born at extremely preterm (<28 weeks). Thus, the prevalence of premature birth in this study was 13.0%. This included spontaneous delivery as well as assisted delivery. The mean gestational age at birth was 38.8±2.4 weeks.

Internationally, an estimated 15 million babies are born preterm every year with the incidence ranging from 9.54% to 10.41% of live births (Word Health Organization (WHO), 2015; Ferre *et al*, 2016)]. Thus the incidence of premature birth among this group seems to be somewhat higher than the global incidence. However, the information on the preterm birth and low birth weight of South Africa and Gauteng province were not available but the percentages of low birth weight of other Southern African countries are: Angola (12%) 2000, Botswana (13%) 2007, Lesotho (11%) 2009, Malawi (14%) 2010, Mozambique (17%) 2011, Namibia (16%) 2006 – 2007, Swaziland (9%) 2010, Zambia (11%) 2007 and Zimbabwe (11%) 2010 – 2011 (UNICEF, 2014).

The results furthermore indicated that 84.1% of the babies had a normal birth weight between 2500 and 3999g, while 12.5% were low birth weight of <2500g and 3.4% were macrosomic (>4000g). The mean birth weight was 2998.2±624.4g, while there were significant difference between the birth weight of boy's birth weight mean 3157.3±571g and girls 2819.3±671g (p=0.015).

With regards to the head circumference, 70.1% of the babies had a head circumference of 31.50 – 35.81cm, 20.7% with a head circumference of >35.81cm and 9.2% of the babies had a head circumference <31.49cm. The head circumference mean was 34.3±3.6cm, with boys head circumference mean of 34.5±4.3cm and girls head circumference mean of 34.1cm. According to Sutan *et al*, (2018) "head circumference is used to monitor the growth of brain volume and is known to be a significant predictor of cognitive and intelligence development of a child".

In this study, 20.7% of the new-borns were above the WHO head circumference-for-age growth standards at the 50th percentile for boys and girls (WHO, 2018); 70.1% between the 5th and 25th percentiles while 9.2% were below the 3rd percentile (WHO, 2018).

Studies have shown that crown heel length was a reliable and universal indicator of linear growth and nutritional status for infants from birth up to 2 years of age (Ismail *et al*, 2016). Likewise crown heel length was a predictor of perinatal mortality, with long infants being at higher risk of perinatal death (Fok *et al*, 2003). The result indicated that 42.2% of the babies had crown heel length of >51cm, 54.2% with a crown heel length of 41 – 50cm and 3.6% of the babies had crown heel length of 31 – 40cm.

4.6 Association between fish consumption and birth results

The results presented in the Table 4.6 contained the information about the association between total fish consumption at early pregnancy and birth results. Correlation analyses were

conducted to examine the associations between total fish consumption in early pregnancy and birth weight, gestational age at birth, head circumference and crown heel length.

There were no statistically significant associations between fish consumption at early pregnancy and birth outcomes such as gestational age at birth (r=0.051; p=0.625), birth weight (r=-0.043; p=0.695) and crown heel length (r=0.008; p=0.943). There was a positive association between maternal fish consumption in early pregnancy and head circumference of the new-born which tends towards statistical significance (r=0.193; p=0.079).

Table 4.6: Association between total fish consumption in early pregnancy and birth outcomes

	Correlation coefficient (r)	p-value
Gestational age at birth (weeks)	0.051	0.625
Birth weight (g)	-0.043	0.695
Head circumference (cm)	0.193	0.079
Crown heel length (cm)	0.008	0.943

4.7 Discussion of findings

This study first examined the types and levels of maternal fish consumption at early pregnancy. The results show that maternal fish intake was generally low and majority of the pregnant women (76.5%) consumed fish rarely (<5g/day). Only 13.3% women consumed fish <1 times per week (5-20 g/day). Of those who consumed lean fish, only 2.0% had a serving of 3 times or more per week and 3.9% had fatty fish of 3 times or more per week. Thus lean fish were consumed more frequently. Even though average per capita fish intake may be little, the small amounts of fish can have a substantial positive nutritional impact by providing essential amino acids, fats and micronutrients.

This study furthermore examined the association between fish consumption during early pregnancy and neonatal birth weight, gestational age, neonatal head circumference and neonatal crown heel length. This study observed a positive association between maternal fish consumption in early pregnancy and head circumference (r=0.193; p=0.079) which tends toward statistical significance. This was in agreement with earlier research of a Norwegian Mother and Child Cohort Study (Brantsæter *et al*, 2012), that increasing maternal fish consumption during pregnancy had showed a consistent increase in new-born head circumference.

This study indicated no statistically significant association between fish consumption at early pregnancy and gestational age at birth (r=0.051; p=0.625), birth weight (r=-0.043; p=0.695) and crown heel length (r=0.008; p=0.943). Likewise, the study of pregnant women in a Massachusetts US cohort study was in agreement with this study that omega-3 fatty acids and fish (seafood) intake were not associated with length of gestation or risk of preterm birth and concluded that fish (seafood) intake during pregnancy was associated with reduced foetal growth (Oken *et al*, 2004).

The study birth outcomes results were not statistically significant, maybe because this study considered maternal fish intake at early pregnancy (<18 weeks) and birth outcomes. It should be considered that fish intake later in pregnancy may have significant effect on foetal growth. Also, it is possible that there was no correlation in the birth outcomes results because the maternal fish intake at <18 weeks gestation was generally very low (total fish 76.5%, fatty fish 81.6% and lean fish 84.7%). The small sample size is a further limitation.

The health benefits of fish intake recognised in human studies (such as reduced risk of coronary heart disease mortality) are mostly related to consumption of species with a high content of omega-3 fatty acids (Mozaffarian, &. Rimm, 2006). The associations between maternal fish consumption and new born measures observed in this study are unlikely to be explained by fish (marine) omega-3 fatty acids because this study considered the total fish intake at early pregnancy. One likely explanation may be the composition of protein in fish. Bioactive peptides released from proteins upon intestinal digestion may modulate specific physiological functions in the human body (Erdmann *et al*, 2008). This study was not able to calculate concentrations of specific amino acids, but experimental studies have revealed that fish proteins fed to pregnant rodents have positively influenced insulin resistance and blood pressure in the offspring (Yahia *et al*, 2003; Tremblay *et al*, 2003). The associations determined by other authors, could therefore be due to the special composition of fish proteins or other substances or a combination of these.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study of generally healthy, non-smoking pregnant women living in Johannesburg with mean BMI of 27.8±5.8kg/m^2, it was found that most women had very low fish consumption. The birth outcomes such as gestational age at birth, birth weight and head circumference did not correlate with fish consumption at early pregnancy. Gestational age at birth and birth weight are significant determinants of neonatal and infant survival.

Even though our study did not show an association between the specific birth outcomes, there are literatures supporting the consumption of fish during pregnancy. The Food-Based Dietary Guidelines, guidelines for Norwegian pregnant women and the American Heart Association recommend including fish and seafood as part of a balanced diet, which may be of benefit to the pregnant women.

3The lack of significant associations between maternal fish intake in early pregnancy and birth outcomes observed in this study could be a result of the very low quantity of fish consumed by the pregnant women as well as the study sample being small.

5.2 Recommendations

Implementation of the Food-Based Dietary Guidelines for South Africans (FBDG-SA), specifically for fish intake, should be encouraged in the context of heavy metal exposure. These recommendations could be emphasised to pregnant women attending antenatal care and even to the general public attending any health facility. People should be encouraged to consume two to three fish servings per week and preferably fatty fish. Equally important, all South Africans should be enlightening on the nutritional health benefits of fish intake.

Likewise, future research into this topic should expand the regions of the data collections so that the results may be used for the general populate.

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APPENDIX 1

NuPED
Nutrition during Pregnancy and Early Development
Quantitative Food Frequency Questionnaire

☐☐☐ *Participant number* ☐☐ *Phase* **Name of fieldworker:** _____

Today's date: 2 0 1☐ ☐☐ ☐☐ *year month day* **Day of the week:** _____

Please think carefully about the food and drink you have consumed during the ***PAST MONTH*** (four weeks). We have divided the foods into different groups for example all the porridges and cereals together. I will go through a list of food groups and drinks with you and I would like you to tell me:

- Which foods you eat in each of the different food groups
- How the food is prepared
- How much of the food you eat at a time
- How many times a day you eat it and if you do not eat it everyday, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food as well as other food models, containers, etc.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

Before we start I would like to find out what type of margarine, oil and milk you **USUALLY** use in your home.

1. What type of **MARGARINE** do you USUALLY use in your home? Give brand name if possible. Mark ONE.
   - ☐ Tub/Soft margarine (brand name) _____
   - ☐ Brick/Hard margarine (brand name) _____
   - ☐ I don't know
   - ☐ Do not use margarine in home
   - ☐ Butter (brand name) _____

2. What type of **OIL** do you USUALLY use in the preparation of food in your home? Mark ONE.
   - ☐ Sunflower oil (give brand name)_____
   - ☐ Canola oil (give brand name) _____
   - ☐ Olive oil (give brand name) _____
   - ☐ Other (give brand name) _____
   - ☐ Oil previously used _____
   - ☐ I don't know
   - ☐ Do not use OIL ever in the home

3. What type of **MILK** do you USUALLY use in your home? Mark only ONE
   - ☐ Full cream milk / Fresh cow's milk/ Box milk full cream
   - ☐ Low fat milk / 2% milk / Box low fat or 2% milk
   - ☐ Fat free milk / Skim milk / Box fat free or skim milk
   - ☐ Powder milk (eg Elite; give brand name) _____
   - ☐ I don't know
   - ☐ Do not use milk

4. What type of **CREAMER** do you USUALLY use in your home?
   - ☐ Cremora, Ellis Brown, Coffee Mate, Tea Mate etc
   - ☐ Cremora Lite
   - ☐ I don't know
   - ☐ Do not use creamer

QUANTIFIED FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the **LAST MONTH**. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

In the last **four weeks**, did you eat…?

MAIZE MEAL, COOKED PORRIDGES AND BREAKFAST CEREALS

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Maize-meal porridge	Stiff (pap)						4401	
	Soft porridge (slappap)						4400	
	Crumbly (phutu)						4402	
Sour porridge (Tini)	Maize meal						9829	
	Mabella						9827	
	Other:							
Mabella	Stiff						3437	
	Soft							
Oats							3239	
Tastee wheat	Soft						3240	
Other cooked porridge	Type							
Morvite	Soft						9804	
Breakfast cereals	All bran flakes						3242	
	Corn flakes plain						3243	
	Weetbix						3244	
	Rice crispies plain						3252	
	Other:							

If yes, in the last **four weeks**, how often did you eat the food?

Do you pour milk on your maize meal (e.g. stiff, phutu soft porridge), cooked porridge or cereal? Yes 1 No 2

If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____

If no, go directly to the "sugar" section.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
If yes, how much milk	Whole milk/full cream milk/ fresh cow's milk						2718	
	Maas/sour milk						2787	
	Low fat / 2% milk						2772	
	Fat free / skim milk						2775	
	Other							

Do you put sugar on your porridge or cereal? Yes 1 No 2

If no, go directly to the next question "do you put anything else in your porridge?".

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
If yes, how much sugar WHITE or BROWN	Cooked porridge						3989	
	Cereal						3989	
	Other porridge / cereal						3989	
	Other							

Do you put anything else in your porridge? Yes 1 No 2

If yes, what? _____ How much? _____

OTHER STARCH

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and other (e.g. peanuts)	Give ratio of samp:other Specify other:						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
	Any fat added?							
Pasta	Macaroni, plain						3262	
	Spaghetti, plain						3262	
	Spaghetti, canned in tomato sauce						3258	
	Macaroni & cheese Cheese: Milk: Fat:							
	Other specify							
Pizza	Home made: Specify topping						3353 (base+ch +tom+oliv)	
	Bought: Specify topping						3353 (base+ch +tom+oliv)	

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH

How many times do you eat meat (beef, mutton, pork, chicken, fish) per week? _____

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Chicken	Meat & skin, boiled						2926	
	Meat without skin, boiled						2963	
	Meat & skin, roasted/ grilled						2925	
	Meat without skin, roasted/ grilled						2950	
	Kentucky / Chicken Licken (Fried in batter/crumbs)						3018	
	Nando's						2925	
	Other							
Chicken stew	With potato and onion WITH skin						9813	
	With tomato and onion WITH skin						2985	
	With vegetables WITH skin						3005	
	With tomato and onion NO skin						4379	
	With vegetables NO skin						4378	
Chicken BONE stew	With potato, onion and tomato						9814	
	Other							
Chicken feet	Nothing added						2997	
	Stew with potato, onion and tomato						9815	
Chicken head							2999	
Chicken offal	Stew with tomato and onion and sunflower oil						9816	
	Liver, cooked						2970	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
RED MEAT	How do you like your meat? With fat **OR** Fat trimmed							
Red meat BEEF	BRISKET, boiled/fried without added fat						4363	
	BRISKET, fried in added fat Type of fat:						4363	
	Beef, stewed with cabbage						3006	
	Beef, stewed with potato, onion and tomato						9817	
	Beef, stewed with vegetables						3020	
	Mince (lean/ topside), nothing added						2921	
	Mince (regular), nothing added						4363	
	Mince, tomato & onion added						2987	
	Beef **BONE** stew with potato and onion and oil						9819	
	Other							
MUTTON	Meat, with fat, cooked						2947	
	Mutton, no fat, cooked						3036	
	Mutton, chop, grilled						2927	
	Mutton, stewed with vegetables						2916	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Beef/mutton Offal	Offal, cooked						3003	
	Stewed with vegetables							
	Liver, beef, fried/cooked						2920	
	Liver, sheep, fried/cooked						2955	
	Kidney, beef, cooked						2923	
	Kidney, sheep, cooked						2956	
	Brain, sheep, cooked						2952	
	Lung, beef, cooked						3019	
	Lung, sheep, cooked						4337	
	"Gemaldes" (lung & fat)						4409	
	Heart, beef, cooked						2968	
	Heart, sheep, cooked						2969	
	Other							
Goat meat	Grilled/roasted/cooked						4281	
	Stewed with vegetables							
	Other							
Venison/ Wild buck							2913	
Horse/Donkey							9807	
Rabbit							4327	
Other type of meat	Specify							

What type of vegetables is usually put into meat stews?

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Wors / Sausage	Beef & pork, boerewors						2931	
Bacon							2906	
Patties	Beef, fried						2984	
	Chicken, fried						3011	
Cold meats AND Processed meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Frankfurter, beef & pork						2937	
	Frankfurter/Sausage, chicken						3012	
	Russian/Salami						2948	
	Other							
Canned meat	Bully beef, plain						2940	
	Bully beef with potato & onion & oil						2994	
	Other							
Meat pie BOUGHT Or HOMEMADE	Beef						2939	
	Steak and kidney						2957	
	Sausage roll						2939	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought						9818	
	Other							
Biltong	Beef (with fat OR without fat)						3021	
Dried wors Dried sausage	Beef						2949	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Dried beans	Baked beans in tomato sauce						3176	
	Bean salad / Sousbone						3174	
	Soup with dried beans, beef and vegetables						3145	
	Sugar beans, cooked						3205	
	Other							
Lentils	Whole, cooked						3203	
	Lentil soup with beef and vegetables						3153	
Soya products eg. Imana, Knorr, Jileleke, Toppers	Cooked						3196	
	Soup/Gravy made with soya products						9831	
	Stewed with extra potato, onion and tomato						9830	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Pilchards in tomato sauce or chilli or brine	Whole						3102	
	Mashed with fried onion						3102 (70%) 3730 (30%)	
	With tomato and onion						9820	
	Other							
Fish	Hake, fried with batter/crumbs in sunflower oil						3072	
	Hake, fried in sunflower oil						3060	
	Hake, steamed						4373	
	Moddervis / Yellow fish* fried in oil						3084	
	Moddervis / Yellow fish baked with onion (NO oil added)						3089	
	Other							
Other canned fish	Tuna in oil						3056	
	Sardines in oil						3104	
	Sardines in tomato sauce						3087	
	Other							
Fish cakes	Bought: Fried						3080	
	Home made with potato, fried in sunflower oil						3098	
Fish fingers	Bought (baked)						3081	
Eggs	Boiled/poached						2867	
	Scrambled (full cream milk & brick margarine)						2890	
	Scrambled (NO milk, ONLY oil added)						2869	
	Scrambled (NO oil, ONLY full cream milk)						2872	
	Fried in oil						2869	
	Fried in brick margarine						2877	
	Other							

Moddervis/ yellow fish is a more fatty fish than hake.

VEGETABLES

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Cabbage	**How do you cook cabbage?**							
	Boiled, nothing added						3756	
	Boiled with potato and onion and sunflower **oil**						3815	
	Boiled with potato and onion and **brick margarine**						3813	
	Fried in **oil**						3812	
	Fried in **brick margarine**						3810	
	Boiled with potato, onion and tomato and oil						9821	
	Raw with nothing added						3704	
	Other							
Spinach or morogo or beetroot leaves or other green leafy	**How do you cook spinach?**							
	Boiled, nothing added						3913	
	Boiled with **oil** added							
	Boiled with **brick margarine** added						3898	
	Boiled with **tub margarine** added						3899	
	Boiled with potato, onion and tomato and oil						9822	
	Other							
Tomato and onion gravy	With oil						9823	
	Without fat, without sugar						3925	
	Canned						4192	
	Thickened with packet soup powder						9832	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Pumpkin (yellow) Butternut Hubbard squash Table Queen Etc	Boiled, nothing added						4164	
	Boiled with sugar only (NO fat)						3728	
	Boiled with **brick margarine** & sugar						3893	
	Boiled with **tub margarine** and sugar						9833	
	Boiled with **oil** and sugar						9828	
	Other							
Carrots	Boiled, nothing added						3757	
	Boiled with **oil** added							
	Boiled with **brick margarine** added						3816	
	Boiled with **tub margarine** added						3817	
	Boiled with **sugar** only						3818	
	Boiled with **oil** and sugar							
	Boiled with **brick margarine** and sugar						3819	
	Boiled with **tub margarine** and sugar						3820	
	Boiled with potato, onion and sunflower **oil**						3824	
	Boiled with potato, onion and **brick margarine**						3822	
	Boiled with potato, onion and **tub margarine**							
	Chakalaka						9812	
	Raw, nothing added						3709	
	Other							
Mealies/ Sweet corn	On cob – fat added **Fat:**						3725	
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
	Whole kernel, frozen, boiled						4132	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
How do you cook potatoes?								
Potatoes	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Boiled with sunflower **oil** added						3873	
	Boiled with **brick margarine** added						3867	
	Boiled with **tub margarine** added						3868	
	Mashed with **whole milk** and **brick margarine**						3876	
	Mashed with **whole milk** and **oil**							
	Roasted in beef fat						3878	
	Roasted in sunflower oil						3979	
	French fries (chips) / Fried potatoes						3740	
	Other							
Sweet potatoes	**How do you cook sweet potatoes?**							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Boiled with **sugar** and **oil** added						9834	
	Boiled with **sugar** and **brick margarine** added						3749	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Broccoli	Boiled						3701	
	Raw						3702	
Cauliflower	Boiled						3716	
Green beans	Boiled, nothing added						3696	
	Cooked with potato, onion and sunflower **oil**						3794	
	Cooked with potato, onion and **brick margarine**						3792	
	Other							
Mixed vegetables	Canned						4264	
	Frozen, boiled (carrot, corn, peas, green beans)						3727	
	Frozen, boiled (carrot, cauliflower, green beans)						4265	
	Other							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber (no dressing)						3921	
	Raw tomato						3750	
	Cucumber, raw						4119	
	Coleslaw (cabbage) (mayonnaise)						3705	
	Coleslaw (cabbage) (commercial)						3707	
	Potato salad (mayonnaise)						3928	
	Baked bean salad						9824	
	Other salad vegetables							
Mayonnaise / salad dressing	Mayonnaise						3488	
	Vinegar, oil						3487	
	Low oil salad dressing						3505	
	Salad cream						3489	
	Other: Specify							
Other vegetables (specify prep)								

Now we come to fruit

FRUIT

Do you like fruit? Yes 1 No 2

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Apples							3592	
Banana							3540	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Watermelon	Fresh						3576	
Fruit salad	Fresh						3588	
	Canned						3580	
Fig (Vye)							3544	
Avocado							3656	
Wild fruit/berries	Specify type							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Dried fruit	Apple, dried, raw						3600	
	Peach, dried, raw						3568	
	Mixed fruit, dried, raw						3593	
	Mixed fruit, dried and cooked with sugar						3590	
	Fruit roll, dried (all types)						3655	
	Other							
Other fruit	_____ _____							

Let me ask you about ***Custard***.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Custard	Homemade, full cream milk or fresh cow's milk						2716	
	Homemade, lowfat milk						2779	
	Homemade, skim milk						2717	
	Commercial eg Ultramel						2716	
	Other							
Custard with other food (e.g. with jelly, fruit salad, baked pudding)								

BREAD AND BREAD SPREADS

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN (Complete one column)				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	
Do you spread anything on the bread?			Always 1		Sometimes 2		Never 3	
Margarine	What brand do you have at home now?							
	Tub, regular						3496	
	Tub, medium fat						9806	
	Tub, light/low fat						3524	
	Brick, regular						3484	
	Brick, medium fat						9805	
	Brick, lite/low fat						3528	
	Other							
Peanut butter							3485	
Jam/syrup/ honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Cheddar						2722	
	Gouda						2723	
	Other							
Sandwich spread							3522	
Achaar							3117	
Other spreads	Specify							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Dumpling	White flour						9835	
	Whole wheat flour						3212	
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc	Provita						3235	
	Cream crackers						3230	
	Other savoury biscuits like Bacon kips, wheat crackers, etc						3331	

DRINKS

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
White sugar	Tea						3989	
	Coffee						3989	
Brown sugar	Tea						4005	
	Coffee						4005	
Milk per cup of TEA	Do you use milk in your TEA? Yes / No If YES, What type of milk do you use in TEA? If no, go to milk in coffee.							
	Fresh / long life whole/full cream						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free / skim milk						2775	
	Creamer/whitener like Ellis Brown / Cremora						2751	
	Cremora **Lite**							
	Condensed milk						2714	
	Evaporated milk						2715	
	Other							
	None							
Milk per cup of COFFEE	Do you use milk in your COFFEE? Yes / No If YES, What type of milk do you use in COFFEE? If no, go to milk as such.							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Creamer/whitener like Ellis Brown						2751	
	Cremora **Lite**							
	Condensed milk						2714	
	Evaporated milk						2715	
	Other							
	None							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole / full cream milk						2718	
	Fresh/long life: 2% milk / low fat milk						2772	
	Fresh/long life: fat free / skim milk						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other							
Milk drinks	Flavoured milk						2774	
	Milo made with full cream milk						2735	
	Milo made with skim milk						2747	
	Drinking chocolate made with water						4287	
	Other							
Yoghurt	Drinking yoghurt low fat						2756	
	Plain low fat						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar or other						3982	
	- artificially sweetener						3990	
	KoolAid (powder mixed with water)						4027	
	Other							
Fizzy drinks	Sweetened						3981	
Coke, fanta, etc	Diet							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other							
Mageu/Motogo							4056	
Home brew beer							4039	
Beer							4031	
Cider	Sweet						4057	
Spirits Eg Brandy, gin, vodka, whisky, cane, etc							4035	
Wine red							4033	
Wine White							4033	
Other specify								
WATER	Tap, borehole, dam, river, etc						4042	
	Bottled						4042	

SNACKS AND SWEETS								
FOOD	**DESCRIPTION**	**AMOUNT**	**TIMES EATEN** Complete one column				**CODE**	**AMOUNT / WEEK**
			Daily Times/ day	**Weekly** Times/ week	**Monthly** Times/ month	**No**		
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Other nuts								
Savoury snack e.g. Fritos, Doritos, Cheese curls, Niknaks							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Milk chocolate, plain						3987	
	Kit Kat/ Tex (with wafers) etc						4024	
	Chocolate coated bars like Bar One, TV bar, etc						3997	
	Other							
Popcorn	Plain						3332	
	Sugar-coated/candied						3359	
Candies/Sweets	Sugus, gums, hard sweets, etc						4000	
Toffees / Fudge / caramels							3991	
Biscuits/cookies	Homemade, plain						3233	
	Commercial, plain						3216	
	Commercial, with filling						3217	
	Other							
Cakes	Butter cake, homemade with **whole milk** and **brick margarine NO icing**						3288	
	Chocolate cake, homemade with **whole milk and brick margarine NO icing**						3289	
	Icing for cake made with **brick margarine**						4014	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Tarts	Apple tart with a batter made with whole milk and brick margarine						3327	
	Other							
Scones	Plain made with whole milk and brick margarine						3237	
	Other							
Muffin	Bran						3407	
	Plain						3408	
	Other							
Rusks	Buttermilk, commercial						3329	
	Homemade, white						3222	
	Other							
Savouries	Sausage rolls, small						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips						3331	
	Other							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Jelly	Jelly						3983	
	Custard added made with whole milk	Yes/No					2716	
	Other							
Baked pudding	Baked in a syrup						3312	
	Baked without a syrup						3429	
	Custard added made with whole milk	Yes/No					2716	
	Other							
Instant pudding	Made with whole milk						3266	
	Made with low fat milk						3395	
	Other							
Ice cream	Regular						3483	
	Soft serve						3518	
	Other							
Sorbet							3491	
Other specify								

SAUCES, GRAVIES AND CONDIMENTS

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
Tomato sauce							3139	
Worcester sauce							4309	
Chutney							3168	
Pickles							3866	
White sauce	Made with whole milk and brick margarine						3142	
Packet soups	Dry powder (all types)						3158	
	Made with water (all types)						3165	
Gravy	Made from meat and thickened						3120	
Other								

WILD FRUITS, WILD BIRDS, ANIMALS OR INSECTS (hunted in rural areas or on farms)

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN Complete one column				CODE	AMOUNT / WEEK
			Daily Times/ day	Weekly Times/ week	Monthly Times/ month	No		
MISCELLANEOUS: Please mention ***ANY OTHER FOODS*** used more than once/two times a week which we have NOT talked about								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat PLEASE GIVE DETAILS								

APPENDIX 2

NuPED

Nutrition during Pregnancy and Early Development

Socio-demographic questionnaire

Participant nr: | | | | **Date:** 2 0 Y Y M M D D **Fieldworker:** _____

1. Date of birth: Y Y Y Y M M D D

2. How would you describe yourself in terms of population group?

1	2	3	4	5
Black	Coloured	Indian	White	Other. Specify:

3. What is your home language?

1	2	3	4	5
English	Xhosa	Zulu	Sotho	Other. Specify:

4. In which country were you born?

1	2	3	4	5	6	7
South Africa	Zimbabwe	Lesotho	Swaziland	Botswana	Namibia	Other. Specify:

5. How long have you been staying in South Africa?

1	2	3	4
Less than 1 year	1 – 2 years	2 – 5 years	More than 5 years

6. What is your highest formal educational level?

1	2	3	4	5
None	Primary School	Std 6-8/ Gr 8-10	Std 9-10/ Gr 11&12	Tertiary Education

7. What is your marital status?

1	2	3	4	5	6	7	8
Unmarried	Married	Divorced	Separated	Widowed	Living Together	Traditional Marriage	Other. Specify:

8. What is your employment status?

1	2	3	4	5	6
Unemployed	Homemaker by choice	Self-Employed	Wage-Earner	Self-employed Professional	Other. Specify:

9. How many people live in your household most days of the week (including children and elderly)?

10. Do any members of the household receive any grants?

	1	2	3	4	5	6
	None	Child support	Social relief	Disability	Old age pension	Other. Specify

11. To determine your living standards measure, please indicate which of the following you currently have in your household:

X = Yes ; - = No

X	Metropolitan dweller (250 000+)		DVD Player / Blu Ray Player
	~~Living in a non-urban area~~		Refrigerator or combined fridge/freezer
	House / Cluster House / Town House		Electric Stove
	Tap water in house / on plot		Microwave oven
	Flush Toilet inside house		Deep Freezer - Free Standing
	Hot running water		Washing machine
	Built in Kitchen Sink		Tumble dryer
	No Domestic Workers or Gardeners		Dishwashing Machine
	Home security service		PayTV (M-net / DSTV / TopTV) Subscription
	2 Cell phones in Household		Home Theatre System
	3 or more Cell phones in Household		Vacuum Cleaner
	Zero or One Radio set in Household		Motor Vehicle
	Air conditioner (excl. fans)		Computer - Desktop / Laptop
	Have TV set(s)		Land line (excl. Cellphone)
	Swimming Pool		

APPENDIX 3

NuPED

Nutrition during Pregnancy and Early Development

Obstetric ultrasonography sheet

Participant nr								
	Phase 1 (<18wks)			**Phase 2 (±22wks)**			**Phase 3 (±36wks)**	
DATE								
Name of sonographer:								
Number of foetuses:								
Fetal heart present (Y/N):								
Gestational age (GA):	/	Weeks/days		/	Weeks/days		/	Weeks/days
Crown-rump length (CRL):		mm			mm			mm
Biparietal diameter (BPD):		mm			mm			mm
Head circumference (HC):		mm			mm			mm
Abdominal circumference (AC):		mm			mm			mm
Femur length (FL):		mm			mm			mm
Estimated fetal weight (EFW):		g			g			g
EDD:								
Amniotic fluid volume:					cm			cm
				decreased	adequate	increased	decreased	adequate	increased

Evaluation of the maternal uterus, tubes, ovaries, and surrounding structures:

_____

_____

Comments on fetus and cord:

_____

_____

_____

Plan for follow up scan

_____

_____

Only for phase 2 (22 wks gestation) and phase 3 (36 wks gestation) measurements
Location and appearance of the placenta:

_____

_____

APPENDIX 4

NuPED

Nutrition during Pregnancy and Early Development

General history and routine tests (Phase 1)*

Participant nr: ☐☐☐ **Fieldworker:** _____

Y = Yes
N = No
NA = Not assessed

Confirmation of general information from Maternity Case Record			
Participant' s date of birth:	__ __/__ __/__ __ __ __ DD MM YYYY	Participant's age:	|__|__| years
Are you planning or willing to deliver baby at RMMCH?	☐ Yes ☐ No		
Are you on any medication?	☐ Yes ☐ No		
If YES, please specify:	Name of medication (and obtain from Maternity Case Record):		
Allergies	☐ Yes ☐ No		
If YES, please specify:			
Do you smoke at present?	☐ Yes ☐ No		
Did you smoke in the past year?	☐ Yes ☐ No		
RVD test date: ___/___/201_	☐ Reactive (Tested HIV positive) ☐ Non-reactive (tested HIV negative) ☐ Test declined		
CD4: _____	Therapy: ☐ HAART ☐ DUAL		

History:	
Previous stillbirth or neonatal loss?	☐ Yes ☐ No
History of 3 or more consecutive spontaneous abortions?	☐ Yes ☐ No
History of abnormality in previous pregnancy?	☐ Yes ☐ No
Last pregnancy: hospital admission for hypertension or pre-eclampsia /eclampsia?	☐ Yes ☐ No
Previous surgery on reproductive tract (including caesarean section)?	☐ Yes ☐ No

Obstetric & Neonatal history: *(A= alive; ID= infant death; NND= neonatal death; IUD= intra-uterine death)*

Year	Gestation	Delivery	Weight	Sex	Complications

General medical:	
Diabetes mellitus on insulin or oral hypoglycaemic treatment?	☐ Yes ☐ No
Cardiac disease	☐ Yes ☐ No
Renal disease	☐ Yes ☐ No
Epilepsy	☐ Yes ☐ No
Asthmatic on medication	☐ Yes ☐ No
Tuberculosis	☐ Yes ☐ No
Known "substance" abuse (including heavy alcohol drinking)	☐ Yes ☐ No
Any other severe medical disease or condition. Specify:	☐ Yes ☐ No

*Questions based on clinic checklist according to the National Department of Health, Guidelines for Maternity Care in South Africa, 2015.

APPENDIX 5

NuPED

Nutrition during Pregnancy and Early Development

Anthropometry - Neonatal

Participant nr: | | | | **Date:** | 2 | 0 | Y | Y | M | M | D | D | **Fieldworker:** _____

Gestational Age at birth (in weeks)				**weeks**		**days**
Birth weight (g)	1st measurement					
	2nd measurement					
Crown-heel length (cm)	1st measurement			.		
	2nd measurement			.		
Mid-upper arm circumference (cm)	1st measurement			.		
	2nd measurement			.		
Head circumference (cm)	1st measurement			.		
	2nd measurement			.		
Thoracic circumference (cm)	1st measurement			.		
	2nd measurement			.		

APPENDIX 6

NuPED

Nutrition during Pregnancy and Early Development

Newborn Assessment

Participant number: | | | | **Date:** 2 0 Y Y M M D D **Fieldworker:** _____

Birth date:	YYYY / MM / DD	**Birth time:**	H H / M M
Gender:	☐ Male ☐ Female	**Gestational age:**	_____ weeks/_____ days
Resuscitation:	☐ None ☐ Oxygen ☐ Mask ☐ Intubation		
Total Apgar Score (1min):		**Total Apgar score (5min):**	
Mode of delivery:	☐ NVD ☐ C/S ☐ Vacuum ☐ Forceps		
Problems with delivery:	....		
Vitamin K administration	☐ Yes ☐ No		
Risk factors to baby: *Pregnancy*		***Treatment:***	
RPR positive	☐ No ☐ Yes		
RPR unknown	☐ No ☐ Yes		
Rhesus negative	☐ No ☐ Yes		
HIV positive	☐ No ☐ Yes		
HIV unknown	☐ No ☐ Yes		
Maternal diabetes	☐ No ☐ Yes		
Risk factors to baby: *Labour*		***Treatment:***	
MSL	☐ No ☐ Yes		
Foetal distress	☐ No ☐ Yes		
Preventative care:	☐ Polio ☐ BCG ☐ RtHC filled in		
Feeding at discharge?	☐ EBF ☐ EFF		
First examination of Neonate:			
Temperature:	☐ 36-37ºC ☐ Hypothermic ☐ Hyperthermic		
Resp rate:	☐ 40 – 60 prn ☐ Fast ☐ Slow		
Apex beat:	☐ 120 – 160/min ☐ Tachycardia ☐ Bradycardia		
Any abnormalities or adverse events:			

Page 1 of 1

APPENDIX 7

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT
POTCHEFSTROOM CAMPUS

Health Research Ethics Committee
Faculty of Health Sciences
NORTH-WEST University
(Potchefstroom Campus)
2016 -04- 26
HREC Stamp

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR ASSESSMENTS DURING PREGNANCY AND BIRTH

TITLE OF THE RESEARCH PROJECT:
Nutrition during Pregnancy and Early Development: The NuPED study

REFERENCE NUMBERS: *NWU-00186-15-S1; M150968*

PRINCIPAL INVESTIGATOR: *Prof Marius Smuts*

ADDRESS: *School of Physiology, Nutrition and Consumer Sciences, Potchefstroom Campus, Building G16, Room 157*

CONTACT NUMBER: *018 299 2086 / 082 451 0486*

Good day

You are invited to take part in a research project. Please take some time to read the information about this project. Please ask the researcher any questions if you do not fully understand. Your participation is ***entirely voluntary*** *and you are free to say no. If you say no, this will not affect you negatively in any way. You are also free to withdraw from the study at any point, even if you agreed to take part at first.*

This study has been approved by the ***Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU-00186-15-S1) and the Human Research Ethics Committee of the University of Witwatersrand (M150968)*** *and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records. This study will be used by several students to obtain further academic qualifications.*

What is this research study all about?

A good diet during pregnancy is important for the healthy growth of the baby. A mother's diet can affect her baby's health. It is not very clear how different eating habits affect the baby's health.

The goals of this research are to describe the food intake and health of pregnant women throughout pregnancy and to determine if it relates to the babies' health.

Therefore, we would like to measure your diet (food intake) and health at early pregnancy, mid-pregnancy and late pregnancy. When you give birth, we will also measure how healthy your baby is. We may approach you after the study to further investigate the development and well-being of your baby, but then we will ask you to provide new consent. At least two hundred and fifty women will be included in this study.

Some of the blood that we will collect from you and the cord between you and your baby after birth will be used to look at some factors that you inherited from your parents and your baby inherited from you (genetic factors, such as DNA and RNA). Genetic factors are like a manual that tells your body how to work. Sometimes there are differences or changes that cause people to react differently to nutrients. We want to investigate these genetic changes to better understand how this works. We promise that all genetic tests and experiments will only focus on genetic factors to do with nutrient usage in the body and related to your and your baby's health.

Why have you been invited to participate?

You have been invited to take part because you are attending the antenatal clinic today.

You have also complied with the following inclusion criteria:

- *You are a pregnant woman, born in South Africa, Lesotho, Swaziland, Zimbabwe, Botswana or Namibia.*
- *You are planning to deliver your baby at Rahima Moosa Mother and Child Hospital.*
- *You are able to communicate effectively in English, Afrikaans, Sotho, Xhosa or Zulu.*

You will be excluded if you:

- *Are more than 18 weeks pregnant since we need to know your status in early pregnancy.*
- *Are younger than 18 years of age or older than 39 years of age since age may influence the health of the baby.*
- *Are stating that you are using illicit drugs since this may influence the growth of the baby.*
- *Are carrying a multiple pregnancy, such as twins or triplets, since these babies are usually born smaller.*
- *Have a known lifestyle disease such as diabetes, kidney disease, high blood cholesterol or high blood pressure or using medication for any of these, since this may influence the health of the baby.*
- *Have a known infectious disease such as tuberculosis or hepatitis or using medication for any of these, since this may influence the health of the baby.*
- *Have a known serious illness such as cancer, lupus or psychosis or using medication for any of these, since this may influence the health of the baby.*
- *Are a smoker, or have been smoking in the past year since this influences the growth of the baby.*
- *Note: You will not be excluded from the study if you have HIV, but will be asked if we can include your HIV status in our data.*

What will your responsibilities be?

If you agree to take part in the study, you will be expected to:

- *From here on have all your antenatal visits at Rahima Moosa Mother and Child hospital and not here at the clinic. You will be refunded for your travelling costs.*
- *Attend Rahima Moosa Mother and Child hospital next week, at 22 weeks pregnancy and at 36 weeks pregnancy for this research. The doctors or nurses may request you to attend other days as well for other medical reasons. Dates will be given to you for each visit.*
- *Answer questions about your age, education and living conditions only today.*
- *Answer questions about your diet and supplement use at each visit, as well as phone calls before and after the following visits.*
- *Answer questions about your general health, mood, allergy symptoms and medication usage at each visit.*
- *Indicate on a checklist how healthy you feel every day.*
- *Let us do some body measurements at each visit. We will only measure your weight by asking you to stand on a scale, your standing height against a height measure and your upper arm circumference with a tape measure.*
- *Get an ultrasound screen at each scheduled visit to the hospital.*
- *Give a urine sample at each visit.*
- *Give a blood sample at each visit. A total amount of 42ml (about three tablespoons full) will be drawn from your arm.*
- *Let us take your blood pressure at each visit.*
- *Do a diabetes test at around 24 weeks pregnancy. You have to fast from 10pm the previous night. The next morning you will be asked to drink a sweet drink at the hospital laboratory. Your blood sugar levels will be tested several times.*
- *Go to Rahima Moosa Mother and Child Hospital admissions when you feel labour pains. The nurse will then do some body measurements if possible.*
- *Allow us to take some body measurements of your newborn baby, such as weight, height and head circumference.*
- *Allow us to take some blood of the cord between you and your baby after the baby has been born and after the cord has been cut.*
- *Allow us to use your and your baby's medical records to check your health.*

Will you benefit from taking part in this research?

The direct benefits for you as a participant will be that you will receive the normal medical care from a gynaecologist and hospital staff. You will receive additional medical tests, such as an ultrasound screen and diabetes test. These services are not available at the clinic. You will receive immediate feedback on the measurements where results are available on the same day, such as blood pressure and the ultrasound screen. If there are any concerns, you can discuss this with the nurse or other medical professions. They can support you with the appropriate medical care.

The indirect benefit will be that you help us understand the dietary habits and health of pregnant women in South Africa and how that affects the health of their babies. By understanding more about this, we can help government to create policies that can address the health of South African pregnant women better.

Are there risks involved in you taking part in this research?
Most of the measurements that will be performed won't harm or hurt you in any way, but you might experience the following:

1. *If you give permission to a blood sample, you might feel uncomfortable or scared. This will only last for a short while. We want to make sure that you are not hurt in any way and therefore the qualified professional will draw the blood from your arm. She will talk to you and explain to you everything that she is going to do.*
2. *You may be concerned that the researchers will be testing your HIV status. The research team will not test your blood for HIV. The clinic nurse may test your blood for HIV as part of routine antenatal care. We do ask you permission that we get the result of this test which is transferred to your study number, thus it is anonymously used further on.*
3. *During the body measurements you will be asked to remove some of your clothes keeping on only your underwear or light clothing. This might make you feel uncomfortable or shy. To help you feel less shy and uncomfortable, only females will take these measurements. Also, the area where these measurements will be done will be private and closed off. This means that no one else will be able to see you. Only the person that will take the measurements and someone to help her will be with you.*
4. *When an ultrasound screen of your baby is taken, a clear gel will be squirted onto your belly. This will feel cold, but can do no harm. The medical professional conducting the ultrasound screen will talk you through the process.*
5. *Being part of such a big research study can be frightening and overwhelming. To prevent us from wasting your time and to make sure that you know where to go and what to do, there will be people available at all times to help you and show you where you have to go every time.*
6. *For the diabetes test, you will be asked not to eat or drink anything from 22:00 (ten o'clock) the night before. You will only be allowed to drink water. You should also not eat any breakfast on the morning of the study and not drink coffee, tea, juice or cold drink. Not eating or drinking anything might make you feel uncomfortable or light headed (dizzy or faint). When you arrive at around 7:00 on the day of your booking, the laboratory staff will give you a sweet sugary drink. This may taste too sweet for you, so the laboratory staff will give you diluted lemon juice to combat the sweetness. Your blood sugar levels will be tested first with a finger prick and by drawing about 3 ml blood from your arm at 1 and 2 hours after drinking the sugar drink. You will be provided with a food parcel to eat after the test.*
7. *Doing all of the measurements on the days of the research study, will take most of the day. This might make you feel very tired. You will be provided with refreshments to eat and drink during the day.*
8. *It is important that you indicate whether you have any food allergies. This will help the research team when providing meals to participants on research days.*

There are more benefits than dangers or risks when you take part in the study.

What will happen in the unlikely event of some harm/form of discomfort occurring as a direct result of you taking part in this research study?
Please let us know if you experience any physical or emotional discomfort during or after participating in the study and we will make appropriate arrangements for you to talk to a medical doctor or psychologist.

Who will have access to the data?
We will handle all your information as confidential as possible by allocating a study code to you and your baby when he/she is born. All samples will be labelled with this code and only the principal investigator and co-principal investigator will have access to the records containing your name. Only the researchers will work with your data. Data will be kept safe and secure by locking hard copies in locked cabinets at the clinic, until your baby is born. Thereafter, these documents will be kept secure in locked cabinets in the researcher's office and for electronic data it will be password protected. Reporting of findings will be anonymous.

What will happen with the data/samples?
Blood samples that will be sent overseas for laboratory analysis will be destroyed once all the pre-defined analyses have been completed. Blood and urine samples being analysed at North-West University will be stored for 7 years after completion of the study. Data will be stored for 15 years. There is the possibility that blood samples and data might be analysed by other researchers over time for the purpose as explained to you.
There is enough money to do the study and perform the most important analyses but some of the tests are very expensive and will only be done once more funding is obtained.

Will you be paid to take part in this study and are there any costs involved?
No, you will not be paid to take part in the study but your expenses for travelling to Rahima Moosa Mother and Child Hospital will be paid for study visits at <18, 22 and 36 weeks. At each visit you will receive a R5 cell phone voucher to enable you to make a call to the researchers or fieldworkers if you need to. Furthermore, you will be provided with a snack/lunch pack every two hours during assessments at <18, 22 and 36 weeks. You will receive a gift hamper to a value of R150 with goods for your baby as a token of appreciation.
Thus, if you take part there will be no costs involved for you.

Is there anything else that you should know or do?
- *You can contact Prof. Marius Smuts at 018 299 2086 / 082 451 0486 or Elize Symington at 072 218 2184 if you have any further queries or encounter any problems.*
- *You can contact the Health Research Ethics Committee of North-West University via Mrs Carolien van Zyl at 018 299 2089; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.*
- *You can also contact the Health Research Ethics Committee of the University of Witwatersrand via:*
  - *Prof Peter Cleaton-Jones, Chairperson of HREC (Medical) Tel: 011 717 2301 Email: peter.cleaton-jones1@wits.ac.za*
  - *Ms Zanele Ndlovu, HREC (Medical) Secretariat, Tel: 011 717 1252/2700/ 1234, Email: Zanele.ndlovu@wits.ac.za*
- *You will receive a copy of this information and consent form for your own records.*

How will you know about the findings?
We will give you immediate feedback of results that we determine during the study, such as blood pressure and diabetes tests. However, take note that it will take time to perform the other analyses and that the results will only be available after several months. Once the study is completed and all the results are available, we will distribute the information to the clinics where you will attend for baby clinics. Should we find an abnormal value during our analyses that needs medical attention, we will inform you and the medical staff immediately for the necessary medical treatment.

Few questions (*to be completed by the person obtaining the consent*):

Did the participant understand the following questions?

	YES	NO
If you take part in the study, where will your follow-up antenatal visits be done?		
Will taking part in the study cost you any money?		
If you take part in the study, from where will we take blood from your new born baby?		

Declaration by participant

By signing below, I ..... agree to take part in a research study entitled: *Nutrition during Pregnancy and Early Development: The NuPED study*. I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.
- I understand and agree that blood samples from me and from the umbilical cord may be sent outside South Africa for laboratory analysis.

Do you have any food allergies?

No ☐

Yes ☐ Which allergies? _____

Do you give permission that some of your blood samples may be analysed outside of South Africa?

Yes, I give permission ☐

No, I don't give permission ☐

Do you give permission that we may collect your genetic material?

Yes, I give permission to collect my genetic material ☐

No, I don't give permission to collect my genetic material ☐

Do you give permission that we may collect your baby's genetic material from the cord blood?

Yes, I give permission to collect my baby's genetic material ☐

No, I don't give permission to collect my baby's genetic material ☐

Do you give permission that the researchers have access to your HIV test results from the clinic?

Yes, I give permission ☐

No, I don't give permission ☐

Do you give permission to have access to your and your baby's medical records at hospital or clinic?

Yes, I give permission ☐

No, I don't give permission ☐

Do you give permission for the researchers to contact you after the birth of your baby for possible follow-up tests?

Yes, I give permission ☐

No, I don't give permission ☐

Do you give permission that other researchers use the blood samples and data at a later stage?

Yes, I give permission ☐

No, I don't give permission ☐

Signed at (*place*) ..... on (*date*) ..... 20....

....

Signature of participant **Signature of witness**

Declaration by person obtaining consent:

I *(name)* …… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (*place*) ……..…….. on (*date*) ……..…….. 20....

.....	.....
Signature of investigator/fieldworker	**Signature of witness**

.....
Signature of researcher

APPENDIX 8

R14/49 Prof Marius Smuts et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150968

NAME: (Principal Investigator)	Prof Marius Smuts et al
DEPARTMENT:	Centre of Excellence for Nutrition University of the Witwatersrand and North West University Region B and C, City of Johannesburg Florida Clinic, Bosmont Clinic, Sophiatown and Rahima Moosa Mother and Child Hospital
PROJECT TITLE:	Nutrition during Pregnancy and Early Development: The NuPED Study
DATE CONSIDERED:	02/10/2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/02/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature Date 18/02/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 9

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: http://www.nwu.ac.za

Institutional Research Ethics Regulatory Committee
Tel +27 18 299 4849
Email Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval by **Health Research Ethics Committee (HREC)**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: Nutrition in Pregnancy and Early Development: The NuPED study

Project Leader: Prof CM Smuts

Ethics number: N W U - 0 0 1 8 6 - 1 5 - A 1
(Institution – Project Number – Year – Status)
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2015-11-18 **Expiry date**: 2017-12-30 **Risk** Minimal

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-RERC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-IRERC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU, ou=Vaal Triangle Campus, email=linda.duplessis@nwu.ac.za, c=ZA
Date: 2015.11.20 21:36:38 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

APPENDIX 10

Received: 2015-10-05 08:12:35 AM [South Africa Standard Time] Page 1 of 1 on 346883776

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

To: Head of Departm ent Obstetrics and Gynaecology
Rahima Moosa M other and Child Hospital

From: Prof Marius Smu s, Project Head
Centre of Excelle ce for Nutrition,N orth-West University, Potchefstroom
018 299 2086; M rius.Smuts@nwu.ac.za

3 September 2015

Permission letter for co nducting research in the Department of Obstetrics and Gynaecology

Nutrition du ing Pregnancy and Early Development: The NuPED study

With reference to previo s conversations and the attached permission letter by Dr E Hank, clinical manager of the hospital we are kindly requesting your permission for conduding the research project in your departme t.

The aim of the research project is to assess dietary intake and nutritional status of urban South African pregnant women and to determine associations with birth outcomes, maternal health and offspring health. Using a ongitudinal observational research design, pregnant women (<16 weeks gestation) (min, n=250) will be recruited from primary healthcare clinics in Johannesburg and followed up at RMMCH. Dietary intake and nutrient status will be assessed at <16, 24 and 36 weeks gestation. At birth, maternal and neonatal health will be assessed. The following data will be obtained from medical re ords in your department:

- Medical history (p arity; gravity, previously or currently diagnosed hypertension, diabetes, TB and HIV; bac terial vaginosis; early symptoms of pregnancy such as nausea and vomiting; smoking number of births, etc.)
- Blood pressure
- Ultrasound screen data
- Glucose tolerance test results

Kindly note that nursing staff will draw blood samples (additional tubes to be supplied by the research team) and ana sed by external laboratories. Urine samples will be collected as per standard operating proced ures, however, fieldworkers will take aliquots for research purposes.

The research team (tra ined fieldworkers) will be obtaining the socio-demographic data, anthropometrical measure ments, diet history and general health questionnaires.

On behalf of the research team, we are herewith requesting your permission for conducting the research project in your de partment and obtaining the abovementioned data.

Prof Marius Smuts

	Approved	29.9.15
Head: Department O&G	Outcome	Date

Oct 05 2015 7:59 Obs & Gynae 0114709092 p.1

APPENDIX 11

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

OUTCOME OF PROVINCIAL PROTOCOL REVIEW COMMITTEE (PPRC)

Researcher's Name (Principal investigator)	Prof. Marius Smuts
Organization / Institution	Centre of Excellence for Nutrition: North West University
Research Title	Nutrition During Pregnancy and Early Development (NuPED) study
Contact number	Address: N/A Contact no: 018 299 2086 Cell: Email: Marius.Smuts@nwu.ac.za
Protocol number	GP2015RP 38 473
Date submitted	26/10/2015
Date reviewed	26/11/2015
Outcome	Approved

It is a pleasure to inform you that the Gauteng Health Department has approved your research on "Nutrition During Pregnancy and Early Development (NuPED) study"

Study sites: JHB Metro, Rahima Moosa Hospital, Florida, Bosmont, Sophiatown and Zandspruit Clinics.

The Provincial Protocol Review Committee kindly requests that you to submit a report after completion of your study and present your findings to the Gauteng Health Department.

Recommended/~~Not Recommended~~

Dr. B. Ikalafeng
(on behalf of the PPRC)
Date: 27/11/2015

Approved/Not approved

Dr. LRR. Lebethe
Acting DDG: Clinical Service

Date: 03/12/15

APPENDIX 12

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Joburg
a world class African city

JOHANNESBURG HEALTH DISTRICT

Enquiries:
Hillbrow CHC Administration Building, Klein Street,
Hillbrow, Johannesburg
E-mail:
CoralF@joburg.org.za
Johannesburg.research@gmail.com

10 December 2015

Professor Marius Smuts
Nutrition
Potchefstroom Campus
E-mail: Marius.Smuts@nwu.ac.za

Dear Professor Smuts,

Re: ***Nutrition in Pregnancy and Early Development: The NuPED Study***

Your application dated 8 December 2015 refers. The District Research Committee has reviewed your application. This letter serves as an in-principle approval to access the Districts Health facilities (mentioned below) for the above project subject to following conditions:

- The facility to be visited: Florida, Bosmont, Sophiatown and Zandspruit Clinics
- The research can only commence after you submit an ethics clearance certificate from a recognized institution.
- Please contact the relevant RHDD prior to your visit to the facilities

Region	Regional Health Manager	Contact No.	Cell phone
B	Ms Paulinah Maepa	011 718 9656	082 551 5804
C	Mr. Tebogo Motsepe	011 761 0248	083 421 9405

- You will report to the Facility Manager before initiating the study.
- Participants' rights and confidentiality will be maintained all the time.
- No resources (Financial, material and human resources) from the above facilities will be used for the study. Neither the District nor the facility will incur any additional cost for this study.
- The study will comply with Publicly Financed Research and Development Act, 2008 (Act 51 of 2008) and its related Regulations.
- You will submit a copy (electronic and hard copy) of your final report. In addition, you will submit a six-monthly progress report to the District Research Committee. Your supervisor and University of South Africa will ensure that these reports are being submitted timeously to the District Research Committee.
- The District must be acknowledged in all the reports/publications generated from the research and a copy of these reports/publications must be submitted to the District Research Committee.

We reserve our right to withdraw our approval, if you breach any of the conditions mentioned above.

APPENDIX 13

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

RAHIMA MOOSA MOTHER AND CHILD HOSPITAL

RAHIMA MOOSA MOTHER AND CHILD HOSPITAL
Enquiries: Dr E Hank
Tel: (011) 470 9030/9031
Fax: (011) 477 4117
Email : Edward.Hank@gauteng.gov.za

Ms Elize Symington

Centre of Excellence for Nutrition

North-West University (Potchefstroom Campus)

6 July 2015

Re: Nutrition in Pregnancy and Early Development: The NuPED study

Dear Ms Symington

Permission is granted for you to conduct the research as indicated in your request as per the title above.

The terms under which this permission is granted is contained in the Researcher Declaration form that you signed. Failure to comply with these conditions will result in the withdrawal of such permission.

Note that it is imperative that you notify the hospital of the actual start and end dates of your study by notifying Karen Marshall by email (Karen.Marshall@wits.ac.za).

Should the study commence more than 12 months from receipt of this letter then the Researcher Declaration form needs to be re-signed prior to commencement of the research. Your are strongly advised to keep a signed copy of the declaration form so as to ensure that the terms of this agreement are complied with at all times.

Yours sincerely,

Clinical Manager

ADDRESS: cnr. FUEL & OUDSTHOORN STREET CORONATIONVILLE 2093 / PRIVATE BAG X20 NEWCLARE 2112 JHB

APPENDIX 14

UNISA university of south africa

CAES RESEARCH ETHICS REVIEW COMMITTEE

National Health Research Ethics Council Registration no: REC-170616-051

Date: 04/04/2018

Ref #: **2017/CAES/059**
Name of applicant: **Ms OW Alawode**
Student #: **49371347**

Dear Ms Alawode,

Decision: Ethics Approval Renewal after First Review for period 01/04/2018 to 31/03/2019

Proposal: The association between levels of fish consumption during pregnancy and birth outcomes of pregnant women in Johannesburg, South Africa

Supervisor: Mrs E Symington

Qualification: Postgraduate degree

Thank you for the submission of your progress report to the CAES Research Ethics Review Committee for the above mentioned research. Approval is granted for the continuation of the project.

Please note that the approval is valid for a one year period only. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2019

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Research Ethics Review Committee on 02 March 2017.

The proposed research may now commence with the proviso that:

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*
2) *Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study, as well as changes in the methodology, should be communicated in writing to the CAES Research Ethics Review Committee. An amended application could be requested if there are substantial changes from the existing proposal, especially if those changes affect any of the study-related risks for the research participants.*
3) *The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study.*

Note:
The reference number [top right corner of this communiqué] should be clearly indicated on all forms of communication [e.g. Webmail, E-mail messages, letters] with the intended research participants, as well as with the CAES RERC.

Kind regards,

Signature
CAES RERC Chair: Prof EL Kempen

Signature
CAES Executive Dean: Prof MJ Linington

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