Predictive anthropometric measurements, associated factors, outcomes, and genetic factors involved in maternal overweight and obesity in HIV-infected and HIV-uninfected black South African pregnant women

By

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DECLARATION

I, **Christen Renèe Erasmus**, declare that:

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Signed: 

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Christen R. Erasmus

Date

DEDICATION

To:

My beloved late grandmother, Lorraine. Her love, generosity, kindness, resilience, and bravery will continue to inspire me.

My darling husband, Matt, for his constant encouragement, positivity, and love throughout this journey.

My parents, for the gift of giving me a strong foundation in education.

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3. Erasmus, CR; Chuturgoon, AA; Maharaj NR. **Maternal overweight and obesity compounded by the HIV infection alters the gene expression of *ADIPOQ, LEP, LEPR* and *FTO* in black South African pregnant women**

ABBREVIATIONS

ABW: Actual body weight

ADIPOQ: Adiponectin

AOR: Adjusted odds ratio

ART: Antiretroviral treatment

AT: Adipose tissue

AUC: Area under the curve

BMI: Body mass index

BREC: Biomedical Research Ethics Council

cDNA: Complementary deoxyribonucleic acid

CI: Confidence interval

CVD: Cardiovascular disease

C/S: Caesarean section delivery

DM: Diabetes Mellitus

DNA: Deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

EFV: Efavirenz

FDC: Fixed-dose combination

FTC: Emtricitabine

FTO: Fat mass and obesity-associated

GDM: Gestational diabetes mellitus

GHRL: Ghrelin

Hb: Haemoglobin

HD: Hypertensive disorders

HELLP: Haemolysis, elevated liver enzymes and low platelet counts

HIV: Human immunodeficiency virus

HPT: Hypertension

ISAK: International Society for the Advancement of Kinanthropometry

KZNDOH: KwaZulu-Natal Department of Health

LEP: Leptin

LEPR: Leptin receptor

MAMC: Mid-arm muscle circumference

MetS: Metabolic syndrome

miRNA: Micro ribonucleic acids

MUAC: Mid-upper arm circumference

mRNA: Messenger ribonucleic acid

MW: Maternal weight

NCD: Non-communicable diseases

OR: Odds ratio

PET: Pre-eclampsia toxaemia

PIH: Pregnancy-induced hypertension

PMMH: Prince Mshiyeni Memorial Regional Hospital

PMTCT: Prevention of mother to child transmission

PROM: Preterm rupture of membranes

qRT-PCR: Quantitative real-time polymerase chain reaction

RNA: Ribonucleic acid

ROC: Receiver operator characteristic

RT: Room temperature

SD: Standard deviation

SDG: Sustainable development goal

SH: Standing height

SSF: Subscapular skinfold

TDF: Tenofovir

TSF: Tricep skinfold

UKZN: University of KwaZulu-Natal

VAT: Visceral adipose tissue

WB: Whole blood

WC: Wrist circumference

WHO: World Health Organisation

3TC: Lamivudine

ABSTRACT

Background: The proportion of overweight and obese people living with human immunodeficiency virus (HIV) infection have increased globally and are both epidemics that are endemic to countries like South Africa. Targeting these two epidemics in pregnant women, need to be a priority in maternal health research, with the findings from these studies aimed to eventually translate into improving maternal health outcomes.

Aims and objectives: This study aimed to evaluate the anthropometric differences, factors, outcomes, and epigenetic factors involved in pregnant black South African pregnant women with a body mass index (BMI) ≥25.0 kg/m^2 in comparison to those with a BMI <25 kg/m^2. The specific study objectives were to: (i) investigate the relationship between maternal BMI and maternal anthropometric measurements among black South African pregnant women; (ii) identify what measurement cut-offs accurately predict each nutritional status group; (iii) investigated the anthropometric differences between pregnant women living with and without HIV; (iv) investigate the differences between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to those with a BMI <25.0 kg/m^2; (v) investigate the factors associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant South African women; (vi) investigate the maternal health outcomes associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant South African women; and (vii) investigate whether maternal BMI and HIV status had an effect on the mRNA expression of adiponectin (*ADIPOQ*), leptin (*LEP*), leptin receptor (*LEPR*), fat mass and obesity-associated (*FTO*), and ghrelin (*GHRL*) in visceral adipose tissue (VAT) and in whole blood (WB) obtained from pregnant black South African women.

Method: A cross-sectional study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital, which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. The catchment area for the hospital includes both rural and urban geographical areas. Pregnant women admitted to the labour ward were approached to participate in this study. The inclusion criteria for this study were as follows: (1) ≥ 18 years of age; (2) pregnant females; (3) black South African citizen; (4) clinically stable; (5) able to stand without assistance; (6) given verbal and written consent to participate in the study; and (7) gave consent to obtain a VAT sample during their c-section operation. The participants were categorized

according to BMI (kg/m^2) into two groups: (1) overweight/obese pregnant women (≥25kg/m^2); and (2) non-overweight/non-obese pregnant women (<25kg/m^2). A total of 458 pregnant women were approached to participate in the study, but 245 subjects met the inclusion criteria and of these 45 declined to participate. Hence, a total of 200 subjects met all the inclusion criteria, but of these participants only 79 subjects were able to provide a VAT sample. The statistical tests that were applied included: (i) Fisher's exact test and the χ 2 test; (ii) Pearson correlation coefficient; (iii) the Spearman's rank-order correlation coefficient; (iv) the Mann Whitney t-test; (v) one-way ANOVA; (vi) area under the curve of the receiver operator characteristic curves to determine the cut-off values; and (v) simple logistic regression was performed to select the variables for multiple logistic regression analysis, and only variables with a *p*-value <0.05. A *p*-value of <0.05 was considered statistically significant.

Results: Maternal age was significantly positively associated with changes in maternal anthropometric measurements. Maternal BMI was significantly positively correlated with other maternal anthropometric measurements including mid upper arm circumference (MUAC) (left and right), tricep skinfold (TSF) (right), subscapular skinfold (SSF) (right), mid arm muscle circumference (MAMC) (right), wrist circumference (WC) (right), but significantly negatively correlated with frame size. The anthropometric methods that were accurate for assessing obesity in pregnancy included TSF (right) (cut-off of ≥20.75 mm), SSF (right) (cut-off of ≥21.75 mm), MAMC (right) (cut-off of ≥25.23 cm), and WC (right) (cut-off of ≥16.25cm). Also, SSF (right) (cut-off of ≥15.75mm) and MAMC (right) (cut-off of ≥23.35cm) could be used to assess for overweight nutritional status. Lastly, frame size could be used to assess for underweight (cut-off of ≥10.05) and normal (cut-off of ≥9.95) nutritional status. The HIV-infected pregnant women did not differ anthropometrically to the HIV-uninfected pregnant women. The demographic characteristics, food frequency intake, physical activity and lifestyle characteristics were not significantly different between the participants with a BMI of ≥ 25.0 kg/m^2 compared to those with a BMI of <25 kg/m^2. The dietary pattern of the overweight/obese participants showed that there was a higher intake of saturated fat, higher in salt, higher in sugar, higher in animal protein, lower in dairy, higher in legumes, higher in starch, higher in vegetables, and had a similar intake of fruit in comparison to the non-overweight/non-obese participants. Also, maternal age was significantly different between those with a BMI ≥25 kg/m^2 compared to those with a BMI <25 kg/m^2, where the overweight and obese participants were significantly older (*p*=0.0173). Multiple logistic

regression analysis showed that maternal age (OR:1.061; 95%CI 1.008-1.117; *p*=0.023) and gestational age (OR:1.121; 95%CI 1.005-1.251; *p*=0.041) were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant women. For maternal health outcomes, multiple logistic regression analysis showed that HPT disorders (OR:0.273; 95%CI 0.124-0.601; *p*=0.001) and anaemia (OR:2.420; 95%CI 1.283-4.563; *p*=0.006) were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant women. The overweight and obese HIV-infected pregnant women (OR:0.233; 95% CI 0.075-0.717; *p*=0.011) had increased odds for developing HPT disorders compared to HIV-uninfected overweight and obese pregnant women (OR:0.471; 95% CI 0.172-1.291; *p*=0.143). It was identified that there were statistically significant differences for *ADIPOQ* (*p* <0.001), LEP (*p*=0.0105) and *LEPR (p*=0.0220) where mRNA expression was greater in the VAT compared to WB. The mRNA expression of *FTO* was similar in VAT and WB (*p*=0.4039). There were no significant differences in mRNA expression for *ADIPOQ, LEP, LEPR* and *FTO* between all the BMI and HIV status groups. However, there were patterns identified that allude to BMI, HIV, and the combination of BMI and HIV which showed that there was an effect on the mRNA expression of *ADIPOQ, LEP, LEPR* and *FTO* in the pregnant women. The pregnant women with a BMI ≥25.0 kg/m^2 showed a downregulatory pattern for *ADIPOQ, LEP, LEPR* and *FTO* in VAT and WB. The HIV-infected pregnant women showed a downregulatory pattern for *ADIPOQ, LEP, LEPR* and *FTO* in VAT and WB. The HIV-infected pregnant women with a BMI ≥25.0kg/m^2 had the lowest mRNA expression for *ADIPOQ, LEP, LEPR* and *FTO* in VAT and WB. The mRNA expression of *GHRL* in the VAT and WB samples from the pregnant women was undetectable.

Conclusion: Maternal nutritional status can be accurately predicted by using surrogate maternal anthropometric measurements such as MUAC, TSF, SSF, MAMC, WC, and frame size. Pregnant women living with HIV do not differ anthropometrically to pregnant women living without HIV. Maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women was significantly associated with maternal age, gestational age, HPT disorders and anaemia. Maternal overweight/obesity decreased the odds for anaemia during pregnancy but increased the odds for the development of HPT disorders during pregnancy, especially in the HIV-infected pregnant women. Pregnant black South African women presenting with overweight/obesity and being HIV-infected showed to have the worst downregulatory effect on mRNA expression of *ADIPOQ, LEP, LEPR,* and *FTO*. The downregulation of these genes may

result in the dysregulation of metabolic pathways that usually control weight gain during pregnancy.

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CHAPTER 1

1. INTRODUCTION

1.1 Background to the study

Overweight and obesity can be defined as an abnormal physiological state characterized by the excess deposition of adipose tissue (Chooi, Ding and Magkos, 2019). Nutritional status is commonly classified according to body mass index (BMI), where in overweight and obesity the total body mass exceeds the standard in relation to height; and is defined as having a BMI of more than or equal to 25.0 kg/m^2 and 30 kg/m^2 respectively (Lahner, 2019). The obesity epidemic has become a global public health priority as well as one of the key focus areas for the global action plan for the prevention and control of non-communicable diseases (NCD) (Global Burden of Disease Risk Factor Collaborators, 2018). Recently, the World Health Organisation (WHO) met to discuss the development of an implementation road map for 2023 to 2030 (WHO, 2021). One of the focus areas for this road map was the prevention and management of obesity over the life course, including vulnerable groups like pregnant women (WHO, 2021).

According to the data collected from the global burden of disease study, it was identified that the secular overweight and obesity global trends have increased, especially in women compared to men (Chooi, Ding and Magkos, 2019; Global Burden of Disease study, 2015) (*refer to Figure 1.1*). The global prevalence of overweight has increased by 47.2% from 26.5% in 1980 to 39.0% in 2015 (Chooi, Ding and Magkos, 2019) (*refer to Figure 1.1*). Also, the global prevalence of obesity increased by 78.6% from 7.0% in 1980 to 12.5% in 2015 (Chooi, Ding and Magkos, 2019) (*refer to Figure 1.1*). According to the African regional prevalence of overweight and obesity, the prevalence of overweight in South Africa increased by 17.0% from 49.4% in 1980 to 57.8% in 2015 (Chooi, Ding and Magkos, 2019) (*refer to Figure 1.2*). Whereas the prevalence of obesity in South Africa increased by 36.3% from 22.6% in 1980 to 30.8% in 2015 (Chooi, Ding and Magkos, 2019) (*refer to Figure 1.2*).

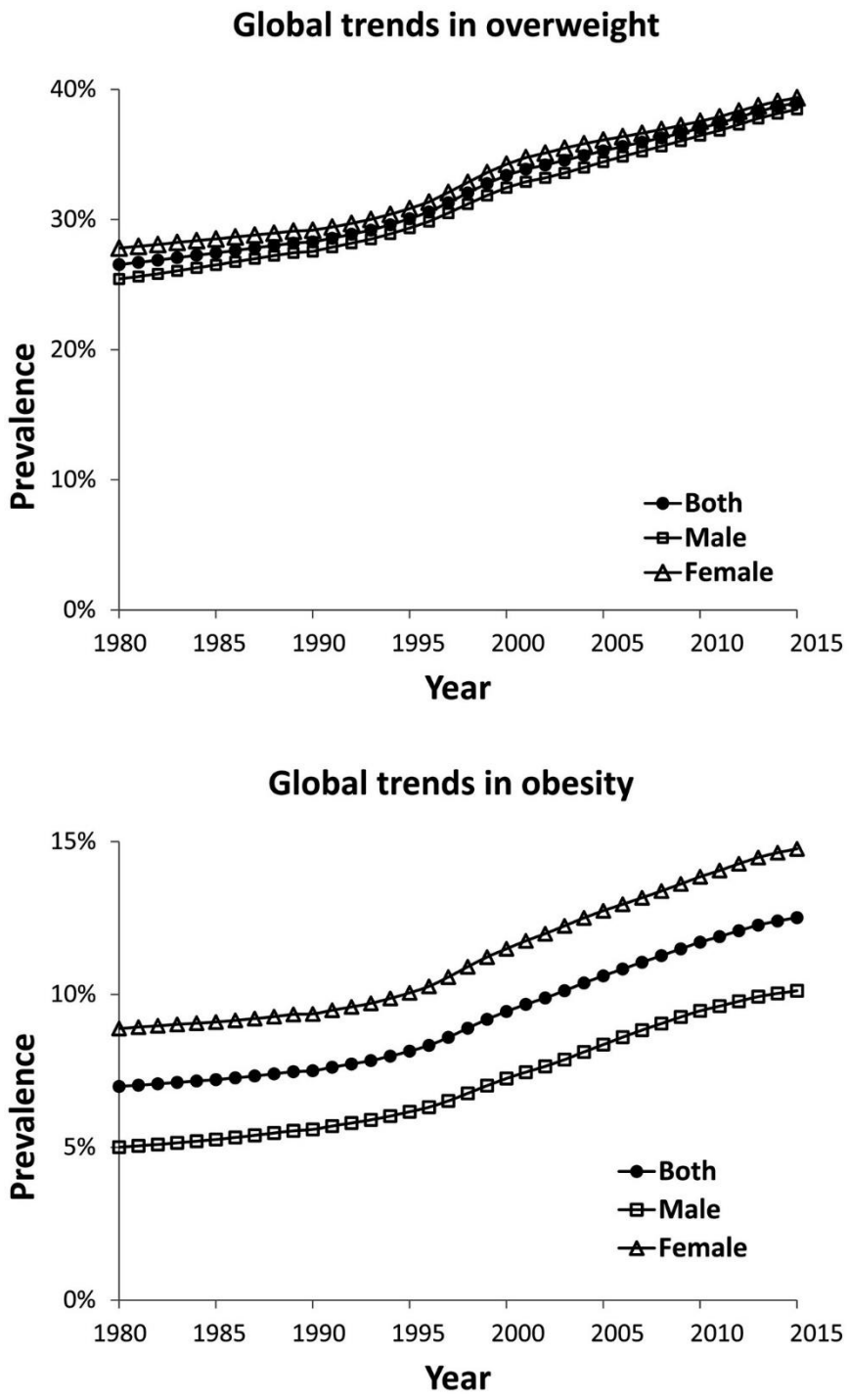

Figure 1.1: Age-standardised global prevalence of overweight (top) and obesity (bottom) in men and women > 20 years old by year (1980–2015) (Chooi, Ding and Magkos, 2019)

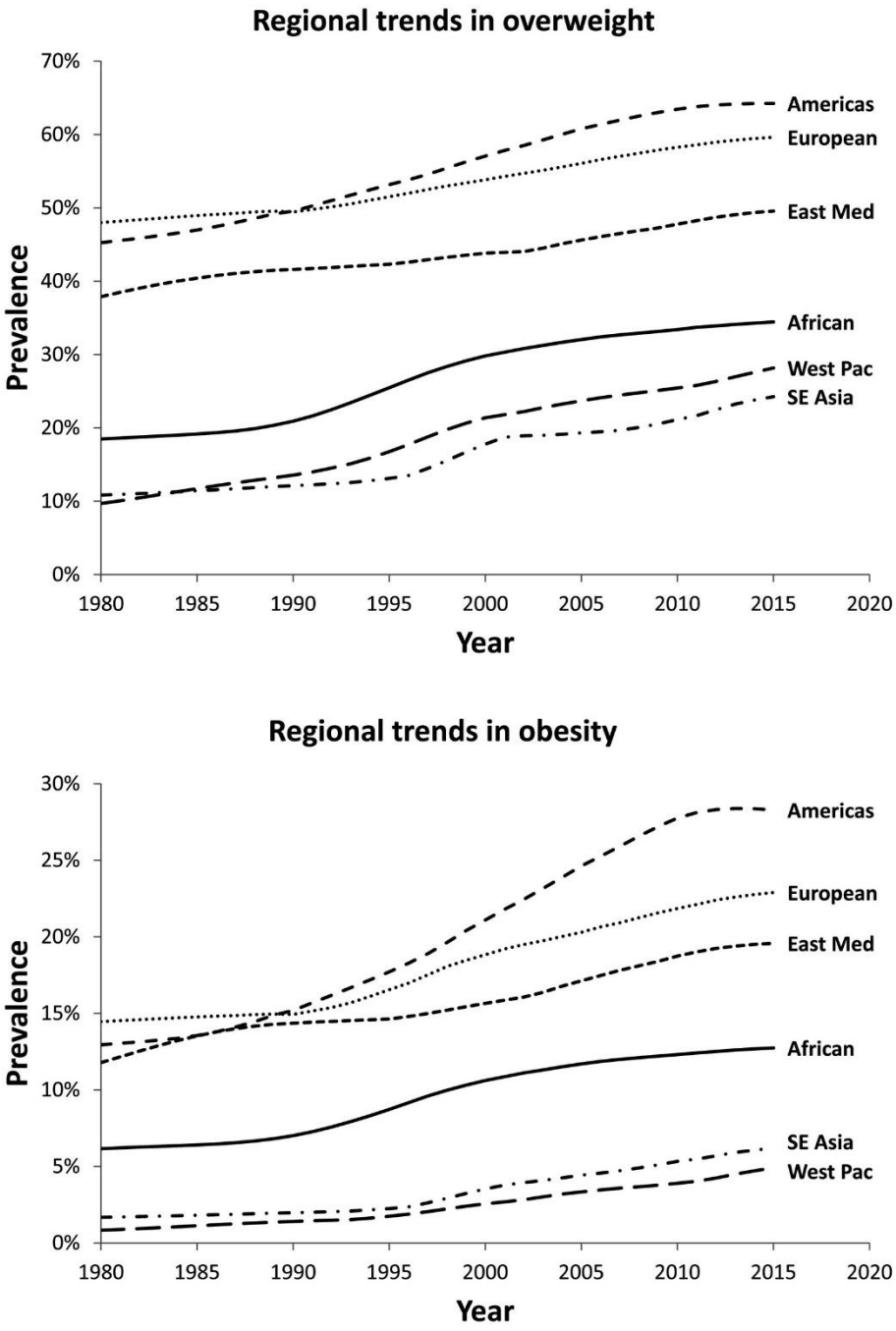

Figure 1.2: Age-standardised prevalence of overweight (top) and obesity (bottom) in adults > 20 years old by geographical region and year (1980–2015) (Chooi, Ding and Magkos, 2019)

According to a study conducted by Chen, Xu and Yan (2018), which estimated the global and country-level burden of overweight and obesity among pregnant women from 2005 to 2014, it was identified that South Africa (low-middle income country) contributed 1.4% to the global burden

of overweight and obesity. Also, the prevalence of overweight and obesity in pregnant women increased by 9.4% from 58.6% in 2005 to 64.1% in 2014 (Chen, Xu and Yan, 2018). Hence, overweight and obesity prevalence in women as well as in pregnant women is on a rising trend in South Africa.

The consequences of an overweight or obese nutritional status during pregnancy is linked to increased risk for metabolic disturbances such as abnormal glucose metabolism e.g., gestational diabetes mellitus (GDM); hypertensive disorders e.g., pregnancy-induced hypertension (PIH) and preeclampsia toxaemia (PET); and respiratory disorders like sleep apnoea, and exacerbation of asthma (Ferraro, *et al*., 2015). In a metanalysis study which investigated the outcomes of overweight and obesity in pregnancy compared to pregnant women of a normal BMI (control group), it was concluded that adverse pregnancy outcomes increased with an increase in BMI (D'Souza, *et al.*, 2019). Pregnant women with a BMI > 40 kg/m^2 were 17% more likely to have GDM compared to 3.9% in control group, pregnant women with a BMI >30 kg/m^2 were 15.9% more likely to have hypertensive disorders in pregnancy compared to 3.5% in the control group, and pregnant women with a BMI >30 kg/m^2 were 47.7% more likely to have a caesarean section birth delivery compared to 26.0% in the control group (D'Souza, *et al*., 2019).

South Africa is considered the epicentre of the human immunodeficiency virus (HIV) infection in the world, with KwaZulu-Natal having the highest disease burden (Hoque, *et al*., 2021). According to Hoque, *et al*., (2021), the prevalence of the HIV infection in pregnant women is at 44.3% in South Africa. It has been identified that the HIV infection and the antiretroviral treatment (ART) thereof are both potential risk factors for causing changes in nutritional status during pregnancy. For example, in Tanzanian study, HIV infection was identified as a significant risk factor for wasting among pregnant women, especially those coming from a low socioeconomic background, where wasting was 34.0% more prevalent in the HIV-infected compared to the HIV-uninfected (Villamor, *et al*., 2002). Also, the type of ART administered has shown to influence the risk for weight gain during pregnancy, for example, in the Tsepamo study conducted in Botswana, it was demonstrated that significantly larger fat mass was gained in pregnant women who were receiving dolutegravir compared to efavirenz (Caniglia, *et al*., 2020).

In South Africa, the impact of obesity prevalence is far-reaching and if it is not addressed, it will not only affect the health of future generations but will continue to have considerable financial

implications. According to Okunogbe, *et al*., (2021), the obesity-associated costs in South Africa was reported at 5.5 billion USD in 2019. It is estimated that should the prevalence of obesity decrease by 5.0%, it will translate to an average annual reduction of 5.2% and 13.2% in economic costs, respectively, between 2020 and 2060 (Okunogbe, *et al.,* 2021). Therefore, obesity centered research should be a priority. Furthermore, there is a need to conduct population-specific research which investigates the unique exposure of the obesogenic environmental factors that will influence the physiological mediators of body weight during pregnancy in South African women (Sartorius, *et al*., 2016). These physiological mediators are largely influenced by genetic and epigenetic mechanisms (Herrera, Keildson and Lindgren, 2011). Whereby, epigenetics' will influence gene expression without changing the deoxyribonucleic acid (DNA) sequence (Herrera, Keildson and Lindgren, 2011). The genetic susceptibility of the black South African pregnant women to obesity is still largely unknown (Yako, *et al.,* 2015) Therefore, this motivates the need to conduct further investigation into the anthropometric assessment, risk factors, and the epigenetic variables involved in the predisposition for obesity in pregnancy in black South African women living with and without HIV.

1.2 Aim of this study

This study aimed to evaluate the anthropometric predictive factors, risk factors, and genetic factors involved in overweight and obese black South African pregnant women in comparison to that of the control group of non-overweight/non-obese black South African pregnant women living with and without HIV.

1.3 Study hypotheses and objectives

1.3.1 Anthropometric differences between different nutritional statuses

One of the most important aspects of obesity-centered research is to be able to classify nutritional status (Gakidou, *et al*., 2017). In pregnancy, the most frequently used anthropometric classifications are pre-pregnancy BMI, gestational weight gain patterns during pregnancy as well as using alternative measurements like mid upper arm circumference (MUAC) (Ferraro, Contador, Tawfiq, Adamo and Gaudet, 2015). However, there is a lack of research on the accuracy of anthropometric measurements for identifying nutritional status in pregnancy in black South African women living with and without HIV.

Therefore, in this study, we hypothesized that:

H_{01}: Maternal BMI is not associated with other maternal anthropometric measurements in pregnant black South African women.

H_{02}: There were no anthropometric differences between HIV-infected pregnant women and HIV-uninfected pregnant women.

Therefore, the corresponding study objectives were to:

(i) Investigate the relationship between maternal BMI and other maternal anthropometric measurements among black South African pregnant women.

(ii) Determine the anthropometric measurement cut-offs for each nutritional status group.

(iii) Determine the anthropometric differences between pregnant women living with and without HIV.

1.3.2 Factors associated with maternal overweight and obesity and its outcomes

Understanding the causes and the effects of overweight and obesity in pregnancy are essential for the development of solutions to prevent and control the overweight and obesity epidemic in South Africa.

Therefore, in this study, it was hypothesized that:

H_{03}: There were no factors associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women

H_{04}: There were no maternal health outcomes associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant South African women.

Therefore, the corresponding objectives were to:

(iv) Investigate the differences between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to those with a BMI <25.0 kg/m^2.

(v) Investigate the factors associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant South African women.

(vi) Investigate the maternal health outcomes associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant South African women.

1.3.3 The mRNA expression patterns in maternal overweight and obesity within the context of HIV

This aspect of the study focused on investigating the interplay between the drivers of maternal overweight and obesity in pregnancy and how they have influenced epigenetics. Therefore, we hypothesized that:

> **H_{05}:** There are no differences in mRNA expression patterns between the pregnant women with a BMI ≥25.0 kg/m^2 compared to pregnant women with a BMI <25.0kg/m^2 living with and without HIV.

Therefore, the corresponding objective was to:

(vii) Investigate whether maternal BMI and HIV status had an effect on the mRNA expression of adiponectin (*ADIPOQ*), leptin (*LEP*), leptin receptor (*LEPR*), fat mass and obesity-associated (*FTO*), and ghrelin (*GHRL*) in visceral adipose tissue (VAT) and in whole blood (WB) obtained from pregnant black South African women.

1.4 Summary of study methodology

A cross-sectional study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital (PMMH), which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. The catchment area for the hospital includes both rural and urban geographical areas. Pregnant women admitted to the labour ward were approached to participate in this study. The inclusion criteria for this study were as follows: (1) ≥ 18 years of age; (2) pregnant females; (3) black South African citizen; (4) clinically stable; (5) able to stand without assistance; and (6) given verbal and written consent to participate in the study. The participants were categorized according to BMI (kg/m^2) into two groups: (1) overweight/obese pregnant women (≥25kg/m^2); and (2) non-overweight/non-obese pregnant women (<25kg/m^2 A total of 458 pregnant women were approached to participate in the study, but 245 subjects met the inclusion criteria and of these 45 declined to participate. Hence, a total of 200 subjects met all the inclusion criteria, but of these participants only 79 subjects were able to provide a VAT sample (refer to *Figure 1.3*).

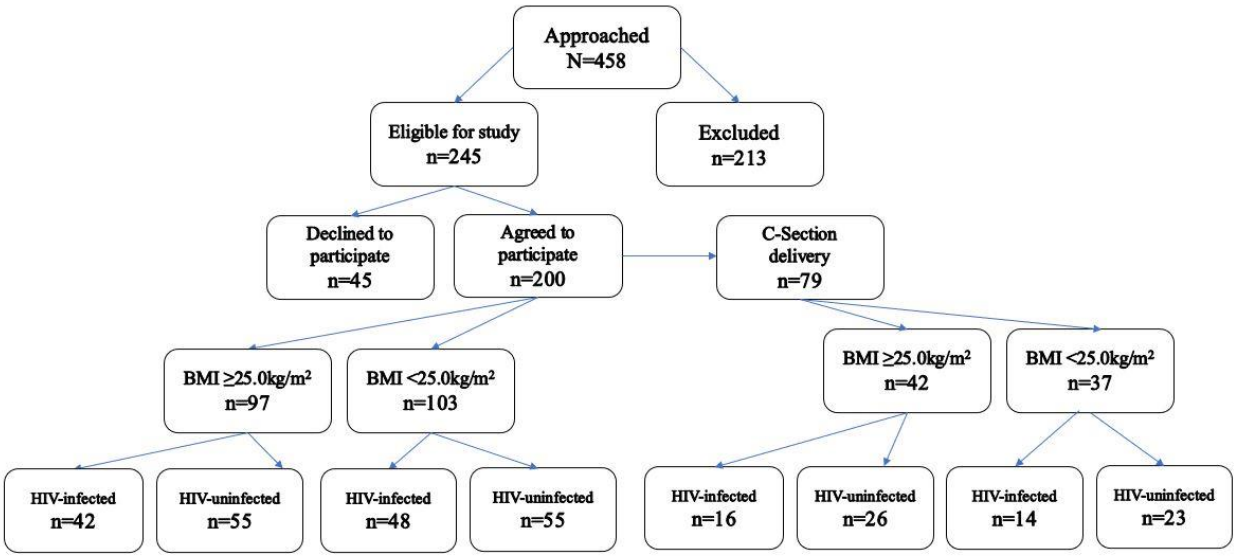

Figure 1.3: Eligibility flow diagram

Quantitative information was collected using a validated questionnaire in an English version (*refer to Appendix 1*) as well as a translated isiZulu version (*refer to Appendix 2*). Anthropometric data were measured by the investigator who is international standards for anthropometric assessment (ISAK) trained level 1. The VAT and blood samples were extracted for quantitative real-time polymerase chain reaction (qRT-PCR) to determine the mRNA expression in overweight/obese versus non-overweight/obese. The prospective power analysis was calculated as a sample size of N=207 with n1 (BMI <25.0 kg/m^2) was 69 and n2 (BMI ≥ 25.0 kg/m^2) was 138. These values were based on a delta value of 20%, power of 0.80, and an alpha value of 0.05.

1.5 Ethics approval and informed consent

The study was approved by the Biomedical Research Ethics Council (BREC) of the University of KwaZulu-Natal (UKZN) (BE269/18) (*refer to Appendix 3 and Appendix 4*), KwaZulu-Natal Department of Health (KZNDOH) (HRKM261/18) (*refer to Appendix 5*), and PMMH (29/RESH/2018) (*refer to Appendix 6*). All the participants in this study had provided verbal and written informed consent (*refer to Appendix 7 to Appendix 9*), participated voluntarily, did not receive incentives, and had the right to withdraw at any stage of the study. All methods were performed following the guidelines and regulations of the declaration of Helsinki.

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CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

Maternal health is defined as the well-being of women of childbearing age before pregnancy, during pregnancy, during childbirth, and during the postpartum period (StatsSA, 2020). According to WHO (2019), adequate and effective maternal health care services form an essential function in achieving the millennium development goals as well as the sustainable development goals (SDG) by 2030. Maternal health targets fall under the third SDG which states that we need to "ensure healthy lives and promote well-being for all at all ages", together with the SDG 3.1 which "aims to reduce the global maternal mortality ratio to less than 70 per 100 000 live births by 2030" (WHO, 2019). Poor maternal and reproductive health outcomes remain a challenge for countries like South Africa, where the estimated maternal mortality rate is 119 per 100 000 live births (WHO, 2019). There is growing evidence to suggest that the nutritional status of a women during pregnancy could influence the risk for maternal complications like maternal death, especially in pregnant women who are overweight or obese. According to a French study (Saucedo, *et al*., 2021), it was reported that the risk for maternal death increased with an increase in BMI, with the odds ratio of death ranging from 1.65 in overweight pregnant women to 3.40 in morbid obesity. Also, it was reported that the cause of death was associated with the complications of overweight/obesity such as cardiovascular disease, venous thromboembolism, hypertensive complications, and stroke (Saucedo, *et al*., 2021). This study highlights an important aspect of having an overweight or obese nutritional status during pregnancy, which is that the excess weight gain has a direct and indirect effect on maternal health outcomes. According to a South African meta-analysis study, the prevalence of overweight in women (aged 15-49 years) increased by 17.0% from 51.3% in 1998 to 60.0% in 2017. Also, the prevalence of obesity in women (aged 15-49 years) increased by 42.5% from 24.7% in 1998 to 35.2% in 2017 (refer to ***Figure 2.1***) (Nglazi and Ataguba, 2022).

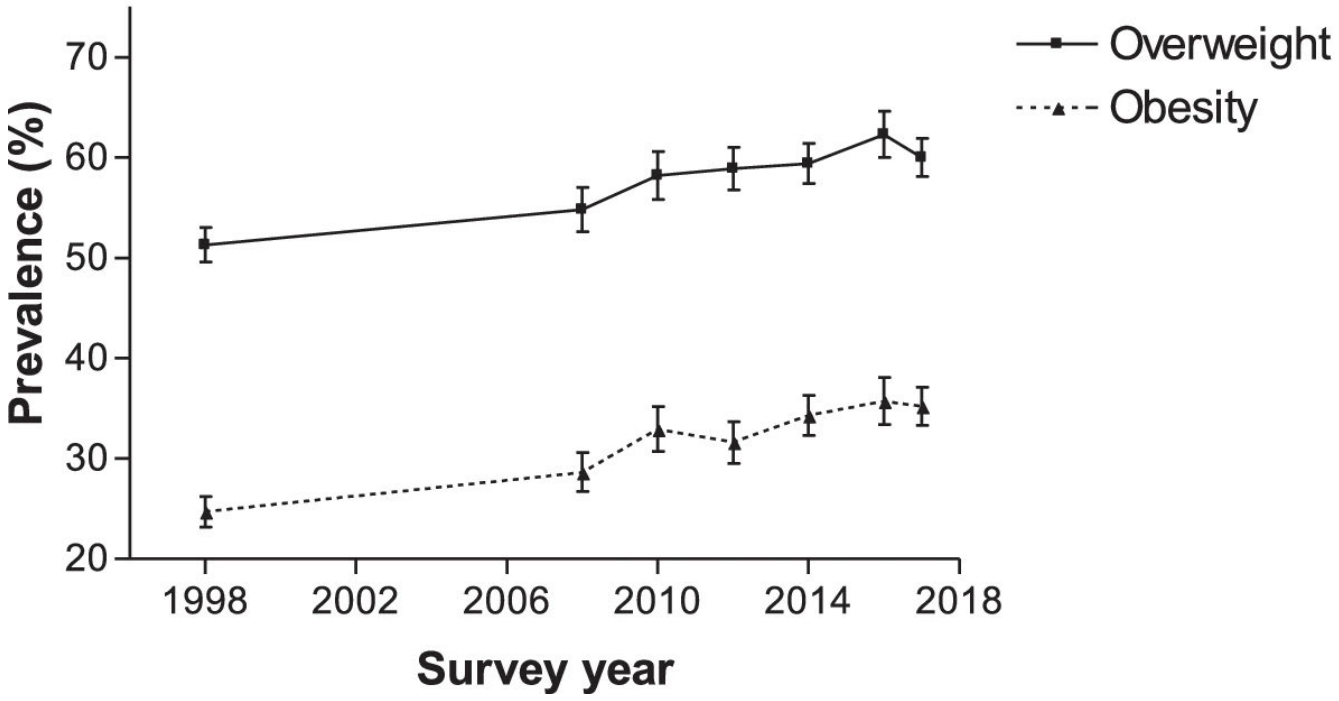

Figure 2.1: Overweight and obesity prevalence for women aged 15–49 years from 1998 to 2017, South Africa (Nglazi and Ataguba, 2022)

More specifically in pregnant women, it has been reported that over 40% of pregnant women at antenatal care entry are classified as obese, including those with HIV on ART (StatsSA, 2020; Bakal, *et al*., 2018; Guehi, *et al*., 2016). In a recent South African study, it was reported that the prevalence of obesity in pregnant women was at 43% (Madlala, *et al*., 2021). This prevalence was supported by an earlier South African study, where the prevalence of obesity in pregnant women was at 44% (Basu, *et al*., 2010). Overall, these studies highlight that there is a high prevalence of overweight and obesity in women and pregnant women living in South Africa. This is a concerning trends because maternal overweight and obesity are both associated with metabolic complications in pregnancy leading to adverse health outcomes (Madlala, *et al*., 2020). Maternal overweight and obesity in South African pregnant women have been linked to an increased risk for adverse maternal health outcomes including: (i) wound infection; (ii) GDM; (iii) PIH; (iv) PET; (v) antepartum haemorrhage; (vi) postpartum haemorrhage; (vii) maternal hospital admissions; (viii) urinary tract infection; (ix) post-date pregnancy; (x) malpresentation; (xi) premature rupture of membranes; (xii) failed induction of labour; and (xiii) c-section delivery (Zar, *et al*., 2019; Onubi, *et al*., 2016; Basu, Jeketera and Basu, 2010;).Therefore, efforts directed at reducing obesity in

pregnancy may have a significant impact in achieving some targets of SDG 3 by 2030. Hence, the focus of this literature review is to highlight the current research available on the assessment of nutritional status during pregnancy using anthropometric measurements, the risk factors associated with overweight and obesity in pregnancy as well as the internal and external factors that affect nutritional status outcome.

2.2 Maternal nutritional status and anthropometric measurements

Nutritional status is a method used to assess the health status of an individual at a given time (Huhmann, 2017). Screening the nutritional status of women of child-bearing age as well as in pregnant women is an essential part of women's primary health care services. The most used nutritional status assessment tools are anthropometric measurements. Anthropometry is defined as the measurements of the body parts, which when compared to standardized or reference measurements, give a reliable indication of the body composition and nutritional status (Lahner, 2019). In pregnancy nutritional status is commonly assessed using methods such as body mass index (BMI), gestational weight gain, and other methods like arm measurements (Chodankar, *et al.*, 2017).

The BMI classification was developed by Ancel Keys, with the function of categorizing the risk associated with weight gain in relation to height (Barnett, 2016). It can be calculated by dividing the weight (kg) of the subject by the height (cm) squared [BMI = weight (kg) /height $(m)^2$ (WHO, 1996). The classification of the BMI score has been defined in *Table 2.1*.

Table 2.1: BMI classification (Cederholm, et al., 2017; WHO, 1996)

BMI value (kg/m^2)	**Interpretation**
< 16.00	Severe malnutrition
16.00 – 17.00	Moderate malnutrition
17.0 – 18.49	Mild malnutrition
18.50 – 24.90	Normal (ideal body weight for height)
≥ 25.00	Overweight
25.00 – 29.90	Pre-obese
30.00 – 34.90	Obese Class I, moderate obesity
35.00 – 40.00	Obese Class II, severe obesity
40.00 – 44.90	Obese Class III, morbid obesity

> 45.00	Obese Class III, super morbid obesity

In pregnancy, overweight and obesity are defined as having a BMI of ≥25 kg/m2 and 30 kg/m2 respectively at the first antenatal visit or post-delivery (Chodankar, *et al*., 2017). Although BMI is a universal tool used to classify obesity, it has been criticized for potentially giving false interpretations as it is not able to discriminate between tissues for example fat mass and muscle mass (Kim, Després and Koh, 2016). This is especially true for pregnancy, where the foetus-associated tissues are included in the mother's body weight measurement. Also, using BMI as the main indicator of health status has been criticized to misclassify cardiometabolic health (Tomiyama, *et al*., 2016). Hence, it is recommended to use other assessment tools in conjunction with BMI. Overall, BMI is an indicator of current nutritional status, but gestational weight gain is another method used for tracking the rate of weight gain or changes in weight during pregnancy.

Gestational weight gain can be defined as the calculated difference between the weight at the first and last prenatal visit just before delivery (Kominiarek and Peaceman, 2017). The overall weight gain is comprised of weight from an increase in water, protein, or fat in the foetus, placenta, uterus, amniotic fluid, maternal blood volume, mammary glands, and maternal AT (refer to ***Figure 2.2***).

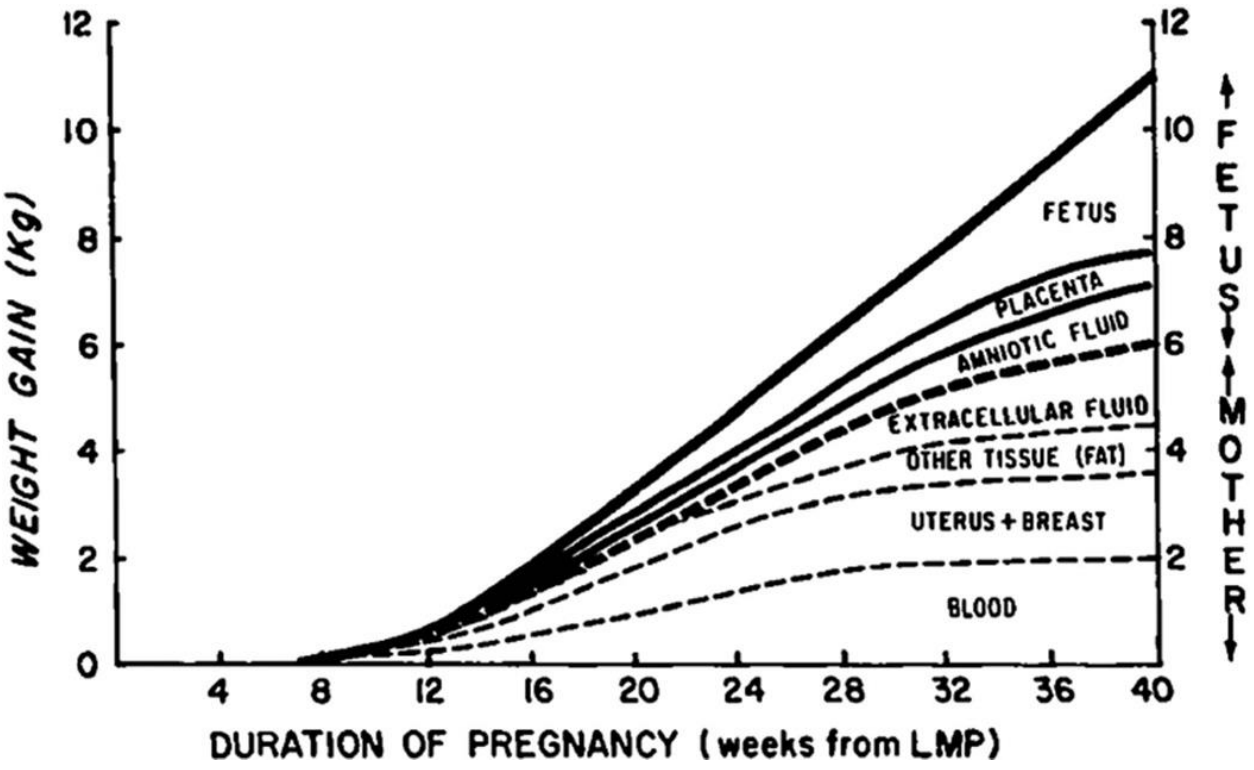

Figure 2.2: Pattern and components of average weight gain in pregnancy (Pitkin, 1976)

It is recommended that gestational weight gain be advised based on the mother's pre-pregnancy BMI (Pitkin, 1976). The recommendations are defined in *Table 2.2* (Pitkin, 1976; Kominiarek and Peaceman, 2017).

Table 2.2: Gestational weight gain recommendations based on BMI (Kominiarek and Peaceman, 2017; Pitkin, 1976)

Pre-pregnancy weight status	**BMI kg/m^2**	**Recommended total weight gain (kg)**	**Recommended rate of weight gain per week in 2nd and 3rd trimester (kg)**
Underweight	< 18.5	12.5 – 18.0	0.51
Normal	18.5 – 24.9	11.5 – 16.0	0.42
Overweight	25.0 – 29.9	7.0 – 11.5	0.28
Obese	≥30.0	5.0 – 9.0	0.22

Weight gain more than the recommended is considered excess weight gain and is associated with increased risk for metabolic disorders such as GDM and PET (Kominiarek and Peaceman, 2017). According to a meta-analysis of 39 cohorts by Santos, *et al*. (2019), it was identified that obese women with high gestational weight gain had the greatest risk of any pregnancy complications in comparison to normal weight women with medium gestational weight gain (OR=2.51; 95%CI 2.31-2.74). Also, it was estimated that 23.9% of the pregnancy complications experienced by the women were associated with overweight or obesity (Santos, *et al*., 2019). Hence, BMI and gestational weight gain are useful indicators of nutritional status when accurate weights can be taken repeatedly. However, in resource limited environments, like South Africa, finding other surrogate measurement methods are important, these include arm measurements.

Longitudinal studies in pregnant women have identified that variable changes can occur across different skinfold thickness (SFT) sites and circumferences throughout pregnancy, and they are useful indicators of nutritional status during pregnancy (Ramlal, *et al*., 2012; Soltani and Fraser, 2000; Adair, Pollitt and Mueller, 1983; Taggart, *et al*., 1967). In a Brazilian study, it was identified that MUAC was strongly correlated (r=0.872) with BMI in pregnant women and could be used as a quick and effective nutritional assessment tool (Miele, *et al.*, 2021). A South African study investigated the correlation between MUAC and BMI in pregnant women which identified that MUAC was strongly correlated with BMI in pregnant women (r=0.92), where a MUAC measurement of ≥ 30.57 cm was classified as obesity and a MUAC measurement of ≥ 22.8 cm was classified as malnutrition (Fakier, Petro and Fawcus, 2017). An earlier study conducted by Kruger (2005), identified that wasting in pregnant women was classified as a MUAC measurement of < 22 cm. Hence, there is a need to further validate the MUAC cut-off measurements that could be used to assess nutritional status in pregnant South African women. Also, circumferences like MUAC are useful for determining overall fat and muscle stores in the designated area by using them in conjunction with skinfolds to differentiate between what is fat versus muscle (Widen and Gallagher, 2014). However, to date, there are no South African based studies which have investigated the correlation between mid-arm muscle area and maternal BMI or their accuracy as predictors of nutritional status in black South African pregnant women.

Using arm measurements like skinfolds are an accurate alternative method to assess the nutritional status of pregnant women. This was confirmed by an Australian study by Kannieappan, *et al*.

(2013), which investigated bicep, tricep and subscapular skinfold thickness measurements in correlation to body fat percentage. It was identified that these skinfold sites were reliable measurements for calculating body fat percentage in pregnant overweight and obese women (Kannieappan, *et al*., 2013). In contrast, a randomized control trial reported that skinfold measurements were not significantly correlated to gestational weight gain during pregnancy (Dodd, *et al*., 2015). However, to date, there are a lack of South African based studies on the correlation between maternal BMI and maternal skinfold measurements or their accuracy as predictors of nutritional status in black South African pregnant women.

2.3 Drivers of overweight and obesity in pregnancy

Maternal overweight and obesity is driven by various modifiable and unmodifiable internal and external stimuli. The interplay between these factors leads to weight gain, excess adiposity, and the associated health risks.

2.3.1 Socio-economic environment

South Africa is a developing country with a high rate of unemployment, with many households experiencing poverty and food insecurity (Kruger, *et al*., 2005). Food insecurity means that households are unable to have adequate access to affordable, nutritious, and culturally appropriate foods (Kruger, *et al*., 2005). According to the SANHANES survey, it was reported that the national prevalence of households experiencing hunger was 26.0% (Shishana, *et al*., 2013). Pregnant women that experience food insecurity, tend to have low dietary diversity, with a high consumption of affordable starchy staples while excluding proteins and other nutrients from their diet (Savy, *et al*., 2005). This high energy and high carbohydrate dietary pattern have been associated with excess adiposity in pregnant women (Coleman, *et al*., 2014). For example, in an Irish cohort, pregnant women from the lower social class group who were experiencing low dietary diversity and food insecurity were associated with having an adverse body composition in the first trimester of pregnancy, most notably increased visceral adiposity (Coleman, *et al*., 2014). According to Kehoe *et al*., (2021), food security was an important determinant of diet quality in low income, urban areas in South Africa. It was reported that food insecure women had the least dietary diversity, however no associations were identified between food security and body size or composition (Kehoe, *et al.,* (2021). Another aspect that effects the socio-economic status, is the educational level of the pregnant women. As stated by Gebremedhin and Bekele (2021), the

gestational weight gain among women who had secondary or above education (9.5, 8.2-10.9 kg) was higher than women with lower education (5.0, 4.3-5.8 kg). Likewise, gestational weight gain in women from the richest households (9.0, 7.2-10.7 kg) was superior to those from poorest households (6.1, 5.3-7.0 kg) (Gebremedhin and Bekele, 2021). In summary, the socio-economic environment that pregnant women are exposed to during pregnancy has the potential to affect their dietary choices which then may affect their gestational weight gain during pregnancy and overall nutritional status.

2.3.2 Nutrition transition

The overweight and obesity epidemic in South Africa has largely been influenced by the process of nutrition transition, which is defined as a shift from a 'end of famine' dietary pattern to a 'energy-dense' dietary pattern (Kruger, *et al.*, 2005). Hence globalization, industrialization and more freedom of movement has exposed the South African population in both rural and urban geographical areas to a unique obesogenic environment which has resulted in changes to the dietary patterns. Namely a shift from traditional foods low in fat and rich in fibre, towards meat and dairy products containing high levels of saturated fats and more highly refined food products (Madlala, *et al*, 2021; Kruger, *et al*., 2005). In a study conducted by Kroll *et al*., (2020), the correlation between neighbourhood food provision with household consumption and poverty was investigated in Khayelitsha, South Africa. It was reported that risky food environments and poverty together promoted obesogenic diets, where 71% of the households consumed obesity-risky foods like bread and processed meat and only 16% of households consumed foods deemed protective of obesity such as fruits and vegetables (Kroll *et al*., 2020). In another South African study by Wrottesley, Pisa and Norris (2017), which investigated the associations between dietary patterns and BMI-specific gestational weight gain in pregnant women, identified that three types of dietary patterns have been found including western, traditional, and mixed. Women that followed a traditional dietary pattern had a reduced risk of excessive gestational weight gain (OR=0.81; 95%CI 0.69-0.94) and the diet was characterized as being high in whole grains, legumes, vegetables, traditional meats, refined sugar, and fat. Whereas the western diet was associated with higher weight gain in normal weight women. The western diet was characterized as energy dense, processed, high in sugar and high fat foods such as white bread, processed and red meat, roast potatoes and chips, sweets and chocolate, soft drinks, and cheese (Wrottesley, Pisa and Norris,

2017). Overall, when pregnant women are exposed to obesogenic environments, it leads to shifts in dietary patterns which can then contribute to diet-induced weight gain during pregnancy.

2.3.3 Diet-induced weight gain

Dietary intake during pregnancy plays a major role in overnutrition. Overnutrition is defined as a positive energy balance that occurs when the total caloric intake from the diet exceeds the energy expenditure (Miller, 2017). According to Soma-Pillay, *et al*., (2016), pregnancy is an anabolic process that affects the metabolism of all nutrients to support maternal metabolic homeostasis, foetal growth and development and to prepares for lactation. These metabolic adjustments lead to an increased deposition of maternal energy stores, production of foetal tissue, redistribution of nutrients, altered nutrient absorption, and an increased basal metabolic rate (Soma-Pillay, *et al.*, 2016). It is well documented that pregnancy is also associated with a change in eating behaviours usually increased hunger, decreased restraint, and increased food intake in comparison to before pregnancy (Clark and Ogden, 1999). In maternal overweight and obesity, there is an associated homeostatic dysregulation of hunger and satiety controlled by signalling pathways from the hypothalamus and the effects of adipokines like leptin (*LEP)* and Ghrelin (*GHRL)* (Fabricatore and Wadden, 2004). Hence, the decreased satiety and increased hunger will encourage an increase in caloric intake through the diet. One of the proposed mechanisms of diet-induced obesity is caused by the initiation of inflammation, oxidative stress, endoplasmic reticulum stress, and adipogenesis caused by high-calorie and high-fat diets, especially saturated fatty acids (Boden, 2011; Guo, *et al.*, 2007). Hence, the high plasma fatty acids are associated with having pro-inflammatory and prooxidant effects which have also been linked to insulin resistance (Samuel, *et al*., 2010). Another mechanism in which diet affects the pathogenesis of maternal overweight and obesity is by the manipulation of the gut microbiome (Ruebel, *et al*., 2021). The gut microbiome is a component of the gut-brain axis which is understood as the interactions among nutrients, enteral neuroendocrine cells, and the autonomic nervous system which are collectively involved in a range of signalling pathways for energy homeostasis (Geurts, *et al.*, 2014). In a recent study by Ruebel *et al*. (2021), the maternal obesity status was associated with changes in the microbiome including an increase in the species *Lachnospiraceae, Bilophila*, *Dialister*, and *Roseburia*. Also, maternal BMI, fat mass, triglyceride, and insulin levels were positively associated with *Bilophila* (Ruebel, *et al*., 2021). Hence, the dietary intake of women during pregnancy can influence gestational weight gain and should therefore be included in maternal overweight and obesity

studies. In a South African study by Madlala *et al*. (2021), the associations between dietary intake and obesity were investigated in pregnant women living with and without HIV. It was reported that in women without HIV who consumed potato (aOR=1.98; 95%CI 1.02-3.84), pumpkin/butternut (aOR=2.13; 95%CI 1.29-3.49), milk in tea/coffee (aOR=6.04; 95%CI 1.37-26.50) all increased the risk for excess gestational weight gain. But the consumption of eggs (aOR= 0.52; 95%CI 0.32-0.86) decreased the risk for overweight and obesity, whereas consumption of peanuts or nuts (aOR=0.34; 95%CI 0.14-0.80) decreased the risk of excessive gestational weight gain. In the pregnant women with HIV who consumed milk/yoghurt/maas to drink/on cereals (aOR 0.35; 95%CI 0.18-0.68), tomato (raw/cooked) (aOR 0.50; 95%CI 0.30-0.84), green beans (aOR 0.41; 95%CI 0.20-0.86), mixed vegetables (aOR 0.49; 95%CI 0.29-0.84) and legumes (aOR 0.50; 95%CI 0.28-0.86) reduced risk of becoming overweight or obese. Also, those that consumed tomato (raw/cooked) (aOR 0.48; 95%CI 0.24-0.96) and mixed vegetables (aOR 0.38; 95%CI 0.18-0.78) had a reduced risk of excessive gestational weight gain (Madlala, *et al*., 2021). In summary, there is evidence suggesting that specific dietary patterns during pregnancy are associated with changes in body composition which can lead to an overweight or obese nutritional status in pregnancy.

2.3.4 Reduced physical activity

According to Galgani and Ravussin (2008), physical exercise is most the effective method to increase the basal metabolic rate, expend energy stores, and prevent excess adiposity. The energy expenditure associated with physical exercise increases based on the intensity of the activity and the amount of cardio-respiratory effort exerted e.g., walking versus running (Galgani and Ravussin, 2008). The benefits of physical exercise in pregnancy include improved insulin sensitivity, improved postprandial blood glucose level, improved psychological well-being, and reduced risk of adverse maternal outcomes like GDM, PET, and an operative birth. (Muhammad, Pramono and Rahman, 2021; Sui and Dodd, 2013). However, despite the benefits of physical exercise there are various barriers that prevent pregnant women from engaging in physical activities such as pregnancy-related symptoms, lack of time, access to childcare, cultural practices, and fears about their safety and that of their unborn infant (Sui and Dodd, 2013). Sedentary behaviours during pregnancy have been associated with excess gestational weight gain (Fazzi, *et al.*, 2017). According to a study by Bacchi, *et al*., (2016), which investigated the physical activity patterns in normal-weight compared to overweight or obese pregnant women, pre-pregnancy

physical activity of >150 minutes per week was an independent predictor of women being physically active during their pregnancy. But physical activity volume was significantly lower in overweight and obese pregnant women in comparison to normal-weight pregnant women (Bacchi, *et al*., 2016). A common pattern identified between studies is that physical activity declines from the first to third trimester (Watson, *et al*., 2017; Bacchi, *et al*., 2016; McParlin, *et al*., 2010). This pattern was reported in South African pregnant women, where a significant decrease in moderate and vigorous physical activity was noted between second and third trimester (Watson, *et al*., 2017). According to van Poppel, *et al*., (2013), this decrease in moderate and vigorous physical activity throughout pregnancy is associated with significantly higher fasting insulin levels, worse insulin sensitivity, increased first- and second-phase insulin response, and higher triglyceride levels in late pregnancy compared to women with smaller decreases in moderate and vigorous physical activity. Hence, physical activity is an important health indicator to monitor throughout pregnancy.

2.3.5 HIV infection and antiretroviral treatment

Across different studies the HIV infection and ART have both been investigated as potential risk factors for causing changes in body composition during pregnancy. In terms of wasting, HIV-infected pregnant women are vulnerable to nutrient deficiencies due to the increased nutritional requirements associated with the combination of HIV infection and pregnancy (Papathakis and Rollins, 2005). HIV-infected individuals have long been associated with wasting syndrome, whereby the depletion of the CD4+ T cell count and raised viral load results in a hypermetabolic response leading to the catabolism of muscle and fat tissue and an overall change in BMI (Erlandson, *et al*., 2016). This was confirmed by Floridia, *et al*., (2020), where a significant positive correlation was found between gestational weight gain and an increase in CD4+ T-cell count during pregnancy. Furthermore, across studies there is a consistent pattern that HIV-infected pregnant women have lower measurements than HIV-uninfected pregnant women. For example, in a South African study by Madlala, *et al*., (2020), HIV-uninfected pregnant women had a median BMI of 29.0 kg/m^2 whereas HIV-infected pregnant women had a median BMI of 28.0 kg/m^2. In a Kenyan cohort, HIV-infected pregnant women had significantly lower measurements in comparison to HIV-uninfected pregnant women including total body weight, fat mass, fat-free mass, tricep skinfold thickness, arm fat area and MUAC (Widen, *et al*., 2019). In study conducted in Uganda, weight and BMI during pregnancy were lower in HIV-infected pregnant women than in comparison HIV-uninfected pregnant women (Ladner, *et al*., 1998). However, there has been

some opposing evidence of the effect that HIV may have on wasting. As reported by another study conducted in Uganda by Widen *et al*., (2017), HIV was not associated with body composition. But the pregnant women's body composition was more effected by their nutritional intake, where food insecurity was significantly inversely associated with body weight and BMI (Widen, *et al*., 2017). In contrast, ART has been associated with increases in body fat mass and increases in BMI during pregnancy.

In South Africa, up to 40% of pregnant women are living with HIV, and of those 30-45% are classified as obese (Bengtson, *et al*., 2020). One of the documented reasons for the weight gain in the HIV-infected is due to the initiation of ART, where there is the restoration of the immune system and subsequently, this results in significant weight gain, irrespective of the baseline weight before initiation (Nduka, *et al.*, 2016; Levitt, *et al*., 2016; Guehi, *et al*., 2016; Nguyen, *et al*., 2016; Lakey, *et al.*, 2013; Huis in 't Veld, *et al.*, 2018; Crum-Cianflone, *et al*., 2010). Consequently, this is reflected in an increase in the BMI (Nduka, *et al.*, 2016; Guehi, *et al*., 2016; Huis in 't Veld, *et al.*, 2018; Crum-Cianflone, *et al*., 2010; Obry-Roguet, *et al*., 2018). Although this immune reconstitution allows for improvement in the nutritional status via weight gain, the concern with this process is related to the amount and site of AT storage (Nguyen, *et al*., 2016). Studies have identified that HIV-infected patients on ART have abnormal body fat distribution called fat redistribution syndrome or lipodystrophy and it occurs as: (i) lipoatrophy defined as the decrease in AT volume in non-ectopic AT deposition sites; and (ii) lipohypertrophy defined as the increase in AT volume in the ectopic AT deposition sites (Deeks, Lewin and Havlir, 2013; Anuurad, Bremer and Berglund, 2010; Lake, *et al.*, 2017). HIV-infected on ART females are at a higher risk for becoming overweight in comparison to HIV-infected on ART males (Guehi, *et al*., 2016; Lakey, *et al.*, 2013; Huis in 't Veld, *et al.*, 2018; WHO, 1996; Wand and Ramjee, 2013; Malaza, *et al.*, 2012). Also, this risk extends to pregnant women. An example of how ART can affect fat mass in pregnant women was demonstrated in the Tsepamo study which was conducted in Botswana (Caniglia, *et al*., 2020). They reported that significantly larger fat mass was gained in pregnant women who were receiving dolutegravir compared to efavirenz (Caniglia, *et al*., 2020). In another cohort by Joseph, *et al*. (2021), it was reported that pregnant women receiving ART with a combined regimen of integrase strand transfer inhibitors (INSTI) and tenofovir alafenamide (TAF) had a 1.7 times increased risk of excess gestational weight gain during pregnancy.

The HIV infection and treatment thereof in combination with pregnancy have been associated with other maternal complications that are also associated with obesity. According to the findings in an 11-year cohort, HIV-infected pregnant women had an increased risk for GDM, PET, preterm contractions, premature rupture of membranes, and postnatal complications (Reitter, *et al.*, 2014). Also, in an Italian study, it was reported that one-quarter of pregnant women infected with HIV were overweight or obese, and they had a significantly increased occurrence of co-morbidities including GDM and PIH (Floridia, *et al*., 2013). Lastly, HIV infection has been associated with anaemia in pregnancy (Dorsamy, Bagwandeen and Moodley, 2020). However, in maternal obesity, an increase in haemoglobin levels has been associated with an increase in BMI (Onubi, *et al.*, 2016). Overall, HIV infection and ART thereof serve as important risk factors for body composition changes in pregnancy as well as increase the risk for co-morbidities.

2.3.6 Genetic and epigenetics factors

Maternal overweight and obesity has a genetic component. Candidate genes increase the risk for weight gain by causing the dysregulation of signalling pathways for example in eating behaviours such as in appetite homeostasis, taste perception, and its role in foods preferences, as well as in the regulation of energy expenditure (Pigeyre, *et al.*, 2016; Cvijanovic, *et al.*, 2015). Epigenetic factors regulate gene expression at the transcriptional and post-transcriptional level which affects the onset of obesity by regulating energy balance even in the absence of gene sequence mutations and polymorphisms (Wankhade, Thakali and Shankar, 2016). Also, circulating or tissue-specific micro ribonucleic acids (miRNAs) and non-coding RNA have been linked to obesity (Engin, 2017; Zaiou, *et al.*, 2018; McGregor and Choi, 2011). Studies have targeted genes like *ADIPOQ*, *LEP*, *LEPR*, *FTO*, and *GHRL* to understand how they influence these signalling pathways in obesity. *ADIPOQ* is a type of adipokine which is produced and secreted by adipocytes (Fang and Judd, 2018). *ADIPOQ* has an anti-inflammatory effect via the signalling mechanisms of its receptors AdipoR1 and AdipoR2 (Fang and Judd, 2018). Obesity is an inflammatory disease (Ellulu, *et al*., 2017) and for this reason, *ADIPOQ* has been associated with providing a protective function against obesity via its antiapoptotic and antilipogenic properties (Nogues, *et al*., 2019). *ADIPOQ* is also involved in energy homeostasis by modulating insulin sensitivity and influencing fatty acid oxidation in skeletal muscle as well as inhibiting the production of glucose in the liver (Fang and Judd, 2018).

LEP is also a type of adipokine which is predominantly produced and secreted by adipocytes and plays an important role in weight loss (Mechanick, Zhao and Garvey, 2018). *LEP* functions by binding to and activating the *LEPR* and signals the hypothalamus to suppress appetite, increase energy expenditure, and consequently result in decreased food intake (Mechanick, Zhao and Garvey, 2018). Regulatory factors of *LEP* may include adiposity, food intake, gender, age, exercise, and glucose uptake (Mechanick, Zhao and Garvey, 2018). In non-obese pregnant women, *LEP* secretion is increased (Faas, Melgert and Paul de Vos, 2010). However, there is growing evidence to suggest that obesity in pregnancy is associated with *LEP* resistance which is characterized by reduced satiety, overnutrition, and increased BMI (Obradovic, *et al*., 2021). *LEP* resistance in obesity occurs due to the *LEP* inability to reach the target cells, a reduction in *LEPR* expression, or a disturbed *LEPR* signalling with the central nervous system (Obradovic, *et al*., 2021). Expression of adipokines like *ADIPOQ* and *LEP* has been studied in human tissues such as placenta and adipose. In a study conducted by Nogues, *et al*., (2019), it was reported that maternal obesity was associated with epigenetic changes in leptin and adiponectin systems characterised by a downregulation of both leptin and adiponectin in placental tissue. However, in a study by Haghiac, *et al.,* (2014), it was identified that maternal adipose tissue, was a prominent site in for ADIPOQ gene expression in comparison to the placenta, with maternal obesity being associated with a decrease in ADIPOQ mRNA expression in adipose tissue. For this reason, adipose tissue should be used as the primary site of adipokine gene expression studies in pregnant women. To date, there has been no South African studies that have investigated adipokine gene expression patterns in the adipose tissue of pregnant women. *GHRL* is a type of orexigenic peptide predominantly produced in the enteroendocrine cells of the stomach, however, in pregnancy, the placenta is known to produce most of the *GHRL* (Abdalla, 2015). It is well known as the hunger hormone due to its effect on the regulation of food intake and energy metabolism by increasing appetite and stimulating the secretion of growth hormone (Abdalla, 2015). In pregnancy, studies have identified that serum *GHRL* levels peak at mid-gestational age and decrease with an increase in body mass, with the lowest levels being in the third trimester (Fuglsang, *et al*., 2005). However, to date, there are no studies that have investigated *GHRL* gene expression in maternal adipose tissue during pregnancy.

The *FTO* gene, which is also an important obesity-associated gene, located on chromosome 16, encodes for the *FTO* protein also known as alpha-ketoglutarate-dependent dioxygenase. The *FTO*

gene is predominantly expressed in the hypothalamus and is involved in mRNA demethylation and energy intake regulation (Frayling, *et al*., 2007). *FTO* is considered a regulatory gene that influences epigenetic control over several key regulatory pathways in obesity (Zhou, Hambly and McLachlan, 2017). The dysregulation of *FTO* is associated with an increased BMI due to mechanisms associated with increased energy intake, impaired satiety response, and increased food responsiveness (Zhou, Hambly and McLachlan, 2017). However, to date, there are no studies that have investigated *FTO* gene expression in maternal adipose tissue during pregnancy.

2.4 Conclusion

As discussed in this review, overweight and obesity in pregnant women is on a rising trend globally as well as within the South African population. The WHO has set out objectives to meet the SDG 3 targets by 2030 (WHO, 2019). Therefore, studies directed at understanding the mediators of overweight and obesity in pregnant women will assist in achieving these goals. The assessment of nutritional status is a fundamental aspect of identifying whether pregnant women are overweight or obese, and this is determined by using anthropometric methodology. It has been highlighted that although anthropometric studies have been conducted in pregnant women, there is a need to further validate the use of surrogate measurement methods to accurately assess and predict nutritional status in pregnancy, especially within the black South African pregnant women population living with and without HIV. Also, currently, there are no South African based studies which have investigated the correlation between mid-arm muscle area, maternal skinfold measurements and maternal BMI or their accuracy as predictors of nutritional status in black South African pregnant women. Overweight and obesity are complex conditions which are driven by various internal and external risk factors such as the socio-economic environment, nutrition transition process, dietary patterns, physical activity, chronic conditions like HIV infection and ART, as well as due to the interplay of genetic and epigenetic factors. Currently, there are no studies to have investigated the mRNA expression of genes like *ADIPOQ*, *LEP*, *LEPR*, *GHRL*, and *FTO* in the adipose tissue of pregnant women within the South African population. Therefore, this study aimed to give population-specific insights into the anthropometric assessment, risk factors and epigenetic factors involved in overweight and obesity in black South African pregnant women living with and without HIV.

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CHAPTER 3

Chapter 2 literature review provided an overview of maternal overweight and obesity in pregnancy covering the nutritional assessment tools used, the pathogenesis, drivers of maternal overweight and obesity within the context of the HIV epidemic, as well as the associated maternal health outcomes.

This chapter responds to the objective i to iii of this study to identify the predictive tools for identifying nutritional status in pregnancy in black South African women living with and without HIV. The chapter is presented in the form of a manuscript entitled “The use of anthropometric measurements as predictors of nutritional status in black South African women during pregnancy”.

3. The use of anthropometric measurements as predictors of nutritional status in black South African women during pregnancy

3.1 Abstract

Background: Nutritional risk assessment is an essential component of primary health care screening especially for pregnant women. This study aimed to: (i) investigate the correlation between maternal BMI and maternal anthropometric measurements among black South African pregnant women; (ii) identify what measurement cut-offs accurately predict each nutritional status group; and (iii) investigated the anthropometric differences between pregnant women living with and without HIV.

Methods: A cross-sectional observational study design was used. Two hundred black South African pregnant women were included, where 90 were human immunodeficiency virus (HIV) infected and 110 were HIV-uninfected. The anthropometric measurements assessed included: (i) left mid-upper arm circumference (MUAC); (ii) right MUAC; (iii) right tricep skinfold (TSF); (iv) right subscapular skinfold (SSF); (v) right mid-arm muscle circumference (MAMC); (vi) wrist circumference (WC); (vii) frame size; and (viii) body mass index (BMI). The statistical tests that were applied included: (i) Fisher's exact test and the χ 2 test; (ii) Pearson correlation coefficient; (iii) the Spearman's rank-order correlation coefficient; (iv) the Mann Whitney t-test; (v) one-way ANOVA; and (vi) area under the curve of the receiver operator characteristic curves to determine the cut-off values.

Results: Maternal age was significantly associated with changes in maternal anthropometric measurements. Maternal BMI was significantly correlated with other maternal anthropometric measurements including MUAC (left and right), TSF (right), SSF (right), MAMC (right), WC (right), and frame size. The anthropometric methods that were accurate for assessing obesity in pregnancy included TSF (right) (cut-off of ≥20.75 mm), SSF (right) (cut-off of ≥21.75 mm), MAMC (right) (cut-off of ≥25.23 cm), and WC (right) (cut-off of ≥16.25cm). Also, SSF (right) (cut-off of ≥15.75mm) and MAMC (right) (cut-off of ≥23.35cm) could be used to assess for overweight nutritional status. Lastly, frame size could be used to assess for underweight (cut-off of ≥10.05) and normal (cut-off of ≥9.95) nutritional status. The HIV-infected pregnant women did not differ anthropometrically to the HIV-uninfected pregnant women.

Conclusion: Maternal nutritional status can be accurately predicted by using surrogate maternal anthropometric measurements such as MUAC, TSF, SSF, MAMC, WC, and frame size. Pregnant women living with HIV do not differ anthropometrically to pregnant women living without HIV.

3.2 Background to the study

Nutritional risk assessment is an essential component of primary health care screening systems, especially for the identification of nutritionally at-risk pregnant women (Heslehurst, 2011). Nutritional status during pregnancy can be classified by using the pregnant women's anthropometric measurements (Onubi, *et al*., 2015). Since overweight and obesity in pregnancy are growing public health concerns in low to middle-income countries like South Africa where pregnant women are living and without the human immunodeficiency virus (HIV) infection, it is a priority to determine how best to assess them (Bengtson, *et al*., 2020; Chen, Xu and Yan, 2018). The definition of overweight and obesity during pregnancy are most frequently assessed using the body mass index (BMI) classification, with a score of $\geq$ 25 kg/m^2 and $\geq$ 30 kg/m^2 respectively (Chodankar, *et al*., 2018; Vidona, Wadioni and Okeke, 2017; Lim and Mahmood, 2015; Fitzsimons, Modder and Greer, 2009). However, in resource limited settings such as in South Africa, surrogate measurement methods are needed to easily assess nutritional status when assessors are unable to measure BMI. Hence, other anthropometric studies have been conducted in pregnant women using other body measurements, such as arm circumferences and skinfold thickness measurements (Miele, *et al.,* 2021; Fakier, Petro and Fawcus, 2017; Dodd, *et al*., 2015; Widen and Gallagher, 2014; Kannieappan, *et al*. 2013; Kruger, 2005). However, there is a need to further validate the use of these surrogate measurement methods to accurately assess and predict nutritional status in pregnancy, especially within the black South African pregnant women population living with and without HIV. Also, currently, there are no South African based studies which have investigated the correlation between maternal BMI and maternal mid-arm muscle area or maternal skinfold measurements and their accuracy as predictors of nutritional status in black South African pregnant women. Therefore, this study sought to provide insight into whether there is an association between maternal BMI and other maternal anthropometric measurements among black South African pregnant women; and identify what measurement cut-offs accurately predict each nutritional status group.

HIV and antiretroviral treatment (ART) in pregnancy are associated with an increased risk for cardio-metabolic abnormalities and changes in body weight (Bengtson, *et al*., 2020). Pregnancy is an anabolic process where there is physiological alterations of the delivery, metabolism, and storage of nutrients to meet the needs of the growing feotus and mother (King, 2000). In combination with HIV, these metabolic processes are further altered which and may cause shifts in fat mass and fat-free mass (Macallan, 1999; Grinspoon, *et al*., 1998). Hence, HIV and ART have been associated with body composition changes in pregnant women such as wasting as well as weight gain (Joseph, *et al*., 2021; Caniglia, *et al*., 2020; Floridia, *et al*., 2020; Madlala, *et al*., 2020; Widen, *et al*., 2019; Erlandson, *et al*., 2016; Ladner, *et al*., 1998).Therefore, since HIV and ART in pregnancy are also associated with an increased risk for changes in body composition, this study also investigated the anthropometric differences between pregnant women living with and without HIV.

3.3 Methods

3.3.1 Sample selection and study population

A cross-sectional observational retrospective study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital (PMMH), which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. The catchment area for the hospital includes both rural and urban geographical areas. Pregnant women admitted to the labour ward were approached to participate in this study from April 2019 to December 2019. The inclusion criteria for this study were as follows: (1) ≥ 18 years of age; (2) pregnant females; (3) black South African citizen; (4) clinically stable; (5) able to stand without assistance; and (6) provided verbal and written consent to participate in the study. A total of 458 pregnant women participated in the study, but 245 subjects met the inclusion criteria and of these 45 declined to participate. Hence, a total of 200 subjects met all the inclusion criteria, where 90 were HIV-infected and 110 were HIV-uninfected. The HIV-infected pregnant women were compliant with the prescribed fixed-dose combination (FDC) ART. The prescribed treatment was in accordance with the South African prevention of mother to child transmission (PMTCT) protocol and the South African ART guidelines. The HIV-infected pregnant women received tenofovir (TDF) + emtricitabine (FTC) or lamivudine (3TC) + efavirenz (EFV) as FDC (DOH, 2014; DOH, 2013). Most of the women had a singleton pregnancy (98.0%), and others were a twin pregnancy (2.0%).

3.3.2 Maternal anthropometric assessment

Anthropometric measurements were conducted by a level 1 dietician who was International Society for the Advancement of Kinanthropometry (ISAK) trained. According to Marfell-Jones, Stewart and de Ridder (2012) and Ulijaszek and Kerr (1991), to avoid anthropometric measurement errors, all measurements were conducted by the same researcher, taken three times, and recorded to the nearest 0.1 cm/mm/kg. The mean of the two closest values was recorded. All measurements were conducted on the right side of the body unless indicated (Marfell-Jones, Stewart and de Ridder, 2012; Ulijaszek and Kerr, 1991).

3.3.2.1 Body Mass Index (BMI)

The standing height (SH) measurement was measured via stretch stature methodology using a calibrated portable stadiometer (Seca) with a sliding headboard (Lahner, Kassier and Veldman, 2017). The weight measurement was taken using the actual body weight (ABW) methodology (Lahner, 2019), using a portable calibrated scale (Seca, with a maximum weight threshold of 250 kg). The scale was calibrated before the commencement of the study. BMI was calculated using the weight of the mother post-delivery. The BMI was then interpreted, and the pregnant women were categorized according to the following classifications: (1) underweight, BMI of <18.5 kg/m^2; (2) normal, BMI of between 18.5 and 24.9 kg/m^2; (3) overweight, BMI of between 25.0 and 29.9 kg/m^2; and (3) obese, BMI of $\geq$ 30.0 kg/m^2 (Weir and Jan, 2020).

3.3.2.2 Mid upper arm circumference (MUAC)

The MUAC measurement was assessed using a Seca measuring tape. The MUAC was measured on the left and right arm and was determined by measuring the linear distance between the acromial and radial sites, with the arm muscle relaxed and the arm extended to their side. The midpoint between these two sites was called the mid-acromial-radiale. The circumference of the arm was measured at the level of the mid-acromial-radiale, perpendicular to the long axis of the arm. The MUAC is the sum of muscle tissue and subcutaneous fat, and it can be used as an indicator of maternal nutritional status, body composition, and arm thickness (Eaton-Evans, 2013; Fakier, Petro and Fawcus, 2017). The MUAC (right) was interpreted according to percentile readings *(refer to Table 1)* (McDowell, *et al*, 2005; Frisancho, 1974).

Table 3.1: Summary of the interpretation of percentile readings for MUAC, TSF, SSF, and MAMC (Frisancho, 1974; McDowell, Fryar, Hirsch, and Ogden, 2005; Frisancho, 1981).

Percentile reading:	**MUAC**	**TSF**	**SSF**	**MAMC**
$\leq 5^{th}$	Very thin arm size	Very low-fat stores	Very low-fat stores	Very low muscle stores
$\leq 10^{th}$	Very thin arm size	Very low-fat stores	Very low-fat stores	Very low muscle stores
$\leq 25^{th}$	Thin arm size	Low-fat stores	Low-fat stores	Low muscle stores
$\leq 50^{th}$	Normal arm size	Low-fat stores	Low-fat stores	Low muscle stores
$\leq 75^{th}$	Thick arm size	High-fat stores	High-fat stores	High muscle stores
$\leq 90^{th}$	Thick arm size	High-fat stores	High-fat stores	High muscle stores
$\leq 95^{th}$	Very thick arm size	Very High-fat stores	Very High-fat stores	Very High muscle stores
$\geq 95^{th}$	Very thick arm size	Very High-fat stores	Very High-fat stores	Very High muscle stores

Abbreviations: MUAC: Mid-upper arm circumference; TSF: Tricep skinfold; SSF: Subscapular skinfold; MAMC: Mid-arm muscle circumference

3.3.2.3 Tricep skinfold thickness (TSF) and Subscapular skinfold thickness (SSF)

The skinfold thickness measurements were assessed using a calliper. The TSF site is the point on the posterior surface of the right arm, in the mid-line, at the level of the mid-acromial-radiale landmark. The TSF measurement was taken parallel to the long axis of the arm at the TSF site. The SSF site was located by palpating the inferior angle of the scapula with the left thumb in order to find the under most tip. The subject must assume a relaxed standing position with their arms hanging by their sides. The SSF was taken on the right-hand side with the fold running obliquely downwards at the SSF site. The TSF (right) and SSF (right) measurements were interpreted according to percentile readings to determine the fat stores *(refer to Table 3.1)* (Frisancho, 1974; McDowell, Fryar, Hirsch and Ogden, 2005).

3.3.2.4 Mid-arm muscle circumference (MAMC)

The MAMC was used for the muscle associated measurement, which was determined by plotting the right TSF and right MUAC measurement on the adult nomogram (Gurney and Jeliffe, 1973). The value obtained was interpreted using percentile readings to determine the muscle stores *(refer to Table 3.1)* (Frisancho, 1974; Frisancho, 1981).

3.3.2.5 Frame size

The frame size was determined using the frame size calculation (frame size (cm): [height (cm)/ WC (cm)]) (Mahan, Escott-Stump and Raymond, 2012). The frame size ranges from small to large *(refer to Table 3.2)* (Chumlea, Wisemandle, Guo and Siervogel, 2002). The WC was measured amongst participants in the relaxed standing position. The WC site was determined by measuring the minimal circumference of the right wrist perpendicular to the long axis of the forearm, distal to the styloid processes (Mahan, Escott-Stump and Raymond, 2012).

Table 3.2: Interpretation of frame size for females (Mahan, Escott-Stump and Raymond, 2012)

Classification:	**Calculated measurement (cm/cm) in females**
Small body frame	>11.0
Medium body frame	10.1 – 11.0
Large body frame	<10.1

3.3.3 Statistical analysis

Data was captured using Microsoft Excel and continuous variables were represented as arithmetic mean ($\bar{x}$) and standard deviation (SD). Categorical data were presented as percentages (%). Data was categorised in relation to HIV status i.e. (HIV-infected or HIV-uninfected) and nutritional status (underweight, normal, overweight, and obese). Statistical analysis was performed using the statistical software packages, GraphPad Prism 5, and IBM SPSS for Windows version 27. The level of significance (α) used in the statistical analysis was $p<0.05$. The parameters that were included in the statistical analysis were: (i) BMI; (ii) MUAC (right); (iii) TSF (right); (iv) SSF (right); (v) MAMC (right); (vi) WC (right); and (vii) frame size. The statistical tests included: (i) Fisher's exact test (two categories) and the χ 2 test (more than two categories) to investigate the

comparison between categories; (ii) the Pearson correlation coefficient for data with normal distribution was used to identify the strength of association between variable means; (iii) the Spearman's rank-order correlation coefficient for data with non-normal distribution was used to identify the strength of association between variable means (normality was tested using the Kolmogorov–Smirnov test or the Shapiro–Wilk test); (iv) the Mann Whitney *t*-test was used for comparison between two variable means; (v) One-way ANOVA was used for the analysis of variance between the variable means; and (vi) area under the curve (AUC) of the receiver operator characteristic (ROC) curves was used to investigate the accuracy of using the anthropometric measurements and defining nutritional status cut-off points. The interpretation of the AUC was as follows: (i) 0.5 equal to chance; (ii) <0.6 was an inaccurate test of no diagnostic value; (iii) >0.6 and <0.7, interpreted as a poor test; (iv) ≥0.7 and <0.8, interpreted as an acceptable or fair test; (v) ≥ 0.8 and <0.9, interpreted as an excellent test; and (vi) ≥0.9, interpreted as an outstanding test (Mandrekar, 2010). The appropriate cut-off point for each anthropometric measurement was defined by using Youden's index and calculating the Youden's J statistic (sensitivity+specificity-1) for each cut-off measure (Kallner, 2018). The absolute value of correlation coefficient was interpreted as follows: (i) ≥0.0 and <0.2, interpreted as a very weak relationship; (ii) ≥ 0.2 and <0.4, interpreted as a weak relationship; (iii) ≥0.4 and <0.6, interpreted as a moderate relationship; (iv) ≥0.6 and <0.8, interpreted as a strong relationship; and (v) ≥0.8 and ≤1.0, interpreted as a very strong relationship.

3.3.4 Ethics approval and informed consent

The study was approved by the Biomedical Research Ethics Council (BREC) of the University of KwaZulu-Natal (UKZN) (BE269/18), KwaZulu-Natal Department of Health (KZNDOH) (HRKM261/18), and PMMH (29/RESH/2018). All the participants in this study had provided verbal and written consent, participated voluntarily, did not receive incentives, and had the right to withdraw at any stage of the study.

3.4 Results

3.4.1 Comparison of parameters between the nutritional status groups

Two hundred pregnant black South African women were included in this study, having a mean gestational age of 37.7 weeks and a mean age of 27.0 years. The anthropometric parameters that were investigated included MUAC (right), MUAC (left), TSF (right), SSF (right), MAMC (right),

WC (right) and frame size. The characteristics of the pregnant women were categorised according to their nutritional status *(refer to Table 3.3)*.

Table 3.3: Anthropometric data for pregnant black South African females, categorized according to nutritional status

Characteristics	**All pregnant females**	**Maternal BMI[a]**				
		Underweight	**Normal**	**Overweight**	**Obese**	**p-value**
Age	**n=200**	**n=19**	**n=84**	**n=52**	**n=45**	
Mean (SD) (years)	27.0 (5.6)	24.9 (5.0)	26.3 (5.6)	27.5 (5.4)	28.3 (5.5)	0.0778
Gestational age	**n=200**	**n=19**	**n=84**	**n=52**	**n=45**	
Mean (SD) (weeks)	37.7 (2.8)	35.2 (4.0)	37.7 (2.5)	37.8 (2.6)	38.4 (2.0)	0.0004
MUAC[b] (left)	**n=199**	**n=19**	**n=83**	**n=52**	**n=45**	
Mean (SD) (cm)	30.6 (5.1)	24.1 (1.6)	28.0 (2.3)	31.3 (2.4)	37.2 (4.9)	<0.0001
MUAC[b] (right)	**n=198**	**n=19**	**n=82**	**n=52**	**n=45**	
Mean (SD) (cm)	30.7 (5.1)	24.4 (1.8)	28.1 (2.4)	31.4 (2.3)	37.4 (4.7)	<0.0001
Percentile Reading:						
<5th (very thin) (%)	4 (2.0)	4 (21.1)	0 (0.0)	0 (0.0)	0 (0.0)	<0.0001
< 10th (very thin) (%)	1 (0.5)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.0237
< 25th (thin) (%)	11 (5.6)	6 (31.6)	5 (6.1)	0 (0.0)	0 (0.0)	<0.0001
< 50th (normal) (%)	39 (23.2)	7 (36.8)	30 (36.6)	2 (3.8)	0 (0.0)	<0.0001
<75th (thick) (%)	39 (23.2)	1 (0.1)	28 (34.1)	10 (19.2)	0 (0.0)	<0.0001
< 90th (thick) (%)	59 (29.8)	0 (0.0)	17 (20.7)	31 (59.6)	11 (24.4)	<0.0001
< 95th (very thick) (%)	20 (10.1)	0 (0.0)	1 (1.2)	8 (15.4)	11 (24.4)	0.0001
>95th (very thick) (%)	25 (12.6)	0 (0.0)	1 (1.2)	1 (1.9)	23 (51.1)	<0.0001
TSF[c] (right)	**n=198**	**n=19**	**n=82**	**n=52**	**n=45**	
Mean (SD) (mm)	19.5 (6.7)	11.1 (2.5)	16.8 (4.3)	20.3 (4.6)	26.9 (6.4)	<0.0001
Percentile Reading:						
<5th (very low) (%)	10 (5.1)	8 (42.1)	2 (2.4)	0 (0.0)	0 (0.0)	<0.0001

< 10th (very low) (%)	20 (10.1)	5 (26.3)	14 (17.1)	1 (1.9)	0 (0.0)	0.0003
< 25th (low) (%)	60 (30.3)	5 (26.3)	32 (39.0)	20 (38.5)	3 (6.7)	0.0008
< 50th (normal) (%)	62 (31.3)	1 (5.3)	28 (34.1)	16 (30.8)	17 (37.8)	0.0663
<75th (high) (%)	36 (18.2)	0 (0.0)	6 (7.3)	15 (28.8)	15 (33.3)	<0.0001
< 90th (high) (%)	4 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (8.9)	0.0031
< 95th (very high) (%)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.7)	0.0158
>95th (very high) (%)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.7)	0.0158
SSF[d] (right)	**n=197**	**n=18**	**n=82**	**n=52**	**n=45**	
Mean (SD) (mm)	20.6 (7.7)	11.6 (2.4)	17.2 (5.1)	22.7 (5.7)	28.1 (7.5)	<0.0001
Percentile Reading:						
<5th (very low) (%)	13 (6.6)	7 (38.9)	5 (6.1)	1 (1.9)	0 (0.0)	<0.0001
< 10th (very low) (%)	10 (5.1)	5 (27.8)	4 (4.9)	0 (0.0)	1 (2.2)	<0.0001
< 25th (low) (%)	84 (42.6)	5 (27.8)	48 (58.5)	23 (44.2)	8 (17.8)	<0.0001
< 50th (normal) (%)	53 (26.9)	0 (0.0)	18 (22.0)	21 (40.4)	14 (31.1)	0.0050
<75th (high) (%)	10 (5.1)	1 (5.6)	5 (6.1)	2 (3.8)	2 (4.4)	0.9430
< 90th (high) (%)	15 (7.6)	0 (0.0)	1 (1.2)	3 (5.8)	11 (24.4)	<0.0001
< 95th (very high) (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
>95th (very high) (%)	12 (6.1)	0 (0.0)	1 (1.2)	2 (3.8)	9 (20.0)	0.0009
MAMC[e] (right)	**n=198**	**n=19**	**n=82**	**n=52**	**n=45**	
Mean (SD) (cm)	24.4 (2.9)	21.1 (1.4)	22.9 (1.6)	24.9 (1.5)	28.1 (2.2)	<0.0001
Percentile Reading:						
<5th (very low) (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
< 10th (very low) (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
< 25th (low) (%)	4 (2.0)	3 (15.8)	0 (0.0)	0 (0.0)	1 (2.2)	<0.0001
< 50th (normal) (%)	18 (9.1)	5 (26.3)	11 (13.4)	2 (3.8)	0 (0.0)	0.0019

<75th (high) (%)	39 (19.7)	8 (42.1)	29 (35.4)	2 (3.8)	0 (0.0)	<0.0001
< 90th (high) (%)	53 (26.8)	3 (15.8)	28 (34.1)	19 (36.5)	3 (6.6)	0.0016
< 95th (very high) (%)	30 (15.2)	0 (0.0)	10 (12.2)	15 (28.8)	5 (11.1)	0.0070
>95th (very high) (%)	54 (27.3)	0 (0.0)	4 (4.9)	14 (26.9)	36 (80.0)	<0.0001
Wrist circumference (right)	**n=198**	**n=19**	**n=82**	**n=52**	**n=45**	
Mean (SD) (cm)	15.8 (1.1)	14.7 (0.8)	15.4 (0.8)	15.9 (0.8)	16.9 (0.9)	<0.0001
Frame size	**n=198**	**n=19**	**n=82**	**n=52**	**n=45**	
Mean (SD) (cm/cm)	10.1 (0.65)	10.5 (0.51)	10.3 (0.54)	10.0 (0.63)	9.6 (0.62)	<0.0001
Small frame (%)	10 (5.1)	3 (15.8)	5 (6.1)	2 (3.8)	0 (0.0)	0.0627
Medium frame (%)	92 (46.5)	11 (57.9)	52 (63.4)	22 (42.3)	7 (15.6)	<0.0001
Large frame (%)	96 (48.5)	5 (26.3)	25 (30.5)	28 (53.8)	38 (84.4)	<0.0001
Notes: [a]BMI calculated by using maternal body weight post birth; [b]Mid upper arm circumference; [c]Tricep skinfold; [d]Subscapular skinfold; [e]Mid arm muscle circumference						

3.4.2 Maternal age

There was no significant difference (p=0.0778) in the mean maternal age of the participants across all the nutritional status groups. However, there were significant positive correlations identified between age and the maternal anthropometric parameters including BMI (r=0.2607;95%CI 0.1266-0.3856; p=0.0002), MUAC (left) (r=0.2846; 95%CI 0.1515-0.4076; p<0.0001), MUAC (right) (r=0.2775; 95%CI 0.1436-0.4014; p<0.0001), TSF (right) (r=0.2377; 95%CI 0.1016-0.3651; p=0.0007), SSF (right) (r=0.2123; 95%CI 0.07473-0.3420; p=0.0027), MAMC (right) (r=0.2458; 95%CI 0.1101-0.3725; p=0.0005), and WC (right) (r=0.1412; 95%CI 0.0018-0.2753; p=0.0472). These associations identified that a change in the maternal age was associated with a change in the maternal BMI, with the overall pattern being that advanced maternal age was associated with an increase in the size of all anthropometric measurements.

3.4.3 Mid-upper arm circumference (left and right)

There were significant positive correlations identified between maternal BMI and MUAC (left) (r=0.9144; 95%CI 0.8883 – 0.9346; p<0.0001), as well as between maternal BMI and MUAC

(right) (r=0.9106; 95%CI 0.8833-0.9317; p<0.0001). Hence, an increase in the maternal BMI was associated with an increase in the size of both maternal MUAC measurements. These findings suggest that the MUAC measurements were able to reflect when there was a change in the maternal BMI. Furthermore, either MUAC measurement side (right or left) could be used to identify possible changes in the maternal BMI because a significant positive correlation was identified between MUAC (right) and MUAC (left) in the study population (r=0.9747; 95%CI 0.9667-0.9809; *p*<0.0001*) (refer to Figure 1.1).* Hence, MUAC (right) and MUAC (left) had consistently shown to produce similar measurements. But it is important to note that the MUAC (right) measurements were consistently larger than the MUAC (left) measurements (*p*<0.0001).

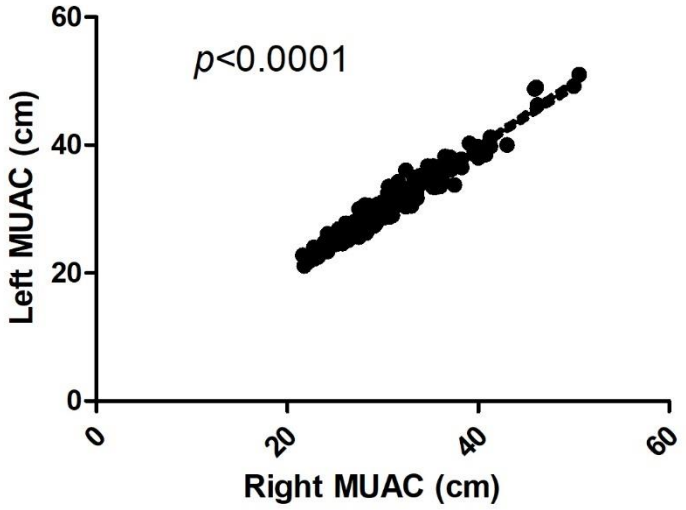

Figure 3.1: Pearson's correlation coefficient between MUAC (right) and MUAC (left), in pregnant black South African women

Right tricep skinfold thickness

There was a significant positive correlation identified between maternal BMI and TSF (right) measurements (r=0.8065; 95%CI 0.7516 – 0.8503; *p*<0.0001) among the pregnant women. Hence, an increase in the maternal BMI was associated with an increase in the size of the maternal TSF (right) measurements further indicating that a change had occurred in the arm associated fat stores. Therefore, the maternal TSF measurements were able to reflect changes in maternal fat stores and nutritional status (*p*<0.0001).

3.4.4 Right subscapular skinfold thickness

There was a significant positive correlation between maternal BMI and SSF (right) measurements (r=0.7375; 95%CI 0.6663 – 0.7953; p<0.0001) among the pregnant women. Hence, an increase in the maternal BMI was associated with an increase in the size of the maternal SSF (right) measurements further indicating that a change had occurred in the maternal fat stores. Therefore, the maternal SSF measurements were able to reflect changes in maternal fat stores and nutritional status (p<0.0001).

3.4.5 Right mid-arm muscle circumference

There was a significant positive correlation between maternal BMI and MAMC (right) measurements (r=0.7964; 95%CI 0.7390 – 0.8423; *p*<0.0001) among the pregnant women. Hence, an increase in the maternal BMI was associated with an increase in size of the maternal MAMC (right) measurements further indicating that a change had occurred in the maternal muscle stores. Therefore, the maternal MAMC measurements were able to reflect changes in maternal arm associated muscle stores and nutritional status (p<0.0001).

3.4.6 Right wrist circumference and frame size

There was a significant positive correlation between maternal BMI and WC (right) measurements (r=0.6822; 95%CI 0.5998 – 0.7503; p<0.0001) and a significant negative correlation between maternal BMI and frame size (r=-0.6016; 95%CI -0.6837 – -0.5044; *p*<0.0001) among the pregnant women. Hence, an increase in the maternal BMI was associated with an increase of the size of the maternal WC (right) measurements and a decrease in the maternal frame size value. Therefore, the maternal WC measurements and frame size were able to reflect changes in maternal nutritional status (p<0.0001).

3.4.7 Accuracy of anthropometric measurements in identifying nutritional status

In this study, ROC curves were used to test the accuracy of using anthropometric measurements for identifying the nutritional status of black South African pregnant women, as summarised in *Table 3.4* below.

Table 3.4: Summary of the accuracy of the anthropometric indicators for assessing nutritional status in pregnant black South African women

Anthropometric Indicator:	Normal	Underweight	Overweight	Obese
MUAC (left) (cm)	X	X	≥28.55*	≥32.30***
MUAC (right) (cm)	X	X	≥28.75*	≥31.95***

TSF (right) (mm)	X	X	X	≥20.75**
SSF (right) (mm)	X	X	≥15.75*	≥21.75**
MAMC (right) (cm)	X	X	≥23.35*	≥25.23***
WC (right) (cm)	X	X	X	≥16.25**
Frame size (cm/cm)	≥9.95*	≥10.05*	X	X

Note: MUAC: Mid-upper arm circumference; TSF: Tricep skinfold; SSF: Subscapular skinfold; MAMC: Mid-arm muscle circumference; WC: Wrist circumference; BMI: body mass index
**poor accuracy, AUC ≥0.60 and <0.70*
***excellent accuracy, >0.80 and <0.90*
****outstanding accuracy, AUC ≥0.90*
X: inaccurate, AUC <0.60

3.4.8 Left mid-upper arm circumference

MUAC (left) was an inaccurate predictive tool for underweight nutritional status (AUC 0.233; 95%CI 0.165-0.300; p<0.0001), and for normal nutritional status (AUC 0.233; 95%CI 0.165-0.300; p<0.0001). MUAC (left) was a poor predictive tool for overweight nutritional status (AUC 0.634; 95%CI 0.560-0.708; p = 0.004*) (refer to Figure 3.2).*

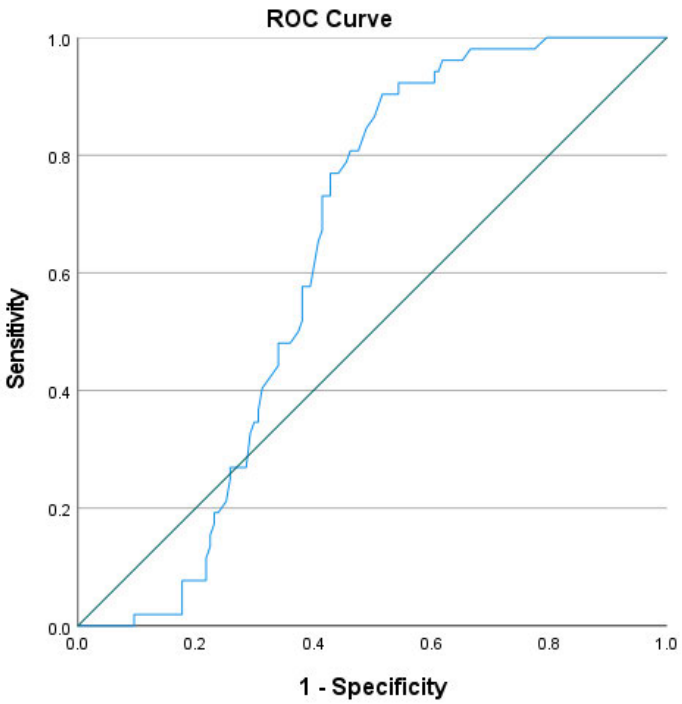

Figure 3.2: MUAC (left) accuracy in predicting overweight nutritional status in pregnant black South African women using ROC curve

The cut-off value of MUAC (left), when compared to overweight nutritional status, was 28.55cm (sensitivity 0.904, specificity 0.517, Youden's J statistic 0.421). Hence, pregnant women that have a MUAC (left) measurement of ≥28.55cm can be classified as having an overweight nutritional status. MUAC (left) was an outstanding predictive tool for obese nutritional status (AUC 0.952; 95%CI 0.924-0.980; *p*<0.0001) *(refer to Figure 3.3)*.

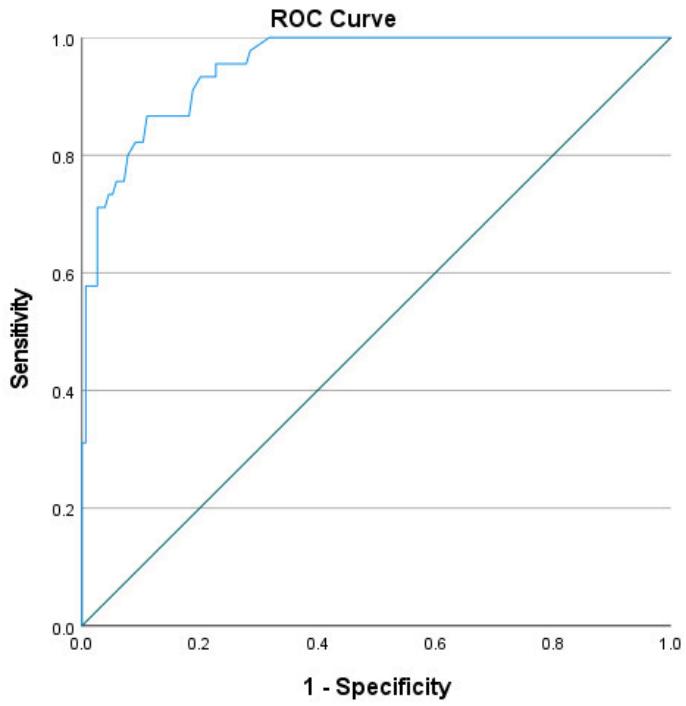

Figure 3.3: MUAC (left) accuracy in predicting obese nutritional status in pregnant black South African women using ROC curve

The cut-off value of MUAC (left), when compared to obese nutritional status, was 32.30 cm (sensitivity 0.867, specificity 0.149, Youden's J statistic 0.016). Hence, pregnant women that have a MUAC (left) measurement of ≥32.30 cm can be classified as having an obese nutritional status.

3.4.9 Right mid-upper arm circumference

MUAC (right) was an inaccurate predictive tool for underweight nutritional status (AUC 0.222; 95%CI 0.156-0.289; p<0.0001) as well as for normal nutritional status (AUC 0.222; 95%CI 0.156-0.289; p<0.0001). MUAC (right) was a poor predictive tool for overweight nutritional status (AUC 0.635; 95%CI 0.560-0.709; *p* = 0.004) *(refer to Figure 3.4)*.

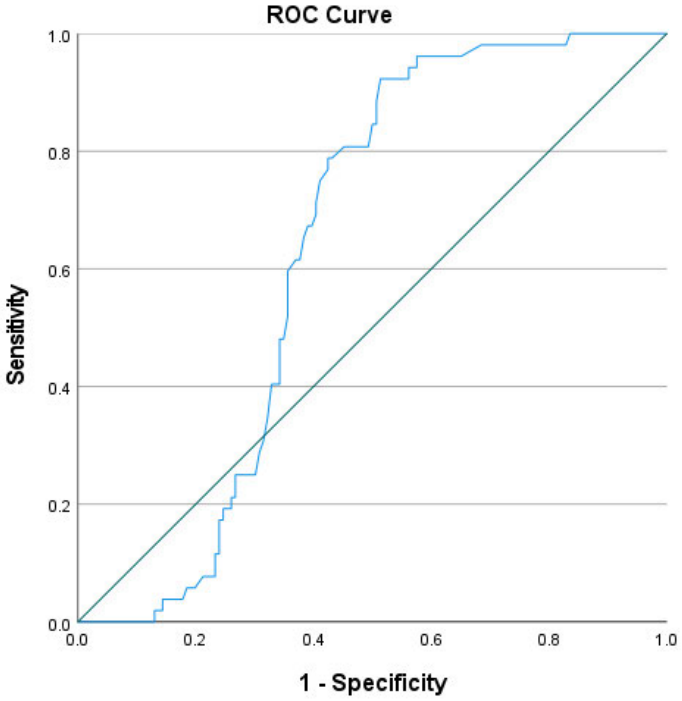

Figure 3.4: MUAC (right) accuracy in predicting overweight nutritional status in pregnant black South African women using ROC curve

The cut-off value of MUAC (right), when compared to overweight nutritional status, was 28.75cm (sensitivity 0.923, specificity 0.514, Youden's J statistic 0.437). Hence, pregnant women that have a MUAC (right) measurement of ≥28.75cm can be classified as having an overweight nutritional status. MUAC (right) was an outstanding predictive tool for obese nutritional status (AUC 0.958; 95%CI 0.932-0.983; $p<0.0001$) *(refer to Figure 3.5)*.

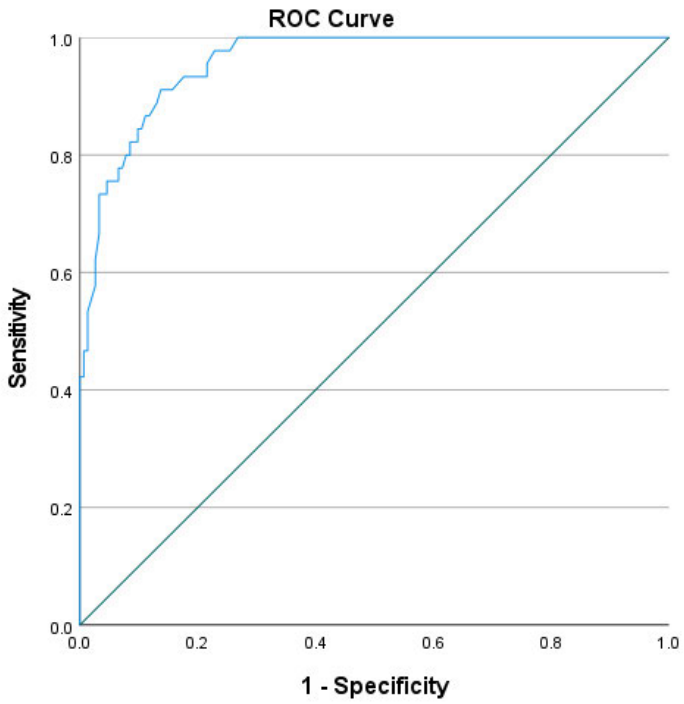

Figure 3.5: MUAC (right) accuracy in predicting obese nutritional status in pregnant black South African women using ROC curve

The cut-off value of MUAC (right), when compared to obese nutritional status, was 31.95 cm (sensitivity 0.933, specificity 0.176, Youden's J statistic 0.109). Hence, pregnant women that have a MUAC (right) measurement of ≥31.95 cm can be classified as having an obese nutritional status.

3.4.10 Right tricep skinfold thickness

TSF (right) was an inaccurate predictive tool for underweight nutritional status (AUC 0.305; 95%CI 0.233-0.378; p<0.0001) as well as for normal nutritional status (AUC 0.305; 95%CI 0.233-0.378; p<0.0001), and for overweight nutritional status (AUC 0.584; 95%CI 0.504-0.664; p = 0.041). TSF (right) was an excellent predictive tool for obese nutritional status (AUC 0.888; 95%CI 0.839-0.936; *p*<0.0001) *(refer to Figure 3.6)*.

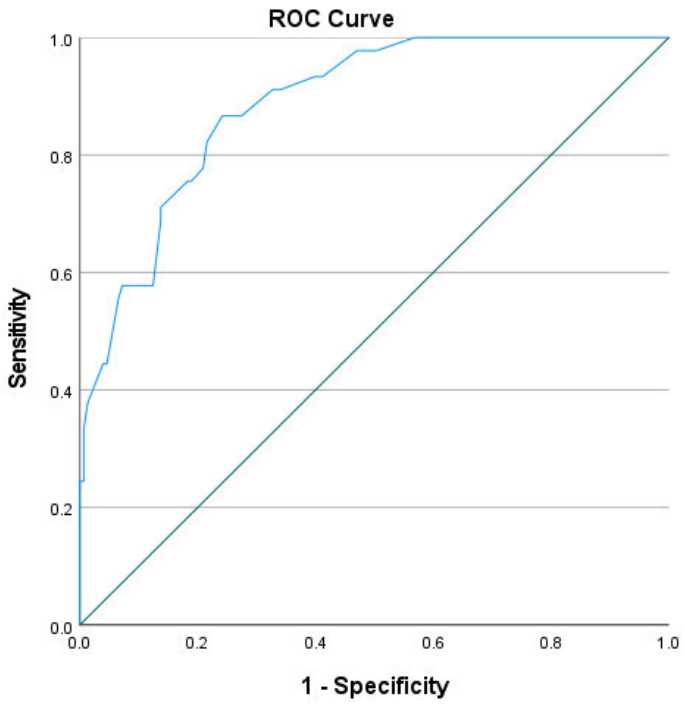

Figure 3.6: TSF (right) accuracy in predicting obese nutritional status in pregnant black South African women using ROC curve

The cut-off value of TSF (right), when compared to obese nutritional status, was 20.75 mm (sensitivity 0.867, specificity 0.242, Youden's J statistic 0.109). Hence, pregnant women that have a TSF (right) measurements of ≥20.75 mm can be classified as having an obese nutritional status.

3.4.11 Right subscapular skinfold thickness

SSF (right) was an inaccurate predictive tool for underweight nutritional status (AUC 0.278; 95%CI 0.207-0.349; p<0.0001) as well as for normal nutritional status (AUC 0.278; 95%CI 0.207-0.349; p<0.0001). SSF (right) was a poor predictive tool for overweight nutritional status (AUC 0.648; 95%CI 0.570-0.726; *p* = 0.040) *(refer to Figure 3.7)*.

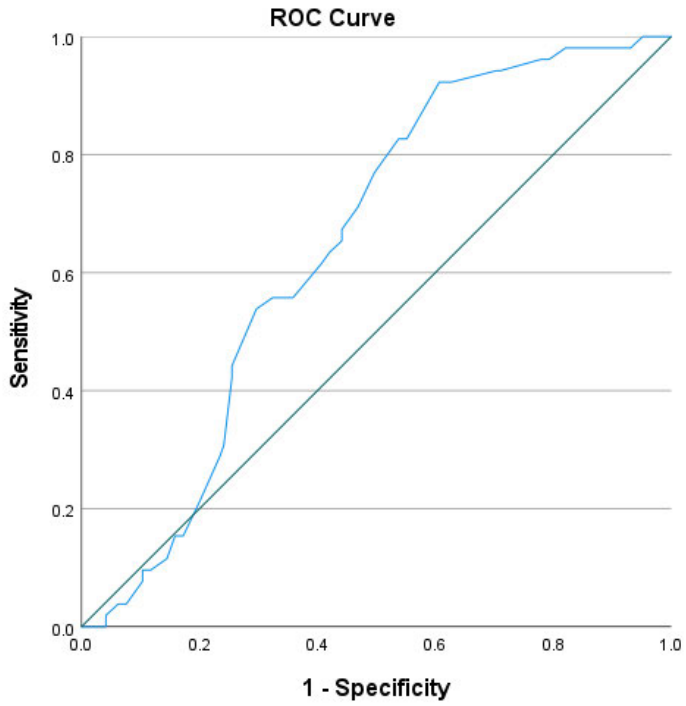

Figure 3.7: SSF (right) accuracy in predicting overweight nutritional status in pregnant black South African women using ROC curve

The cut-off value of SSF (right), when compared to overweight nutritional status, was 15.75cm (sensitivity 0.923, specificity 0.607, Youden's J statistic 0.53). Hence, pregnant women that have a SSF (right) measurement of ≥15.75cm can be classified as having an overweight nutritional status. SSF (right) was an excellent predictive tool for obese nutritional status (AUC 0.888; 95%CI 0.839-0.936; p<0.0001) *(refer to Figure 3.8)*.

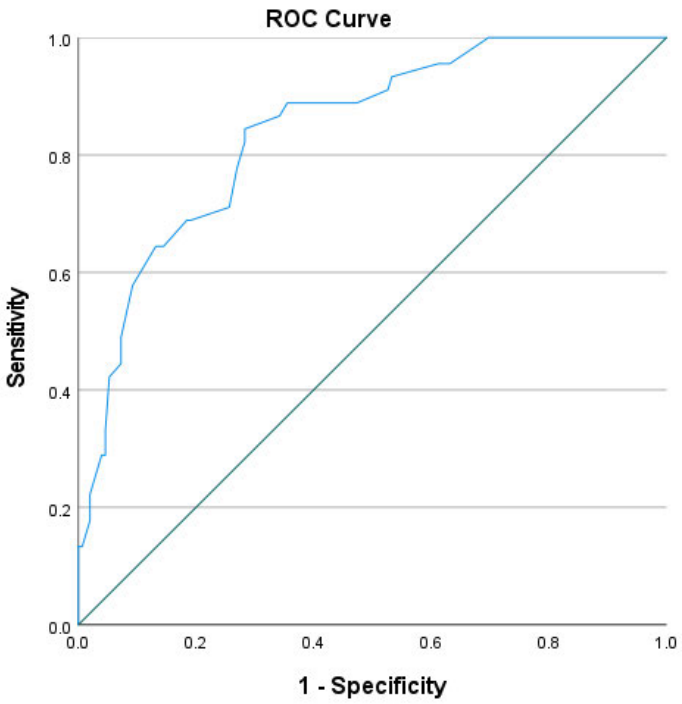

Figure 3.8: SSF (right) accuracy in predicting obese nutritional status in pregnant black South African women using ROC curve

The cut-off value of SSF (right), when compared to obese nutritional status, was 21.75 mm (sensitivity 0.822, specificity 0.283, Youden's J statistic 0.105). Hence, pregnant women that have a SSF (right) measurement of ≥21.75 mm can be classified as having an obese nutritional status.

3.4.12 Right mid-upper arm muscle circumference

MAMC (right) was an inaccurate predictive tool for underweight nutritional status (AUC 0.214; 95%CI 0.149-0.278; p<0.0001) as well as for normal nutritional status (AUC 0.214; 95%CI 0.149-0.278; p<0.0001). MAMC (right) was a poor predictive tool for overweight nutritional status (AUC 0.638; 95%CI 0.563-0.714; p = 0.038) *(refer to Figure 3.9).*

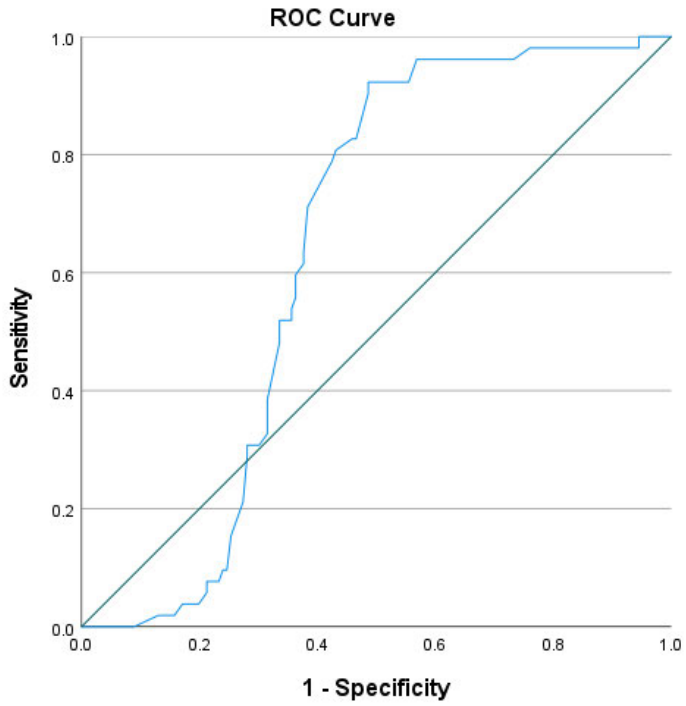

Figure 3.9: MAMC (right) accuracy in predicting overweight nutritional status in pregnant black South African women using ROC curve.

The cut-off value of MAMC (right), when compared to overweight nutritional status, was 23.35cm (sensitivity 0.923, specificity 0.486, Youden's J statistic 0.409). Hence, pregnant women that have a MAMC (right) measurement of ≥23.35cm can be classified as an overweight nutritional status. MAMC (right) was an outstanding predictive tool for obese nutritional status (AUC 0.935; 95%CI 0.895-0.976; p<0.0001) (*refer to Figure 3.10).*

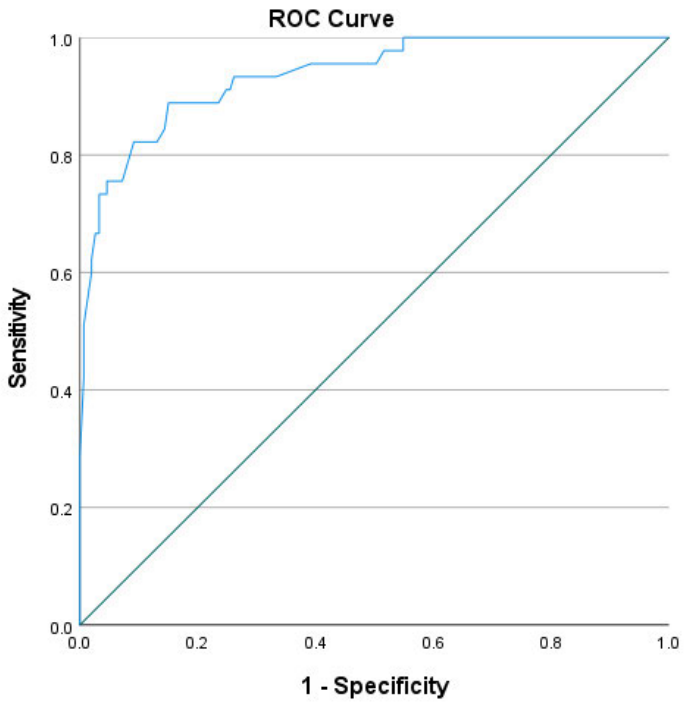

Figure 3.10: MAMC (right) accuracy in predicting obese nutritional status in pregnant black South African women using ROC curve

The cut-off value of MAMC (right), when compared to obese nutritional status, was 25.23 mm (sensitivity 0.889, specificity 0.170, Youden's J statistic 0.059). Hence, pregnant women that have a MAMC (right) measurement of ≥25.23 mm can be classified as having an obese nutritional status.

3.4.13 Right wrist circumference

WC (right) was an inaccurate predictive tool for underweight nutritional status (AUC 0.303; 95%CI 0.230-0.376; p<0.0001) as well as for normal nutritional status (AUC 0.303; 95%CI 0.230-0.376; p<0.0001), and overweight nutritional status (AUC 0.572; 95%CI 0.490-0.654; p = 0.125). WC (right) was an excellent predictive tool for obese nutritional status (AUC 0.844; 95%CI 0.782-0.906; *p*<0.0001), (*refer to Figure 3.11).*

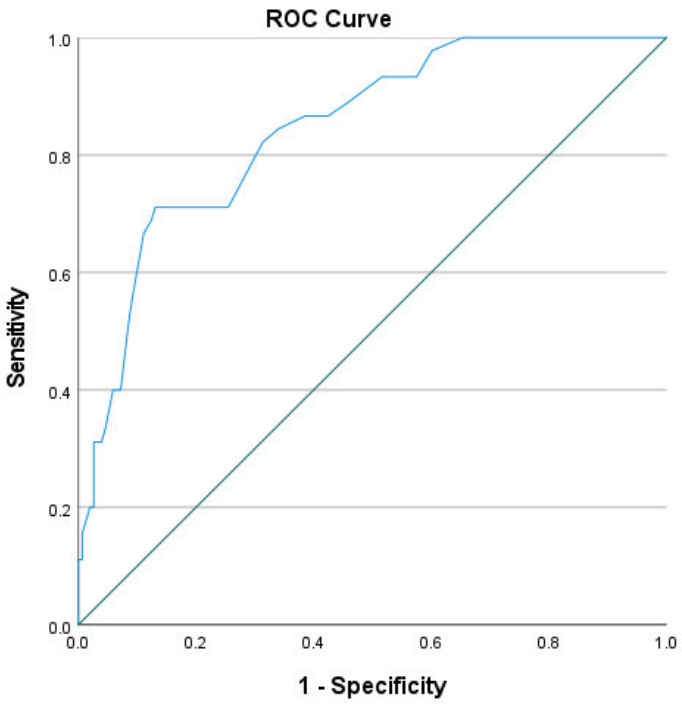

Figure 3.11: WC (right) accuracy in predicting obese nutritional status in pregnant black South African women using ROC curve

The cut-off value of WC (right), when compared to obese nutritional status, was 16.25 cm (sensitivity 0.711, specificity 0.131, Youden's J statistic -0.158). Hence, pregnant women that have a WC (right) measurement of ≥16.25 cm can be classified as having an obese nutritional status.

3.4.14 Frame size

Frame size (cm/cm) was a poor predictive tool for underweight nutritional status (AUC 0.680; 95%CI 0.606-0.754; p<0.0001) (*refer to Figure 3.12).*

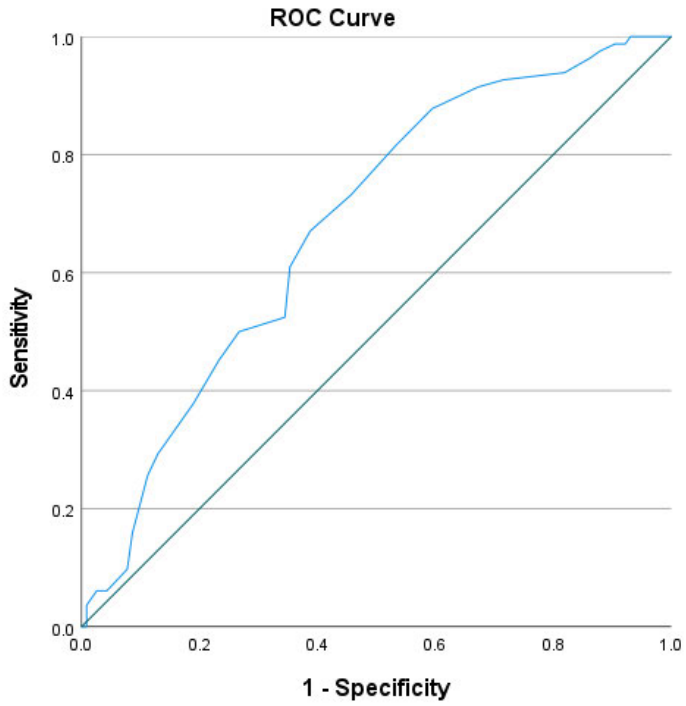

Figure 3.12: Frame size accuracy in predicting Underweight nutritional status in pregnant black South African women using ROC curve

The cut-off value of frame size when compared to underweight nutritional status was 10.05 (cm/cm) (sensitivity 0.671, specificity 0.388, Youden's J statistic 0.059). Therefore, pregnant women that have a calculated frame size of ≥10.05 cm/cm can be classified as having an underweight nutritional status. Frame size was a poor predictive tool for normal nutritional status (AUC 0.680; 95%CI 0.606-0.754; p<0.0001) *(refer to Figure 3.13).*

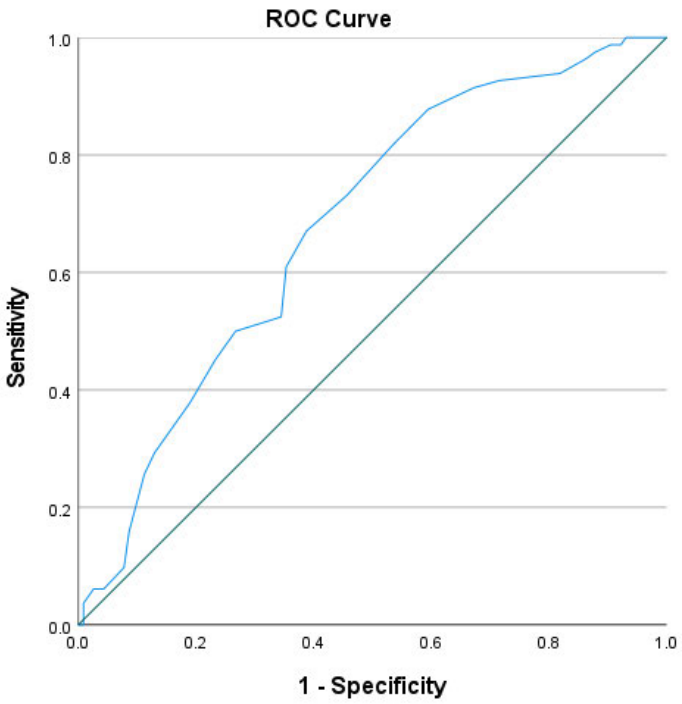

Figure 3.13: Frame size accuracy in predicting normal nutritional status in pregnant black South African women using ROC curve

The cut-off value of frame size, when compared to normal nutritional status, was 9.95 (cm/cm) (sensitivity 0.732, specificity 0.457, Youden's J statistic 0.189). Hence, pregnant women that have a calculated frame size of ≥9.95 cm/cm can be classified as having a normal nutritional status. Frame size was an inaccurate predictive tool for overweight nutritional status (AUC 0.437; 95%CI 0.349-0.526; p = 0.181) as well as for obese nutritional status (AUC 0.213; 95%CI 0.140-0.287; p<0.0001).

3.5 Anthropometric differences between pregnant women living with and without HIV

The characteristics of the pregnant women were categorised according to HIV status *(refer to Table 3.5)*. Of the 200 pregnant women in the study, 45.0% (n=90) were HIV-infected and 55.0% (n=110) were HIV-uninfected. The HIV-infected pregnant women were older than the HIV-uninfected pregnant women (p<0.0001).

Table 3.5: Anthropometric data for all pregnant females, categorized according to HIV status

Characteristics	HIV-infected		HIV-uninfected	p-value
Age	**n=90**		**n=110**	<0.0001
Mean (SD) (years)	29.0 (5.5)		25.3 (5.1)	
Gestational age (SD)	**n=90**		**n=110**	0.0894
Mean (SD) (weeks)	37.4 (2.9)		37.9 (2.7)	
BMI[a] Mean (SD) (kg/m^2)	**All pregnant women (n=200)**	**n=90**	**n=110**	0.6540
		31.7 (6.1)	32.5 (7.2)	
	Obese (n=45)	**n=18**	**n=27**	0.8258
		40.4 (5.0)	42.0 (7.1)	
	Overweight (n=52)	**n=24**	**n=28**	0.6529
		33.7 (1.9)	33.3 (1.6)	
	Normal (n=84)	**n=38**	**n=46**	0.7226
		28.5 (2.1)	28.2 (2.4)	
	Underweight (n=19)	**n=10**	**n=9**	0.7802
		22.9 (2.2)	23.3 (1.4)	
MUAC[b] (left) Mean (SD) (cm)	**All pregnant women (n=200)**	**n=90**	**n=109**	0.9605
		30.4 (4.6)	30.8 (5.5)	
	Obese (n=45)	**n=18**	**n=27**	
		36.7 (3.9)	37.5 (5.7)	0.8894
	Overweight (n=52)	**n=24**	**n=28**	0.5943
		31.4 (2.5)	31.3 (2.5)	
	Normal (n=83)	**n=38**	**n=45**	0.2647
		28.3 (2.3)	27.7 (2.3)	
	Underweight (n=19)	**n=10**	**n=9**	0.9024
		24.2 (1.5)	24.1 (1.8)	
MUAC[b] (right) Mean (SD) (cm)	**All pregnant women (n=200)**	**n=89**	**n=109**	0.7103
		30.4 (4.7)	31.0 (5.4)	
	Percentile Reading:			
	<5th (very thin) (%)	3 (3.4)	1 (0.9)	0.3281
	< 10th (very thin) (%)	1 (1.1)	0 (0.0)	0.4495
	< 25th (thin) (%)	8 (9.0)	3 (2.8)	0.0674
	< 50th (normal) (%)	16 (18.0)	23 (21.1)	0.5962
	<75th (thick) (%)	15 (16.9)	24 (22.0)	0.3767
	< 90th (thick) (%)	30 (33.7)	29 (26.6)	0.3488
	< 95th (very thick) (%)	8 (9.0)	12 (11.0)	0.8133

	>95th (very thick) (%)	8 (9.0)	17 (15.6)	0.2819
	Obese (n=45)	**n=18**	**n=27**	0.7457
		36.9 (3.9)	37.8 (5.3)	
	Overweight (n=52)	**n=24**	**n=28**	0.8328
		31.3 (2.1)	31.5 (2.5)	
	Normal (n=82)	**n=37**	**n=45**	0.3810
		28.3 (2.4)	27.9 (2.4)	
	Underweight (n=19)	**n=10**	**n=9**	0.6532
		24.2 (1.8)	24.6 (1.9)	
TSF[c] (right) Mean (SD) (mm)	**All pregnant women (n=200)**	**n=89**	**n=109**	0.5303
		18.9 (6.0)	19.9 (7.2)	
	Percentile Reading:			
	<5th (very low) (%)	6 (6.7)	4 (3.7)	0.3495
	< 10th (very low) (%)	9 (10.1)	11 (10.1)	1.000
	< 25th (low) (%)	25 (28.1)	35 (32.1)	0.6412
	< 50th (normal) (%)	32 (36.0)	30 (27.5)	0.2205
	<75th (high) (%)	14 (15.7)	22 (20.2)	0.4628
	< 90th (high) (%)	2 (2.2)	2 (1.8)	1.0000
	< 95th (very high) (%)	1 (1.1)	2 (1.8)	1.0000
	>95th (very high) (%)	0 (0.0)	3 (2.8)	0.2539
	Obese (n=45)	**n=18**	**n=27**	
		25.1 (5.2)	28.0 (7.0)	0.2321
	Overweight (n=52)	**n=24**	**n=28**	0.9707
		20.2 (4.6)	20.3 (4.7)	
	Normal (n=82)	**n=37**	**n=45**	0.4865
		17.2 (4.5)	16.5 (4.2)	
	Underweight (n=19)	**n=10**	**n=9**	0.5925
		10.8 (2.7)	11.5 (2.5)	
SSF[d] (right) Mean (SD) (mm)	**All pregnant women (n=200)**	**n=89**	**n=108**	0.5531
		20.4 (8.1)	20.8 (7.4)	
	Percentile Reading:			
	<5th (very low) (%)	9 (10.1)	4 (3.7)	0.0874
	< 10th (very low) (%)	6 (6.7)	4 (3.7)	0.3519
	< 25th (low) (%)	33 (37.1)	51 (47.2)	0.1926
	< 50th (normal) (%)	27 (30.3)	26 (24.1)	0.3374
	<75th (high) (%)	3 (3.4)	7 (6.5)	0.5165
	< 90th (high) (%)	6 (6.7)	9 (8.3)	0.7903
	< 95th (very high) (%)	0 (0.0)	0 (0.0)	-
	>95th (very high) %	5 (5.6)	7 (6.5)	1.000

	Obese (n=45)	**n=18**	**n=27**	0.7454
		27.9 (8.2)	28.3 (7.3)	
	Overweight (n=52)	**n=24**	**n=28**	0.3772
		23.5 (6.2)	22.0 (5.4)	
	Normal (n=82)	**n=37**	**n=45**	0.8923
		17.2 (5.8)	17.2 (4.6)	
	Underweight (n=18)	**n=10**	**n=8**	0.5611
		11.1 (1.8)	12.3 (3.1)	
MAMC[e] (right) Mean (SD) (cm)	**All pregnant women (n=200)**	**n=89**	**n=109**	0.9781
		24.4 (2.8)	24.5 (2.9)	
	Percentile Reading:			
	<5th (very low) (%)	0 (0.0)	0 (0.0)	-
	< 10th (very low) (%)	0 (0.0)	0 (0.0)	-
	< 25th (low) (%)	3 (3.4)	1 (0.9)	0.3281
	< 50th (normal) (%)	12 (13.5)	6 (5.5)	0.0798
	<75th (high) (%)	13 (14.6)	26 (23.9)	0.1103
	< 90th (high) (%)	24 (27.0)	29 (26.6)	1.0000
	< 95th (very high) (%)	16 (18.0)	14 (12.8)	0.3273
	>95th (very high) %	21 (23.6)	33 (30.3)	0.3374
	Obese (n=45)	**n=18**	**n=27**	0.5463
		28.4 (1.8)	27.9 (2.5)	
	Overweight (n=52)	**n=24**	**n=28**	
		25.0 (1.3)	25.0 (1.7)	0.9634
	Normal (n=81)	**n=37**	**n=44**	0.4900
		23.0 (1.7)	22.8 (1.6)	
	Underweight (n=19)	**n=10**	**n=9**	0.8377
		21.0 (1.6)	21.2 (1.3)	
Wrist circumference (right) Mean (SD) (cm)	**All pregnant women (n=200)**	**n=89**	**n=109**	0.5361
		15.7 (1.0)	15.9 (1.2)	
	Obese (n=45)	**n=18**	**n=27**	
		16.7 (0.7)	17.0 (1.3)	0.9260
	Overweight (n=52)	**n=24**	**n=28**	0.2900
		15.8 (0.8)	16.0 (0.9)	
	Normal (n=82)	**n=37**	**n=45**	0.9368
		15.4 (1.0)	15.4 (0.7)	
	Underweight (n=19)	**n=10**	**n=9**	0.9674
		14.8 (0.9)	14.7 (0.8)	
Frame size	**All pregnant women (n=200)**	**n=89**	**n=109**	0.6128
Mean (SD) (cm)		10.13 (0.58)	10.08 (0.71)	
Small frame		3 (3.4)	7 (6.4)	0.5165
Medium frame		44 (49.4)	48 (44.0)	0.4762
Large frame		42 (47.2)	54 (49.5)	0.7762

[a]BMI: body mass index; [b]Mid upper arm circumference; [c]Tricep skinfold; [d]Subscapular skinfold; [e]Mid arm muscle circumference

Overall, there were no significant differences between the HIV-infected pregnant women and the HIV-uninfected pregnant women across all the anthropometric parameters. Therefore, HIV status and ART did not show to have any significant effect on the maternal anthropometric measurements and nutritional status during pregnancy in the study population.

3.6 Discussion

Pregnancy is a dynamic anabolic process associated with various longitudinal changes to the body proportions in specific anatomic regions including the abdomen, gluteal, thoracic, femoral, and arm areas (Balasubramanian and Robinette, 2020). These changes are measured by using anthropometric measurements, which can then be used to assess changes in body composition and interpret the nutritional status (Gueri, Jutsum and Sorhaindo, 1982). This study identified that maternal BMI was significantly associated with other maternal anthropometric measurements such as MUAC, TSF, SSF, MAMC, WC, and frame size. These differences in body measurements among the pregnant women of different nutritional statuses were able to reflect differences in fat mass and muscle mass. For example, with the obese pregnant women having the highest fat stores and highest muscles stores in comparison to the other nutritional statuses. These findings were supported by other studies, which identified that as women gained weight during pregnancy, they also gained fat mass with obese pregnant women having the largest fat stores compared to other nutritional statuses (Most, *et al*., 2020; Okereke, *et al*., 2004). In terms of other correlation studies, a Brazilian study identified that MUAC was strongly correlated (r=0.872) with BMI in pregnant women (Miele, *et al.*, 2021). Also, a South African study investigated the correlation between MUAC and BMI in pregnant women which identified that MUAC was strongly correlated with BMI in pregnant women (r=0.92) (Fakier, Petro and Fawcus, 2017). In an Australian study by Kannieappan, *et al*. (2013), which investigated bicep, tricep and subscapular skinfold thickness measurements in correlation to body fat percentage. It was identified that these skinfold sites were reliable measurements for calculating body fat percentage in pregnant women (Kannieappan, *et al*., 2013). Hence, our study's findings show that because the maternal measurements were significantly correlated with maternal BMI, they were able to be used as surrogate measurements to give insight into the nutritional status of black South African pregnant women. For example, in

our study findings, MUAC measurements were able to accurately assess nutritional status in overweight and obese pregnant women. Overweight pregnant women had a MUAC cut-off of ≥28.55 cm (left) and ≥28.75 cm (right), whilst obese women had a MUAC cut-off of ≥32.30 cm (left) and ≥31.95 cm (right). In comparison to other African studies, obese Nigerian pregnant women had a cut-off of 33.0 cm (Okereke, *et al*., 2013), and in a Cape Town study obese pregnant women had a cut-off of 30.57cm and malnourished pregnant women had a cut-off of ≥ 22.8 cm (Fakier, Petro and Fawcus, 2017). An earlier study conducted by Kruger (2005), identified that wasting in pregnant women was classified as a MUAC measurement of < 22 cm. Hence, our study findings added more insight into the population-specific classifications of defining overweight and obesity in black South African pregnant women using the MUAC measurement. Also, our study findings gave new insight into using skinfolds thickness measurements, MAMC, WC, and frame size to assess nutritional status in pregnancy in black South African women. We identified that obesity in pregnancy was defined as having a TSF (right) cut-off measurement of ≥20.75 mm, SSF (right) cut-off measurement of ≥21.75 mm, MAMC (right) cut-off measurement of ≥25.23 cm, and a WC (right) cut-off measurement of ≥16.25cm. Overweight nutritional status in pregnancy could be defined as having a SSF (right) cut-off measurement of ≥15.75mm and a MAMC (right) cut-off measurement of ≥23.35cm. Lastly, frame size could be used to accurately assess for an underweight nutritional status (cut-off of ≥10.05) and normal nutritional (cut-off of ≥9.95) in black South African pregnant women.

Advanced maternal age has been linked to an increased risk for adverse pregnancy outcomes (Frick, 2021). Our findings identified that a rise in maternal age was associated with an increase in BMI as well as an associated increase in all other anthropometric measurements including MUAC (left), MUAC (right), TSF (right), SSF (right), MAMC (right), and WC (right). Hence, advanced maternal age was linked to an increased accumulation of body mass, fat mass and muscle mass. Another study explains that with an advance in maternal age there is an associated reduction in the resting metabolic rate which in turn leads to body composition changes, such as an increase in fat stores and decrease in muscle stores (St-Onge and Gallagher, 2010).

Despite the large numbers of women of reproductive age in Sub-Saharan Africa who living with HIV, few studies have investigated the relationship between HIV infection during pregnancy and maternal anthropometric parameters of nutritional status. Initiation of ART has in previous studies

been associated with adipose tissue weight gain and consequently an increase in BMI but did not impact lean body mass (muscle) (Esposito, *et al*., 2008). Studies have documented that HIV affects the distribution of adipose tissue deposition, without having major shifts in BMI (Brown, *et al*., 2009). In younger HIV-infected women, the median BMI, WC, and fat percentage were significantly lower than in HIV-uninfected women (Hattingh, Walsh and Bester, 2011). In this study, the HIV-infected pregnant women consistently had lower measurements in comparison to the HIV-uninfected pregnant women, however, there were no statistically significant differences between any of the anthropometric measurements. Overall, HIV-infected pregnant women did not differ anthropometrically from their HIV-uninfected counterparts. One of the potential reasons for these results could be due to the ART compliance of the study population, which then prevents HIV replication and prevention of the associated changes in nutritional status such as in HIV-associated wasting syndrome. Wasting syndrome is defined as a complication of HIV infection, where a specific combination of internal factors leads to a hypermetabolic response and initiates a catabolic effect, breaking down muscle and fat tissue (Weinroth, Parenti and Simon, 1995). The internal factors that mediate this process will vary from patient to patient but may include compliance to ART program, dietary patterns, malabsorption, physical activity, metabolic derangements, epigenetics, and cytokine activity (Weinroth, Parenti and Simon, 1995).

3.7 Conclusion

Anthropometric measurements are simple and effective predictive tools for the assessment of nutritional status in pregnant women. Maternal age was significantly associated with changes in maternal anthropometric measurements. Maternal BMI was significantly correlated with other maternal anthropometric measurements including MUAC (left and right), TSF (right), SSF (right), MAMC (right), WC (right), and frame size. The anthropometric methods that were accurate for assessing obesity in pregnancy included TSF (right) (cut-off of ≥20.75 mm), SSF (right) (cut-off of ≥21.75 mm), MAMC (right) (cut-off of ≥25.23 cm), and WC (right) (cut-off of ≥16.25cm). Also, SSF (right) (cut-off of ≥15.75mm) and MAMC (right) (cut-off of ≥23.35cm) could be used to assess for overweight nutritional status. Lastly, frame size could be used to assess for underweight (cut-off of ≥10.05) and normal (cut-off of ≥9.95) nutritional status. The HIV-infected pregnant women did not differ anthropometrically to the HIV-uninfected pregnant women. This

study provides an insight into the various anthropometric measurements that can be used to assess and define the nutritional status of black South African women during pregnancy.

3.8 List of abbreviations

ABW: actual body weight

ART: Antiretroviral treatment

AUC: Area under the curve

BMI: Body mass index

BREC: Biomedical Research Ethics Council

EFV: Efavirenz

FDC: Fixed-dose combination

FTC: Emtricitabine

HIV: Human immunodeficiency virus

ISAK: International Society for the Advancement of Kinanthropometry

KZNDOH: KwaZulu-Natal Department of Health

MAMC: Mid-arm muscle circumference

MUAC: Mid-upper arm circumference

MW: Maternal weight of the mother

PMMH: Prince Mshiyeni Memorial Regional Hospital

PMTCT: Prevention of mother to child transmission

ROC: Receiver operator characteristic

SD: Standard deviation

SH: Standing height

SSF: Subscapular skinfold

TDF: Tenofovir

TSF: Tricep skinfold

UKZN: University of KwaZulu-Natal

WC: Wrist circumference

3TC: Lamivudine

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CHAPTER 4

This chapter responds to the objective iv to vi of this study to identify the associated factors and outcomes for overweight and obesity in pregnancy within the context of HIV. The chapter is presented in the form of a manuscript entitled “Maternal overweight and obesity and its associated factors and outcomes in HIV-infected and HIV-uninfected black South African pregnant women”. This manuscript has been accepted to the Journal of Obstetrics and Gynaecology Research (article DOI: https://doi.org/10.1111/jog.15392) (Appendix 10).

4. Maternal overweight and obesity and its associated factors and outcomes in HIV-infected and HIV-uninfected black South African pregnant women

4.1 Abstract

Introduction: Maternal overweight and obesity within the context of HIV presents significant health risks to the mother. The study aimed to investigate maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women and determine its associated factors and outcomes, as well as determine whether overweight and obese HIV-infected pregnant South African women have a significantly increased risk for maternal health outcomes.

Methods: A cross-sectional study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital, which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. Two hundred black South African pregnant women were enrolled in the study. The participants were categorized according to BMI (kg/m^2) into two groups: (1) overweight/obese ($\geq 25kg/m^2$) (n=97); and (2) non-overweight/non-obese ($<25kg/m^2$) (n=103), where 90/200 were HIV-infected and 110/200 were HIV-uninfected. Anthropometric measurements were conducted by a trained dietician. BMI was calculated using the weight of the mother post-delivery. Quantitative data was collected with the use of a validated questionnaire. Descriptive statistics were performed for demographic, clinical, and laboratory data. Descriptive statistics with mean and standard deviation, frequency, and percentages were calculated. The differences between the maternal BMI categories were assessed using Fisher's exact t-test (two categories) and the χ 2 test (more than two categories). Simple and multiple logistic regression analyses were used to determine factors associated with maternal overweight and obesity. The independent variables were HIV status, type of pregnancy, gestational age, maternal age, marital status, job status, number of people living at home, geographic position, living conditions, education, and maternal health outcomes like C-section, preterm delivery, hypertensive disorders, and anaemia. In this study, the dependent variable was the BMI category, and the single dichotomous outcome was coded as 0 for non-overweight/non-obese and 1 for overweight/obese. Simple logistic regression was performed to select the variables for multiple logistic regression analysis, and only variables with a *p*-value <0.05. The factors included in the multiple logistic regression were gestational age, maternal age, hypertensive (HPT) disorders and anaemia. The

adjusted odds ratio was estimated with a 95% confidence interval. A *p*-value of <0.05 was considered statistically significant.

Results: The demographic characteristics, food frequency intake, physical activity and lifestyle characteristics were not significantly different between the participants with a BMI of ≥ 25.0 kg/m^2 compared to those with a BMI of <25 kg/m^2. The dietary pattern of the overweight/obese participants showed that there was a higher intake of saturated fat, higher in salt, higher in sugar, higher in animal protein, lower in dairy, higher in legumes, higher in starch, higher in vegetables, and had a similar intake of fruit in comparison to the non-overweight/non-obese participants. Also, maternal age was significantly different between those with a BMI ≥25 kg/m^2 compared to those with a BMI <25 kg/m^2, where the overweight and obese participants were significantly older (*p*=0.0173). Multiple logistic regression analysis showed that maternal age (OR:1.061; 95%CI 1.008-1.117; *p*=0.023) and gestational age (OR:1.121; 95%CI 1.005-1.251; *p*=0.041) were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant women. For maternal health outcomes, multiple logistic regression analysis showed that HPT disorders (OR:0.273; 95%CI 0.124-0.601; *p*=0.001) and anaemia (OR:2.420; 95%CI 1.283-4.563; *p*=0.006) were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant women. The overweight and obese HIV-infected pregnant women (OR:0.233; 95% CI 0.075-0.717; *p*=0.011) had increased odds for developing HPT disorders compared to HIV-uninfected overweight and obese pregnant women (OR:0.471; 95% CI 0.172-1.291; *p*=0.143).

Conclusion: Maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women was significantly associated with maternal age, gestational age, HPT disorders and anaemia. Maternal overweight/obesity decreased the odds for anaemia during pregnancy but increased the odds for the development of HPT disorders during pregnancy, especially in the HIV-infected pregnant women.

4.2 Background to the study

Maternal overweight and obesity in pregnancy has largely been investigated for its association with unfavourable clinical outcomes for both mother and child. With a focus on the mother, the health risks associated are dependent on the linearity of the body mass index (BMI) of the mother before, during and after pregnancy (Stubert, *et al*., 2018). Hence, in terms of the risks for adverse maternal health outcomes during pregnancy these may include metabolic conditions like gestational diabetes mellitus (GDM), cardiovascular disease like hypertensive (HPT) disorders, as well as other complications like caesarean section (C-section) birth, failed induction of labour, preterm rupture of membranes, venous thromboembolism, sepsis, and postpartum haemorrhage (Basu, Jeketera and Basu, 2010). Therefore, there is a need to better understand how to prevent overweight and obesity in pregnancy so that the risks for these adverse maternal health outcomes might be mitigated. In South Africa, up to 40% of pregnant women are living with human immunodeficiency virus (HIV) infection, and of those 30-45% are classified as obese (Bengtson, *et al*., 2020). Hence, population-specific methodology should be applied to investigate the unique risk factors that exist to encourage overweight and obesity in pregnant women within a South African context, and these factors may include the diet, physical activity, lifestyle choices and demographic characteristics (Madlala, *et al*., 2021; Madlala, *et al*., 2020; Basu and Basu, 2010). Therefore, this study sought to investigate maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women and determine its associated factors and outcomes, as well as determine whether overweight and obese HIV-infected pregnant South African women have a significantly increased risk for maternal health outcomes.

4.3 Methods

4.3.1 Sample selection and study population

A cross-sectional study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital (PMMH), which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. The catchment area for the hospital includes both rural and urban geographical areas. Pregnant women admitted to the labour ward were approached to participate in this study. The inclusion criteria for this study were as follows: (1) ≥ 18 years of age; (2) pregnant females; (3) black South African citizen; (4) clinically stable; (5) able to stand without assistance; and (6) given verbal and written consent to participate in the study. The participants were categorized according to BMI (kg/m^2) into two groups: (1) overweight/obese

pregnant women (≥25kg/m^2); and (2) non-overweight/non-obese pregnant women (<25kg/m^2). A total of 458 pregnant women were approached to participate in the study, but 245 subjects met the inclusion criteria and of these 45 declined to participate. Hence, a total of 200 subjects met all the inclusion criteria.

4.3.2 Maternal anthropometric assessment

Anthropometric measurements were conducted by an international society for the advancement of kinanthropometry level 1 trained dietician. According to Marfell-Jones, Stewart and de Ridder (2012) and Ulijaszek and Kerr (1991), to avoid anthropometric measurement errors, all measurements were conducted by the same researcher, taken three times, and recorded to the nearest 0.1 cm/kg. The mean of the two closest values was recorded. The standing height (SH) measurement was measured via. stretch stature methodology using a portable calibrated stadiometer (Seca) with a sliding headboard (Lahner, Kassier and Veldman, 2017). The weight measurement was taken using actual body weight (ABW) methodology (Lahner, 2019), using a portable calibrated scale (Seca, with a maximum weight threshold of 250 kg). The scale was calibrated before the commencement of the study. BMI was calculated using the weight of the mother (MW) post-delivery. The BMI was interpreted according to the BMI (MW/SH^2 = kg/m^2) classifications: (1) overweight/obese pregnant women (≥25kg/m^2); and (2) non-overweight/non-obese pregnant women (<25kg/m^2). (Weir and Jan, 2020).

4.3.3 Maternal interviews

A trained dietician conducted a one-on-one interview with each participant using an adapted version of a validated questionnaire which was made available in English and isiZulu (an indigenous South African language) (DOH, MRC and OrcMacro, 2007; *Appendix* 1-2). The isiZulu version was translated from English and then back translated by a second translator to ensure that the interpretation was correct. The questionnaire covered the following topics: (i) demographic information (age, marital status, employment, number of people living at home, geographic position, water source, fuel source, type of housing, housing materials, and education); (ii) physical activity during pregnancy (sitting, walking, moderate and vigorous exercise); (iii) smoking during pregnancy; (iv) drug abuse during pregnancy; (v) alcohol consumption during pregnancy; and (vi) dietary intake during pregnancy assessed using a 48-item unquantified food

frequency questionnaire as well as a food group-specific questionnaire on saturated fat intake, salt intake, and sugar intake.

4.3.4 Medical information

Medical data was also collected from the participant's medical records including blood results, medication, and prevalence of co-morbidities such as GDM, HPT disorders, and anaemia.

4.3.4.1 Gestational diabetes

Gestation diabetes is defined as any degree of glucose intolerance with onset or first diagnosis during pregnancy (Kampmann, *et al*., 2015). The participants were categorized as having GDM based on whether they were diagnosed during their pregnancy.

4.3.4.2 Hypertensive disorders

Hypertension is defined as having a continuous systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥ 90 mmHg (Berhe, *et al*., 2020). The participants were categorized as having a HPT disorder with the presence of either: (i) chronic hypertension defined as hypertension pre-dating pregnancy or diagnosed before 20 weeks of gestational age during pregnancy (Seely and Ecker, 2014) ; (ii) pregnancy-induced hypertension (PIH) defined as high blood pressure that occurs at ≥20 weeks of gestational age during pregnancy in a previously normotensive woman (Berhe, *et al.*, 2020; Seely and Ecker, 2014); (iii) pre-eclampsia toxaemia (PET) defined as blood pressure of ≥140/90 mmHg accompanied by proteinuria or evidence of organ dysfunction at ≥20 weeks of gestational age during pregnancy (Seely and Ecker, 2014); (iv) late-onset PET defined as PET diagnosed ≥34 weeks of gestational age during pregnancy (Erez, *et al*., 2017); and (v) PET characterized by the presence of haemolysis, elevated liver enzymes and low platelet counts (HELLP) (Haram, Svendsen and Abildgaard, 2009).

4.3.4.3 Anaemia

Pregnancy-associated anaemia is caused by a physiological change in the vascular system whereby haemodilution occurs with an increase in intravascular blood volume without an equivalent increase in red blood cells (Chen, *et al*., 2018). The haemoglobin (Hb) blood count (g/dL) was measured on admission to the hospital. Anaemia was defined as having a Hb (g/dL) value of less than 11g/dL (Stephen, *et al*., 2018).

4.3.4.4 Caesarean section

According to Torloni, *et al*., (2011), a C-Section is a surgical delivery method, whereby the baby is removed from the mother's womb by making a surgical incision in the abdomen and uterus. This procedure is indicated when natural vaginal delivery is not feasible (Torloni, *et al*., 2011).

4.3.4.5 Preterm delivery

A normal human pregnancy lasts 40 weeks, and a preterm birth is defined as a baby delivered before 37 weeks of gestation (Slattery and Morrison, 2002).

4.3.4.6 Geriatric pregnancy

A geriatric pregnancy is defined as a pregnancy in women of ≥35 years of age (Correa-de-Araujo and Yoon, 2021).

4.3.5 Statistical analysis

To determine the factors associated with maternal overweight and obesity and its outcomes the BMI classification was used where overweight/obese pregnant women were classified as having a BMI of ≥25kg/m^2 and non-overweight/non-obese pregnant women having a BMI of <25kg/m^2 (Weir and Jan, 2020). Data entry and statistical analysis was performed using the statistical software packages, GraphPad Prism 5, and IBM SPSS for Windows version 27. Descriptive statistics were performed for demographic, clinical, and laboratory data. Descriptive statistics with mean ($\bar{x}$) and standard deviation (SD), frequency, and percentages were calculated. The differences between the maternal BMI categories were assessed using Fisher's exact t-test (two categories) and the χ 2 test (more than two categories). Simple and multiple logistic regression analyses were used to determine factors associated with maternal overweight and obesity. Multiple logistic regression was performed using the forward selection and backward elimination method, followed by the manual retention or removal of the independent variables remaining in the model based on their clinical importance. The output between models was then compared and the best model was selected. The independent variables were HIV status, type of pregnancy, gestational age, maternal age, marital status, job status, number of people living at home, geographic position, living conditions, education, and maternal health outcomes like C-section, preterm delivery, hypertensive disorders, and anaemia. In this study, the dependent variable was the BMI category, and the single dichotomous outcome was coded as 0 for non-overweight/non-obese and 1 for overweight/obese. Simple logistic regression was performed to select the variables for multiple logistic regression

analysis, and only variables with a p-value <0.05. The factors included in the multiple logistic regression were gestational age, maternal age, HPT disorders and anaemia. Multicollinearity between the different predictor variables was checked using the variance inflation factor (VIF). Notably, a VIF value of <5.0 indicates no multicollinearity. All possible two-way interaction terms between significant variables were checked individually. The adjusted odds ratio was estimated with a 95% confidence interval. A *p*-value of <0.05 was considered statistically significant.

4.3.6 Ethics approval and informed consent

The study was approved by the Biomedical Research Ethics Council (BREC) of the University of KwaZulu-Natal (UKZN) (BE269/18), KwaZulu-Natal Department of Health (KZNDOH) (HRKM261/18), and PMMH (29/RESH/2018). All the participants in this study had provided verbal and written consent, participated voluntarily, did not receive incentives, and had the right to withdraw at any stage of the study.

4.4 Results

4.4.1 Maternal demographic characteristics

The 200 pregnant women were categorized into overweight/obese (n=97) and non-overweight/non-obese (n=103), where 45.0% (n=90) were HIV-infected and 55.0% (n=110) were HIV-uninfected. The demographic characteristics for the both HIV-infected and HIV-uninfected pregnant black South African women are represented in *Table 4.1*, which includes the following variables: (i) HIV status; (ii) type of pregnancy; (iii) gestational age; (iv) maternal age; (v) marital status; (vi) job status; (vii) number of people living at home; (viii) geographic position; (ix) living conditions; and (x) education. Our study findings identified that study participants (N=200) were experiencing various social challenges including being single (without the support of a partner) (88.0%), being unemployed (82.5%), living in informal housing (66.5%), having a large household size ($\bar{x}$±SD=6.6±3.9), having a lack of access to safe running water (5.0%), no access to electricity (11%), and a lack of education with 50% of the pregnant women not having achieved matriculation. Notably, the demographic characteristics were not significantly different between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to the pregnant women with a BMI of < 25 kg/m^2. However, the overweight/obese pregnant women were significantly older than the pregnant women with a BMI of < 25 kg/m^2. However, the overweight/obese pregnant women were significantly older ($\bar{x}$±SD=27.9±5.6) than the pregnant women with a BMI of <25 kg/m^2

($\bar{x}$±SD=26.1±5.5), where 18% of the pregnant women were categorised as having a geriatric pregnancy.

Table 4.1: Demographic characteristics for both HIV-infected and HIV-uninfected pregnant black South African women categorised according to the maternal BMI (kg/m^2)

Variables	**All pregnant females (n=200)**	**Maternal BMI (kg/m^2)**		***p*-value**
		≥ 25.0 (n=97)	**< 25.0 (n=103)**	
HIV Status:				
HIV-infected[c] (%)	90 (45.0)	42 (43.3)	48 (46.6)	0.6713[a]
HIV-uninfected[d] (%)	110 (55.0)	55 (56.7)	55 (53.4)	
Type of pregnancy:				
Single (%)	196 (98.0)	95 (97.9)	101 (98.1)	1.000[a]
Twin (%)	4 (2.0)	2 (2.1)	2 (1.9)	1.000[a]
Gestational age (weeks):				
Mean (SD)	37.7 (2.8)	38.1 (2.4)	37.2 (3.1)	0.0982[b]
Age groups:				
Mean age in years (SD)	27.0 (5.6)	27.9 (5.6)	26.1 (5.5)	0.0173[b*]
18-34 (%)	178 (89.0)	84 (86.6))	94 (91.3)	0.3674[a]
≥35 (%)	18 (11.0)	13 (13.4)	9 (8.7)	0.3674[a]
Marital status:				
Single (%)	176 (88.0)	82 (84.5)	94 (91.3)	0.1915[a]
Married (%)	10 (5.0)	8 (8.2)	2 (1.9)	0.0528[a]
Divorced (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
Engaged (%)	14 (7.0)	7 (7.2)	7 (6.8)	1.0000[a]
Job-status:				
Employed (%)	35 (17.5)	18 (18.6)	17 (15.5)	0.7141[a]
Unemployed (%)	165 (82.5)	79 (81.4)	86 (83.5)	
Number of people living at home:				
Mean Total (SD)	6.6 (3.9)	6.1 (3.3)	7.1 (4.3)	0.0741[b]
Mean Adult (SD)	3.9 (2.3)	3.6 (1.9)	4.2 (2.6)	0.0666[b]
Mean Children (SD)	2.7 (2.5)	2.5 (2.1)	3.0 (2.9)	0.4947[b]
Geographic position:				
Rural (%)	100 (50.0)	51 (52.6)	49 (47.5)	0.5715[a]
Urban (%)	100 (50.0)	46 (47.4)	54 (52.4)	
Living conditions:				
Water source:				
Inside Tap (%)	93 (46.5)	43 (44.3)	50 (48.5)	0.5730[a]
Outside Tap (%)	86 (43.0)	44 (45.4)	42 (40.8)	0.5684[a]
Pump (%)	11 (5.5)	6 (6.2)	5 (4.9)	0.7610[a]
River (%)	10 (5.0)	4 (4.1)	6 (5.8)	0.7487[a]

Housing material used to make a home:				
Plastic/cardboard (%)	4 (2.0)	1 (1.0)	3 (2.9)	0.6219[a]
Mud (%)	6 (3.0)	2 (2.1)	4 (3.9)	0.6836[a]
Mud and cement (%)	48 (24.0)	27 (27.8)	21 (20.4)	0.2481[a]
Corrugated iron/zinc (%)	118 (59.0)	61 (62.9)	57 (55.3)	0.3151[a]
Bare brick/cement block (%)	107 (53.5)	50 (51.5)	57 (55.3)	0.6707[a]
Plaster/finished (%)	57 (28.5)	26 (26.8)	31 (30.1)	0.6406[a]
Housing type:				
Formal (%)	67 (33.5)	34 (35.1)	33 (32.0)	0.6566 [a]
Informal (%)	133 (66.5)	63 (64.9)	70 (68.0)	
Fuel source:				
Electricity (%)	178 (89.0)	88 (90.7)	90 (87.4)	0.5034[a]
Paraffin (%)	22 (11.0)	8 (8.2)	14 (13.6)	0.2635[a]
Gas (%)	21 (10.5)	6 (6.2)	15 (14.6)	0.0657[a]
Wood (%)	64 (32.0)	28 (28.9)	36 (35.0)	0.3677[a]
Education:				
≤ Grade 7 (%)	5 (2.5)	2 (1.0)	3 (1.5)	1.0000[a]
Grade 8 – 11 (%)	95 (47.5)	43 (21.5)	52 (26.0)	0.3988[a]
Grade 12 (%)	56 (28.0)	26 (26.8)	30 (29.1)	0.7543[a]
Tertiary studies-incomplete (%)	29 (14.5)	17 (17.5)	12 (11.7)	0.3153[a]
Tertiary studies-diploma (%)	13 (6.5)	7 (7.2)	6 (5.8)	0.7783[a]
Tertiary studies-degree (%)	2 (1.0)	2 (2.1)	0 (0)	0.2340[a]

**results are statistically significant p<0.05; a: Fisher's exact test; b: Mann Whitney t-test; c: pregnant women have been tested for HIV infection and results are positive, they are receiving antiretroviral treatment; d: Pregnant women have been test for the HIV infection and results are negative. HIV: Human immunodeficiency virus*

4.4.2 Maternal physical activity and lifestyle characteristics

The maternal physical activity and lifestyle characteristics in both HIV-infected and HIV-uninfected pregnant black South African women are represented in *Table 4.2*, which includes the following variables: (i) physical activity; (ii) vigorous physical activity; (iii) moderate physical activity; (iv) travel by walking; (v) smoking; (vi) drugs; and (vii) alcohol consumption during pregnancy. This study identified that 20.5% of the pregnant women (N=200) engaged in physical exercise during their pregnancy, which is one in five pregnant women. Our study findings investigated whether the pregnant women engaged in risky behaviour during pregnancy such as alcohol consumption, drug abuse, and smoking during pregnancy. It was identified that 24.0% of the pregnant women (N=200) consumed alcohol during pregnancy, which is one in four pregnant

women. Only 1.0% of the pregnant women (N=200) participated in drug abuse during pregnancy. Two percent (2.0%) of the pregnant women (N=200) chose to smoke during their pregnancy. Whereas 29.0% of the pregnant women (N=200) were unwillingly exposed to second hand smoke during their pregnancy, which is 1 in 3 pregnant women. Notably, the physical activity and lifestyle characteristics were not significantly different between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to the pregnant women with a BMI of < 25 kg/m^2.

Table 4.2: *Physical activity and lifestyle characteristics in both HIV-infected and HIV-uninfected pregnant black South African women categorised according to the maternal BMI (kg/m^2)*

Variables	**All pregnant females (n=200)**	**Maternal BMI (kg/m^2)**		***p*-value**
		< 25.0	**≥ 25.0**	
Physical activity:	**(n=200)**	**(n=103)**	**(n=97)**	
Exercise during pregnancy (%)	41 (20.5)	25 (24.3)	16 (16.5)	0.2201[a]
Vigorous activity (%)	**11 (6.0)**	**9** (56.3)	**2** (12.5)	0.0595[a]
Mean number of days per week (SD)	4.0 (2.5)	4.4 (2.3)	2.0 (1.0)	-
Mean min per day (SD)	51.0 (36.9)	54 (36.8)	37.5 (22.5)	-
Moderate activity (%)	**34 (17.0)**	**19** (76.0)	**15** (93.8)	0.7069[a]
Mean number of days per week (SD)	3.1 (1.8)	3.3 (1.9)	2.9 (1.5)	0.8027[b]
Mean min per day (SD)	38.0 (35.9)	35.0 (32.5)	44.3 (39.6)	0.2260[b]
The mean number of hours spent sitting in the previous week before admission (SD)	25.8 (23.5)	4.7 (2.8)	5.4 (3.0)	0.2542[b]
Travel by walking (%)	**175 (87.5)**	**90** (87.4)	**85** (87.6)	1.000[a]
Mean number of days per week (SD)		3.0 (1.9)	3.0 (2.0)	0.8650[b]
Mean min per day (SD)		28.8 (23.6)	30.7 (22.0)	0.1899[b]
Smoking/ exposure:	**(n=200)**	**(n=103)**	**(n=97)**	
Currently smoking (%)	4 (2.0)	1 (1.0)	3 (3.1)	0.3567[a]
Smoked before pregnancy (%)	11 (6.0)	6 (5.8)	5 (5.2)	1.000[a]
Exposure to in-house second-hand smoking (%)	58 (29.0)	34 (33.0)	24 (24.7)	0.2152[a]
Exposure to second-hand smoking at work (%)	6 (3.0)	4 (23.5)	2 (11.1)	0.4018[a]
Exposure to industrial smoke at work (%)	4 (2.0)	2 (11.8)	2 (11.1)	1.0000[a]
Drugs (%)	2 (1.0)	1 (0.5)	1 (0.5)	1.0000[a]
Alcohol intake during pregnancy (%):	48 (24.0)	27 (13.5)	21 (10.5)	0.5091[a]
Note: a: Fisher's exact test; b: Mann Whitney t-test				

4.4.3 Maternal food frequency intake during pregnancy

This study investigated the retrospective food frequency intake of obesity in both HIV-infected and HIV-uninfected pregnant black South African women during their pregnancy. The food groups that were assessed were: (i) saturated fat; (ii) cooking fat; (iii) salt; (iv) sugar; (v) animal protein; (vi) dairy; (vii) legume; (viii) starch; (ix) vegetables; and (x) fruit (refer to *Table 4.3; and Appendix 1-2*).

Table 4.3: Maternal food frequency intake in both HIV-infected and HIV-uninfected pregnant black South African women divided into high, moderate, and low, and further categorised according to the maternal BMI (kg/m^2)

Food group:	**Maternal BMI (kg/m^2)**		***p*-value[a]**
	<25.0	**≥25.0**	
Saturated fat intake:	**(n=97)**	**(n=97)**	0.616
Low (%)	2 (2.1)	1 (1.0)	
Moderate (%)	48 (49.5)	43 (44.3)	
High (%)	47 (48.5)	53 (54.6)	
Cooking fat intake:	**(n=93)**	**(n=92)**	0.608
Low (%)	1 (1.1)	0 (0.0)	
Moderate (%)	57 (61.3)	57 (62.0)	
High (%)	35 (37.6)	35 (38.0)	
Salt intake:	**(n=96)**	**(n=96)**	0.275
Low (%)	6 (6.3)	3 (3.1)	
Moderate (%)	50 (52.1)	60 (62.5)	
High (%)	40 (41.7)	33 (34.4)	
Sugar intake:	**(n=97)**	**(n=97)**	0.284
Low (%)	11 (11.3)	5 (5.2)	
Moderate (%)	21 (21.6)	21 (21.6)	
High (%)	65 (67.0)	77 (79.4)	
Animal protein intake:	**(n=94)**	**(n=93)**	0.424
Low (%)	29 (30.9)	22 (23.7)	
Moderate (%)	56 (59.6)	58 (62.4)	
High (%)	9 (9.6)	13 (14.0)	
Dairy intake:	**(n=94)**	**(n=93)**	0.872
Low (%)	35 (37.2)	38 (40.9)	
Moderate (%)	35 (37.2)	32 (34.4)	
High (%)	24 (25.5)	23 (24.7)	
Legume intake:	**(n=94)**	**(n=92)**	0.138
Low (%)	64 (68.1)	50 (54.3)	
Moderate (%)	22 (23.4)	33 (35.9)	
High (%)	8 (8.5)	9 (9.8)	
Starch intake:	**(n=94)**	**(n=92)**	0.210
Low (%)	12 (12.8)	9 (9.8)	

Moderate (%)	43 (45.7)	54 (58.7)	
High (%)	39 (41.5)	29 (31.5)	
Vegetable intake:	**(n=93)**	**(n=91)**	0.185
Low (%)	11 (11.8)	7 (7.7)	
Moderate (%)	50 (53.8)	41 (45.0)	
High (%)	32 (34.4)	43 (47.3)	
Fruit intake:	**(n=95)**	**(n=91)**	0.963
Low (%)	27 (28.4)	27 (29.7)	
Moderate (%)	50 (52.6)	48 (52.7)	
High (%)	18 (18.9)	16 (17.6)	
a: Pearson chi-square test			

Overall, the dietary pattern identified for the overweight and obese pregnant women in comparison to the non-overweight/non-obese pregnant women was that their diet was higher in saturated fat, higher in salt, higher in sugar, higher in animal protein, lower in dairy, higher in legumes, higher in starch, higher in vegetables, and had a similar intake of fruit (refer to *Appendix 11*). However, these dietary patterns were not significantly different between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to the pregnant women with a BMI of < 25 kg/m^2.

4.4.4 Factors associated with maternal overweight and obesity

A simple logistic regression showed that maternal age and gestational age were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women (refer to *Table 4.4*). Notably, there were no significant associations between HIV status, type of pregnancy, marital status, job status, number of people living at home, geographic position, water source, housing type, fuel source, education, physical activity, smoking, alcohol, and drugs with maternal overweight and obesity.

Table 4.4: *Simple logistic regression of factors associated with maternal overweight and obesity (n=200) obesity in both HIV-infected and HIV-uninfected pregnant black South African women*

Variables	**Regression coefficient B**	**Crude OR (95% CI)**	**Wald statistic (df)**	***p*-value**
HIV status: Infected[a] Uninfected[b]	 0.134	 1 1.143 (0.654-1.996)	 0.220 (1)	 0.639
Type of pregnancy: Single Twin	-0.61	0.941 (0.130-6.812) 1	0.004 (1)	0.952

Gestational age	0.114	1.121 (1.006-1.248)	4.284 (1)	0.038*
Maternal Age	0.060	1.061 (1.009-1.117	5.274 (1)	0.022*
Geriatric maternal age[#]	-0.480	0.619 (0.252-1.521)	1.095 (1)	0.295
Marital status:				
Single	-0.137	0.872 (0.294-2.591)	0.060 (1)	0.806
Married	1.386	4.000 (0.616-25.964)	2.110 (1)	0.146
Engaged		1		
Job-status:				
Employed		1		
Unemployed	-0.142	0.868 (0.418-1.800)	0.146 (1)	0.703
Number of people living at home:				
Total	-0.073	0.930 (0.861-1.005)	3.353 (1)	0.067
Adults	-0.130	0.878 (0.770-1.001)	3.812 (1)	0.051
Children	-0.073	0.930 (0.830-1.041)	1.582 (1)	0.208
Geographic position:				
Rural		1		
Urban	-0.200	0.818 (0.470-1.426)	0.500 (1)	0.479
Water source:				
Inside Tap	0.255	1.290 (0.341 -4.874)	0.141 (1)	0.707
Outside Tap	0.452	1.571 (0.414-5.965)	0.441 (1)	0.507
Pump	0.588	1.800 (0.318-10.201)	0.441 (1)	0.507
River		1		
Housing type:				
Formal		1		
Informal	-0.135	1.145 (0.636-2.060)	0.203 (1)	0.652
Fuel source:				
Electricity	-0.428	0.652 (0.178-2.389)	0.417 (1)	0.518
Paraffin	-1.099	0.333 (0.051-2.177)	1.317 (1)	0.251
Gas	-21.608	0.000 (0.000-0.000)	0.000 (1)	0.999
Wood		1		
Education:				
≤ Grade 7	-21.608	0.000 (0.000-0.000)	0.000 (1)	0.999
Grade 8 – 11	-21.393	0.000 (0.000-0.000)	0.000 (1)	0.999
Grade 12	-21.346	0.000 (0.000-0.000)	0.000 (1)	0.999
Tertiary studies- incomplete	-20.855	0.000 (0.000-0.000)	0.000 (1)	0.999
Tertiary studies- diploma	-21.049	0.000 (0.000-0.000)	0.000 (1)	0.999
Tertiary studies- degree		1		
Physical activity:				
Yes		1		
No	0.484	1.623 (0.806-3.268)	1.835 (1)	0.176
Vigorous activity:				
Yes	-1.371	0.254 (0.047-1.379)	2.521 (1)	0.112
No		1		

Moderate activity:				
Yes	0.862	2.368 (0.417-13.461)	0.946 (1)	0.331
No		1		
Travel by walking:				
Yes		1		
No	-0.023	0.977 (0.422-2.261)	0.003 (1)	0.957
Currently smoking:				
Yes		1		
No	-1.180	0.307 (0.031-3.005)	1.029 (1)	0.310
Smoked before pregnancy:				
Yes		1		
No	0.129	1.138 (0.336-3.857)	0.043 (1)	0.835
Second-hand smoking (house):				
Yes		1		
No	0.405	1.499 (0.808-2.779)	1.650 (1)	0.199
Second-hand smoking (work):				
Yes		1		
No	0.652	1.919 (0.343-10.724)	0.551 (1)	0.458
Industrial smoke (work):				
Yes	-0.61	1	0.004 (1)	0.952
No		0.941 (0.130-6.812)		
Drugs:				
Yes		1		
No	-0.061	0.941 (0.058-15.259)	0.002 (1)	0.966
Alcohol:				
Yes		1		
No	0.251	1.286 (0.669-2.470)	0.569 (1)	0.451
Saturated fat intake:				
Low		1		
Moderate	0.583	2.255 (0.198-25.679)	0.220 (1)	0.639
High	0.813	1.792 (0.157-20.463)	0.429 (1)	0.512
Cooking fat intake:				
Low		1		
Moderate	21.203	16.5 (0.000-0.000)	0.000 (1)	1.000
High	21.203	16.5 (0.000-0.000)	0.000 (1)	1.000
Salt intake:				
Low		1		
Moderate	0.875	2.400 (0.571-10.087)	1.428 (1)	0.232
High	0.501	1.650 (0.383-7.109)	0.452 (1)	0.502

Sugar intake:				
Low		1		
Moderate	0.788	2.200 (0.651-7.436)	1.610 (1)	0.205
High	0.877	2.403 (0.792-7.287)	2.399 (1)	0.121
Animal protein intake:				
Low		1		
Moderate	0.311	1.365 (0.702-2.654)	0.843 (1)	0.359
High	0.644	1.904 (0.690-5.252)	1.548 (1)	0.213
Dairy intake:				
Low		1		
Moderate	-0.172	0.842 (0.434-1.636)	0.257 (1)	0.612
High	-0.125	0.883 (0.424-1.838)	0.111 (1)	0.739
Legume intake:				
Low		1		
Moderate	0.652	1.920 (0.998-3.693)	3.820 (1)	0.051
High	0.365	1.440 (0.518-4.000)	0.489 (1)	0.484
Starch intake:				
Low		1		
Moderate	0.515	1.674 (0.646-4.341)	1.125 (1)	0.289
High	-0.009	0.991 (0.369-2.665)	0.000 (1)	0.986
Vegetable intake:				
Low		1		
Moderate	0.254	1.289 (0.458-3.623)	0.231 (1)	0.631
High	0.747	2.112 (0.737-6.048)	1.938 (1)	0.164
Fruit intake:				
Low		1		
Moderate	-0.062	0.940 (0.483-1.829)	0.033 (1)	0.855
High	-0.118	0.889 (0.376-2.099)	0.072 (1)	0.788

HIV: Human immunodeficiency virus
*a: Pregnant women have been tested for HIV infection and results are positive, they are receiving antiretroviral treatment; b: Pregnant women have been tested for the HIV infection and results are negative; *results are statistically significant $p<0.05$; $^{\#}\geq 35$ years*

Multiple logistic regression analysis showed that maternal age and gestational age were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant women (refer to *Table 4.5*).

Table 4.5: *Multiple logistic regression of factors associated with maternal overweight and obesity (n=200) in both HIV-infected and HIV-uninfected pregnant women*

Variables	Regression coefficient B	Crude OR[#] (95% CI)	Wald statistic (df)	*p*-value
Gestational age	0.114	1.121 (1.005-1.251)	4.183 (1)	0.041*
Maternal Age	0.059	1.061 (1.008-1.117)	5.189 (1)	0.023*

#Model has been adjusted for gestational age and maternal age
**results are statistically significant p<0.05*

Hence, for every 1-year unit increase in the age of the participants, they were 1.061 times more likely to be overweight/obese. This suggests that as the maternal age increases, so does the weight of the mother. Likewise with gestational age, for every 1-week increase, the participants were 1.121 times more likely to be overweight/obese. This suggests that as the gestational age of the pregnancy increases, so does the weight of the mother.

4.4.5 Association between maternal overweight/obesity and maternal health outcomes

The associations between maternal health outcomes and maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women are summarised in *Table 4.6*. In our study, 39.5% of the pregnant women had a C-section birth, 24.0% had a preterm birth, and a small percentage of 1.5% had GDM. Notably, C-section birth, preterm birth, GDM, PIH, PET, PET and HELLP and late onset PET were not significantly different between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to the pregnant women with a BMI of < 25 kg/m^2. However, HPT disorders (*p*=0.0043), chronic HPT (*p*=0.0003), mean Hb (g/dl) (*p*=0.0409) and anaemia (*p*=0.0381) were significantly different between the pregnant women with a BMI of ≥ 25.0 kg/m^2 compared to the pregnant women with a BMI of < 25 kg/m^2.

Table 4.6: Maternal health outcomes in both HIV-infected and HIV-uninfected pregnant black South African women categorised according to the maternal BMI (kg/m^2)

Variables	All pregnant women (N=200)	Maternal BMI (kg/m^2)		p-value
		n=103	n=97	
		< 25.0	≥ 25.0	
Caesarean section (%)	79 (39.5)	37 (35.9)	42 (43.3)	0.1783[a]
Preterm delivery (%)	48 (24.0)	30 (29.1)	18 (18.6)	0.0562[a]
GDM (%)	3 (1.5)	1 (1.0)	2 (2.1)	0.6120[a]

Hypertensive disorders (%)	39 (19.5)	12 (11.7)	27 (27.8)	0.0043[a*]
Chronic HPT (%)	11 (5.5)	0 (0.0)	11 (11.3)	0.0003[a*]
PIH (%)	13 (6.5)	6 (5.8)	7 (7.2)	0.7783[a]
PET (%)	15 (7.5)	6 (5.8)	9 (9.3)	0.4260[a]
HELLP & PET (%)	1 (0.5)	1 (16.7)	0 (0.0)	1.0000[a]
Late onset PET (%)	2 (1.0)	1 (16.7)	1 (11.1)	1.0000[a]
Anaemia:	**(n=146)**	**(n=77)**	**(n=69)**	
Mean Hb in g/dL (SD)	11.0 (1.6)	10.7 (1.6)	11.2 (1.6)	0.0409[b*]
Prevalence of anaemia (%)	71 (48.6)	44 (57.0)	27 (39.1)	0.0381[a*]

[*]results are statistically significant p<0.05; a: Fisher's exact test; b: Mann Whitney t-test; HPT: hypertension; PET: pre-eclampsia toxaemia; PIH: pregnancy-induced hypertension; GDM: Gestational Diabetes Mellitus; Hb: Haemoglobin

The prevalence of hypertensive disorders in the pregnant women (N=200) was 19.5%, with the highest prevalence being in the overweight and obese group of pregnant women (n=27; 27.8%) (refer to *Figure 4.1).* These hypertensive disorders included chronic HPT (n=11; 5.5%), PET (n=15; 7.5%), and PIH (n=13; 6.5%).

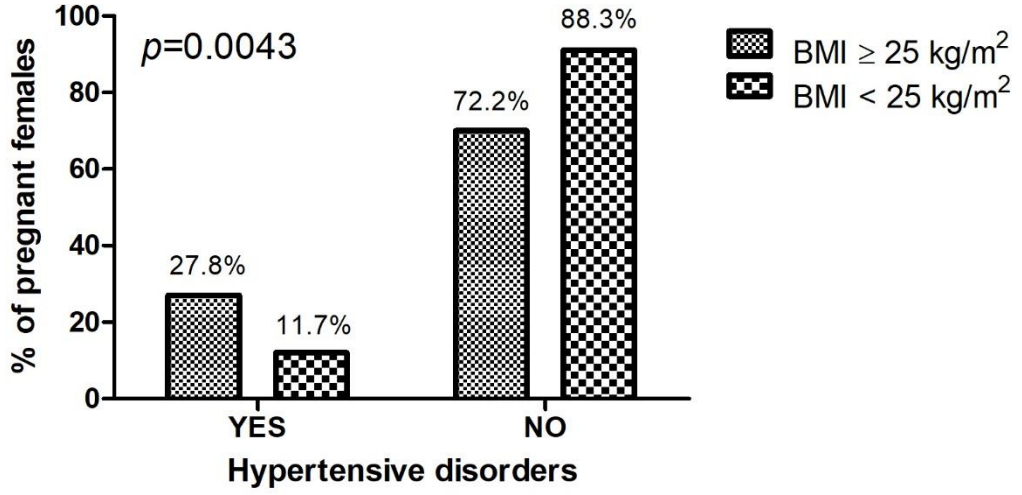

Figure 4.1: Fisher's exact t-test showing the prevalence of hypertensive disorders in both HIV-infected and HIV-uninfected pregnant black South African women categorised according to BMI (kg/^{2})

In the present study, 48.6% of the pregnant women had anaemia, with the highest prevalence in pregnant women with a BMI <25kg/m^2 (n=44; 57.0 %) than compared to overweight and obese

pregnant women (n= 27; 39.1%) (refer to *Figure 4.2*).

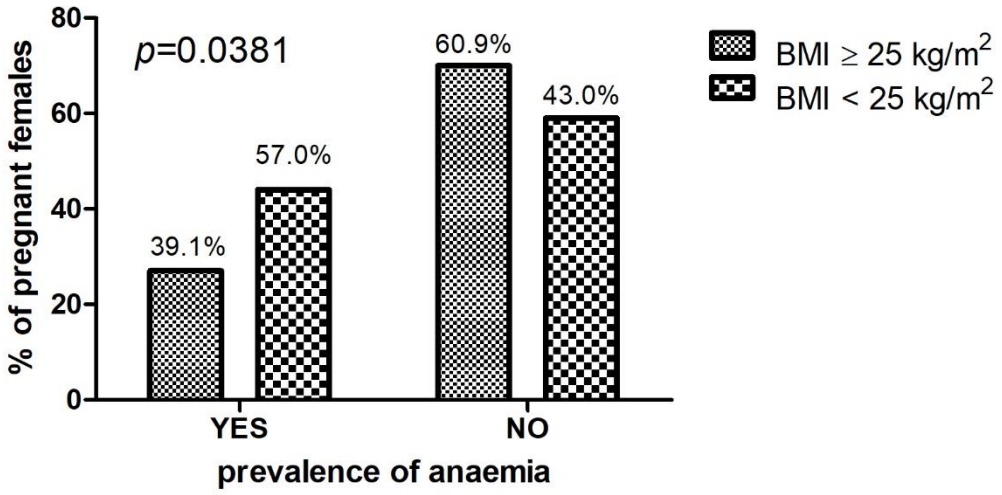

Figure 4.2: Fisher's exact t-test showing the prevalence of anaemia in both HIV-infected and HIV-uninfected pregnant black South African women categorised according to maternal BMI (kg/m^2)

A simple logistic regression showed that hypertensive disorders, anaemia, and Hb (g/dL) were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women (refer to *Table 4.7*). Notably, there were no significant associations with C-section, preterm delivery, GDM and PET.

Table 4.7: Simple logistic regression of maternal health outcomes associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women (N=200)

Variables	**Regression coefficient B**	**Crude OR (95% CI)**	**Wald statistic (df)**	***p*-value**
Caesarean section:				
Yes		1		
No	-0.309	0.734 (0.416-1.296)	1.135 (1)	0.287
Preterm delivery:				
Yes		1		
No	0.590	1.804 (0.927-3.508)	3.019 (1)	0.082
GDM:				
Yes		1		

No	-0.764	0.466 (0.042-5.220)	0.384 (1)	0.535
Hypertensive disorder:				
Yes		1		
No	-1.073	0.342 (0.162-0.722)	7.909 (1)	0.005*
PET:				
Yes		1		
No	-0.503	0.605 (0.207- 1.768)	0.844 (1)	0.358
Anaemia:				
Yes		1		
No	0.659	1.933 (1.070 – 3.492)	4.777 (1)	0.029*
Hb (g/dL)	0.211	1.235 (1.003 – 1.520)	3.961 (1)	0.047*

*HPT: hypertension; PET: pre-eclampsia toxaemia; PIH: pregnancy-induced hypertension; GDM: Gestational Diabetes Mellitus; Hb: Haemoglobin *results are statistically significant p<0.05*

A simple logistic regression showed that hypertensive disorders were significantly associated with maternal overweight and obesity in HIV-infected pregnant black South African women (refer to *Table 4.8*). Notably, there were no significant associations with C-section, preterm delivery, GDM, PET and anaemia.

Table 4.8: *Simple logistic regression of maternal health outcomes associated with maternal overweight and obesity pregnant black South African women, adjusted for HIV status*

Variables	**Regression coefficient B**	**Crude OR (95% CI)**	**Wald statistic (df)**	***p*-value**
Caesarean section:				
HIV-infected:				
Yes		1		
No	-0.402	0.669 (0.277-1.614)	0.800 (1)	0.371
HIV-uninfected:				
Yes		1		
No	-0.221	0.802 (0.377-1.703)	0.331 (1)	0.565
Preterm delivery:				
HIV-infected:				
Yes		1		
No	0.567	1.318 (0.513-3.387)	0.328 (1)	0.567
HIV-uninfected:				
Yes				
No	0.880	2.410 (0.933-6.226)	3.302 (1)	0.069

GDM:				
HIV-infected:				
Yes		1		
No	21.090	144 (0.000-0.000)	0.000 (1)	1.000
HIV-uninfected:				
Yes		1		
No	-21.240	0.0 .000-0.000)	0.000 (1)	0.999
Hypertensive disorder:				
HIV-infected:				
Yes		1		
No	-1.459	0.233 (0.075-0.717)	6.439 (1)	0.011*
HIV-uninfected:				
Yes		1		
No	-0.753	0.471 (0.172-1.291)	2.142 (1)	0.143
PET:				
HIV-infected:				
Yes		1		
No	-0.150	0.860 (0.231-3.206)	0.050 (1)	0.860
HIV-uninfected:				
Yes		1		
No	-1.443	0.236 (0.026-2.184)	1.618(1)	0.203
Anaemia:				
HIV-infected:				
Yes		1		
No	0.504	1.656 (0.709-3.867)	1.359 (1)	0.244
Hb (g/dL)	0.170	1.185 (0.869-1.618)	1.149 (1)	0.284
HIV-uninfected:				
Yes		1		
No	0.794	2.213 (0.956-5.126)	3.437 (1)	0.064
Hb (g/dL)	0.245	1.278 (0.957-1.705)	2.767 (1)	0.096

*HPT: hypertension; PET: pre-eclampsia toxaemia; PIH: pregnancy-induced hypertension; GDM: Gestational Diabetes Mellitus; Hb: Haemoglobin *results are statistically significant p<0.05*

Multiple logistic regression analysis showed that hypertensive disorders and anaemia were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women (refer to *Table 4.9*).

Table 4.9: Multiple logistic regression of maternal health outcomes associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women (n=200)

Variables	Regression coefficient B	Crude OR[#] (95% CI)	Wald statistic (df)	*p*-value
Hypertensive disorder:				
Yes		1		
No	-1.300	0.273 (0.124 – 0.601)	10.401 (1)	0.001*
Anaemia:				
Yes		1		
No	0.884	2.420 (1.283 – 4.563)	7.454 (1)	0.006*

#Model has been adjusted for Hypertensive disorders and anaemia
**results are statistically significant p<0.05*

Hence, overweight and obese pregnant women were 2.420 times more likely to not have anaemia in comparison to those with a BMI < 25 kg/m^2. However, overweight and obese pregnant women were 0.273 times more likely to have hypertensive disorders in comparison to those with a BMI < 25 kg/m^2. Therefore, maternal overweight and obesity increase the risk for hypertensive disorders during pregnancy but decreases the risk for anaemia during pregnancy.

4.5 Discussion

In our cross-sectional study of both HIV-infected and HIV-uninfected pregnant black South African women, we identified that maternal overweight and obesity in pregnancy was significantly associated with maternal age, gestational age, HPT disorders and anaemia. In a setting where there is both a high prevalence of overweight and obesity in women of child-bearing age and a high prevalence of HIV in pregnant women, our findings highlight the need for weight management interventions during pregnancy to minimise the adverse maternal health outcomes (Iyun, *et al*., 2018; Statistics South Africa, 2017).

In the present study, geriatric pregnancy was not associated with maternal overweight and obesity, but it was identified that maternal age was associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women. Similar findings were found in an Australian study, which identified that increasing maternal BMI was associated with increasing maternal age (*p* <0.001*)* and suggested that older women were at a higher risk of becoming overweight and obese in pregnancy (Callaway, *et al*., 2006). In a Lithuanian study, obese pregnant women were also significantly older than the normal weight pregnant women (*p* <0.001) (Ramonienė, *et al*., 2017). Similarly other African studies have identified that the risk for

overweight/obesity was higher among older women (Mosha, *et al*., 2021; Mukora-Mutseyekwa, *et al*., 2019; Mndala and Kudale, 2019; Abrha, *et al*., 2016). The mechanisms responsible for this age-associated risk with gestational weight gain may be linked to metabolic dysfunction and alterations in the deposition of adipose tissue (Pontzer, *et al*., 2021).

Gestational age in this study was also positively associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women. Typically, pregnant women are encouraged to gain weight as their gestational age progresses, with the goal of the total amount of weight gain for the 40 weeks of gestation based on the mother's pre-pregnancy actual body weight (kg) (Kominiarek and Peaceman, 2017; Pitkin, 1976). Therefore, maternal overweight and obesity in pregnancy has been attributed to excessive gestational weight gain, which is defined as maternal weight gain more than the recommended amount over the course of pregnancy (Kominiarek and Peaceman, 2017; Triunfo and Lanzone, 2014). Hence, this excessive gestational weight gain should be avoided because it has been linked with increased risks of delivery complications like C-section births, increased risk of post-partum weight retention for the mother, miscarriage, HPT disorders and GDM (Kominiarek and Peaceman, 2017; Triunfo and Lanzone, 2014). Even more so, for pregnant women who are already overweight/obese at the onset of pregnancy, weight gain should be carefully monitored throughout their pregnancy (Triunfo and Lanzone, 2014).

Interestingly, our study found that modifiable lifestyle factors like physical activity, dietary intake, smoking, alcohol intake and drug abuse were all not associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women. In our study findings, the food frequency intake was not associated with overweight and obesity in pregnancy. But the dietary patterns identified were consistent with other studies that have linked similar dietary patterns to obesity in pregnancy including a high intake of energy-dense foods, especially foods high in saturated fat, salt, and sugar (Moran, *et al*., 2013; Lindsay, *et al*., 2015). We found that alcohol consumption was not associated with overweight and obesity in pregnancy, but other studies have linked alcohol consumption to weight gain due to its energy density and effects on fatty acid metabolism (Mohd-Shukri, *et al*., 2015; Traversy and Chaput, 2015). Also, we found that smoking was not significantly associated with overweight and obesity in pregnancy. However, according to Gaillard *et al*., (2013), smoking during pregnancy was associated with increased risk

of excessive gestational weight gain and should be avoided. For physical activity, as pregnant women progress through their pregnancy it is common to find that their activity level decreases (Anderson-Hall, *et al*., 2021; Okafor and Goon, 2020). For women living in Africa, there are various barriers which prevent them from engaging in physical activity such as lack of time, lack of knowledge, inadequate information from healthcare providers, feelings of tiredness and a lack of social support (Okafor and Goon, 2020). But physical activity or exercise during pregnancy should be encouraged during pregnancy because it has been associated with a reduction in gestational weight gain and inversely associated with adverse maternal health outcomes like HD and GDM (Anderson-Hall, *et al*., 2021; Du, *et al*., 2019).

Hypertension is considered a preventable complication of pregnancy, but it is a life-threatening condition when untreated or mismanaged (Berhe, *et al*., 2020; Moodley, *et al.*, 2019)**.** In South Africa, HPT is considered the most direct cause of maternal mortality and accounts for 18% of maternal deaths (Moodley, *et al*., 2019). The mechanisms involved in the pathogenesis of obesity-related HPT in pregnancy are caused by physiological changes in adiposity, increased blood circulation, sympathetic nervous system overactivation, stimulation of the renin-angiotensin-aldosterone system, alterations in adipose-derived cytokines, insulin resistance, and structural as well as functional renal changes (Shariq and McKenzie, 2020). Overall, we identified that HD disorders were significantly associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women, where one in five pregnant women were affected by HPT disorders. According to Kivelä, *et al*., (2021), maternal overweight and obesity is associated with a significantly different cardiovascular disease profile and an adverse metabolic profile compared to pregnant women with a BMI <25.0 kg/m^2. This altered metabolic profile leads to increased risk for developing HPT disorders in pregnancy (Kivelä, *et al*., 2021). For example, in two retrospective cohort studies it was identified that maternal overweight or obesity in pregnancy was associated with a significantly increased odds of developing hypertensive disorders in comparison to pregnant women with a BMI <25 kg/m^2 (Haugen, *et al*., 2014). In terms of the HIV infection and treatment thereof, it is of particular interest in South African pregnant women, because it has been linked to vascular endothelial dysfunction (Nkeh-Chungag, *et al*., 2021). Our study identified that overweight and obese HIV-infected pregnant women had increased odds for developing HPT disorders. Other studies have supported this, where HIV-infected pregnant women with a BMI ≥ 25 kg/m2 had a significantly increased odds of HPT

disorders like PET (OR = 3.0; 95% CI: 1.5–6.0) (Machado, *et al*., 2014). Hence, this has highlighted that the HIV-infected pregnant women who have a BMI ≥ 25.0 kg/m^2 have a different cardiometabolic risk to the HIV-uninfected pregnant women with a BMI <25 kg/m^2.

Our study findings identified that one in three pregnant women were affected by anaemia. Anaemia is a common blood condition experienced by many pregnant women and is associated with an increased risk for maternal mortality in pregnancy (Tunkyi and Moodley, 2015; Stephen, *et al*., 2018). Iron deficiency is one of the most common causes of anaemia in South Africa, and preventative measures have been routinely implemented to prevent anaemia via prophylactic iron supplementation (Tunkyi and Moodley, 2015). Despite these types of interventions already in place, the present study showed that anaemia is still a cause for concern during pregnancy. However, our study identified that anaemia was inversely associated with maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women. Our study findings are consistent with that of other studies that have investigated the association between maternal BMI and risk for anaemia. In a Chinese study, where women of childbearing age with overweight (OR=0.72; 95% CI: 0.62-0.89) and obesity (OR=0.59; 95% CI:0.43-0.79) were less likely to be anaemic as compared to normal-weight women (Qin, *et al*., 2013). A study conducted in Ghana showed a similar pattern where differences in BMI influenced the risk for anaemia, where pregnant women who were underweight had an increased odds for anaemia compared to the normal weight pregnant women (OR=3.17; 95%CI:1.19-8.32) (Nonterah, *et al*., 2019). Also, in the prospective cohort study by Mocking, *et al*., (2018), a higher BMI in early pregnancy was associated with a higher Hb (g/dL) at the first antenatal booking and with a reduced risk of anaemia in Indonesian and Ghanaian pregnant women. Overall, our study identified that the pregnant women with a BMI ≥25 kg/m2 were less likely to be anaemic compared to those with a BMI <25 kg/m^2. The mechanisms behind this have yet to be investigated, but it could be linked to the overweight/obese pregnant women dietary intake with possible consumption of higher quantities of bioavailable iron-rich foods for example, in South Africa, staple foods such as maize and bread are fortified with iron (van Jaarsveld, Faber and Stuijvenberg, 2015).

4.6 Conclusion

In summary, maternal overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women was significantly associated with maternal age, gestational age, HPT

disorders and anaemia. Also, maternal overweight and obesity decreased the odds for anaemia during pregnancy but increased the odds for the development of HPT disorders during pregnancy, especially in the HIV-infected.

4.7 List of abbreviations

ABW: actual body weight

ART: antiretroviral treatment

BMI: Body mass index

BREC: Biomedical Research Ethics Council

C-section: Caesarean section delivery

CI: Confidence interval

GDM: Gestational diabetes mellitus

Hb: Haemoglobin

HPT: Hypertensive

HELLP: Haemolysis, elevated liver enzymes and low platelet counts

HIV: Human immunodeficiency virus

KZNDOH: KwaZulu-Natal Department of Health

MW: Maternal weight

OR: Odds ratio

PET: Pre-eclampsia toxaemia

PIH: Pregnancy induced hypertension

PMMH: Prince Mshiyeni Memorial: Preterm rupture of membranes

SD: Standard deviation

SH: Standing height

UKZN: University of KwaZulu-Natal

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CHAPTER 5

This chapter responds to the objective vii of this study to identify the effect of overweight and obesity on mRNA expression patterns in black South African pregnant women HIV-infected and HIV-uninfected. The chapter is presented in the form of a manuscript entitled "Maternal overweight and obesity compounded by the HIV infection alters the gene expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in black South African pregnant women". The raw data has been published in the NCBI gene expression omnibus (GEO) repository (GE199833).

5. Maternal overweight and obesity compounded by the HIV infection alters the gene expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in black South African pregnant women

5.1 Abstract

Background: The pathogenesis of maternal overweight and obesity within the context of the human immunodeficiency virus (HIV) epidemic, is multifactorial and involves interactions among genetic and epigenetic, environmental, and behavioural factors. To further understand the pathogenesis, the aim of this study was to investigate whether maternal BMI and HIV status had an effect on the mRNA expression of adiponectin (*ADIPOQ*), leptin (*LEP*), leptin receptor (*LEPR*), fat mass and obesity-associated (*FTO*), and ghrelin (*GHRL*) in visceral adipose tissue (VAT) and in whole blood (WB) obtained from pregnant black South African women.

Methods: A cross-sectional study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital (PMMH), which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. The catchment area for the hospital includes both rural and urban geographical areas. Pregnant women admitted to the labour ward were approached to participate in this study. The inclusion criteria for this study were as follows: (1) ≥ 18 years of age; (2) pregnant females; (3) black South African citizen; (4) clinically stable; (5) able to stand without assistance; (6) given verbal and written consent to participate in the study; and (7) gave consent to obtain a VAT sample during their c-section operation. The participants were categorized according to BMI (kg/m^2) into two groups: (1) overweight/obese pregnant women (≥25kg/m^2); and (2) non-overweight/non-obese pregnant women (<25kg/m^2). A total of 79 subjects met all the inclusion criteria where 42/79 had a BMI ≥25kg/m^2 and 37/79 had a BMI <25 kg/m^2, and 30/79 were HIV-infected and 49/79 were HIV-uninfected.

Results: It was identified that there were statistically significant differences for *ADIPOQ* (p <0.001), *LEP* (p = 0.0105) and *LEPR* (p=0.0220) where mRNA expression was greater in the VAT compared to WB. The mRNA expression of *FTO* was similar in VAT and WB (p=0.4039). There were no significant differences in mRNA expression for *ADIPOQ*, *LEP*, *LEPR* and *FTO* between all the BMI and HIV status groups. However, there were patterns identified that allude to BMI, HIV, and the combination of BMI and HIV which showed that there was an effect on the mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in the pregnant women. The pregnant women with

a BMI ≥25.0 kg/m^2 showed a downregulatory pattern for *ADIPOQ*, *LEP*, *LEPR* and *FTO* in VAT and WB. The HIV-infected pregnant women showed a downregulatory pattern for *ADIPOQ*, *LEP*, *LEPR* and *FTO* in VAT and WB. The HIV-infected pregnant women with a BMI ≥25.0kg/m^2 had the lowest mRNA expression for *ADIPOQ*, *LEP*, *LEPR* and *FTO* in VAT and WB. The mRNA expression of *GHRL* in the VAT and WB samples from the pregnant women was undetectable.

Conclusion: Pregnant black South African women presenting with overweight/obesity and being HIV-infected showed to have the worst downregulatory effect on mRNA expression of *ADIPOQ*, *LEP*, *LEPR*, and *FTO*. The downregulation of these genes may result in the dysregulation of metabolic pathways that usually control weight gain during pregnancy.

5.2 Introduction

The proportion of overweight and obese women living with human immunodeficiency virus (HIV) infection have increased globally and are both epidemics that are endemic to countries like South Africa (Bailin, Gabriel, Wanjalla and Koethe, 2020). The pathogenesis of obesity is multifactorial and involves the interactions among genetics and epigenetics, environmental, and behavioural factors (Reichetzeder, 2020). Likewise, HIV and the treatment thereof have been linked to the pathogenesis of changes in fat metabolism, adipocyte function, and fat deposition that are similarly associated with obesity (Bailin, Gabriel, Wanjalla and Koethe, 2020). Currently, there is a gap in the knowledge as to whether obesity and/or HIV effects the expression of genes in the adipose tissue of pregnant women. This epigenetic regulation involves the intersection between genetics and the pregnant woman's obesogenic environment, which determines whether genes are turned on or off (Reichetzeder, 2020). Overweight and obesity in pregnancy are characterized by the excess accumulation of adipose tissue before and/or during pregnancy (Reichetzeder, 2020). Adipose tissue, especially visceral adipose tissue (VAT), may be considered as an endocrine organ metabolically involved in the synthesis and secretion of many different adipokines and cytokines and is therefore an important site to investigate for gene expression (Shuster, Patlas, Pinthus and Mourtzakis, 2012). Overall, little is known about whether maternal BMI and the HIV infection alter the messenger ribonucleic acid (mRNA) expression of adiponectin (*ADIPOQ*), leptin (*LEP*), leptin receptor (*LEPR*), fat mass and obesity-associated (*FTO*), and ghrelin (*GHRL*) in VAT and in whole blood (WB) human pregnancy. Therefore, the aim of this study was to investigate whether maternal BMI and/or HIV status had an effect on the mRNA expression of *ADIPOQ*, *LEP*, *LEPR*, *FTO*, and *GHRL* in VAT and WB obtained from pregnant black South African women.

5.3 Materials and methods

5.3.1 Sample selection and study population

A cross-sectional study design was employed. Sample selection was conducted at Prince Mshiyeni Memorial Regional Hospital (PMMH), which is situated in Umlazi within the eThekweni municipality, KwaZulu-Natal, South Africa. The catchment area for the hospital includes both rural and urban geographical areas. Pregnant women admitted to the labour ward were approached to participate in this study. The inclusion criteria for this study were as follows: (1) ≥ 18 years of age; (2) pregnant females; (3) black South African citizen; (4) clinically stable; (5) able to stand

without assistance; (6) given verbal and written consent to participate in the study; and (7) gave consent to obtain a VAT sample during their c-section operation. The participants were categorized according to BMI (kg/m^2) into two groups: (1) overweight/obese pregnant women ($\geq 25kg/m^2$); and (2) non-overweight/non-obese pregnant women ($<25kg/m^2$). A total of 458 pregnant women were approached to participate in the study, but 245 subjects met the inclusion criteria and of these 45 declined to participate. Hence, a total of 200 subjects met all the inclusion criteria, but of these participants only 79 subjects were able to provide a VAT sample. Therefore, a total of 79 subjects met all the inclusion criteria where 42/79 had a BMI $\geq 25kg/m^2$ and 37/79 had a BMI <25 kg/m^2, and 30/79 were HIV-infected and 49/79 were HIV-uninfected.

5.3.2 Maternal anthropometric assessment

Anthropometric measurements were conducted by an international society for the advancement of kinanthropometry level 1 trained dietician. According to Marfell-Jones, Stewart and de Ridder (2012) and Ulijaszek and Kerr (1991), to avoid anthropometric measurement errors, all measurements were conducted by the same researcher, taken three times, and recorded to the nearest 0.1 cm/kg. The mean of the two closest values was recorded. The standing height (SH) measurement was measured via. stretch stature methodology using a portable calibrated stadiometer (Seca) with a sliding headboard (Lahner, Kassier and Veldman, 2017). The weight measurement was taken using actual body weight (ABW) methodology (Lahner, 2019), using a portable calibrated scale (Seca, with a maximum weight threshold of 250 kg). The scale was calibrated before the commencement of the study. BMI was calculated using the weight of the mother (MW) post-delivery. The BMI was interpreted according to the BMI ($MW/SH^2 = kg/m^2$) classifications: (1) overweight/obese pregnant women ($\geq 25kg/m^2$); and (2) non-overweight/non-obese pregnant women ($<25kg/m^2$) (Weir and Jan, 2020).

5.3.3 HIV status

The HIV status was determined from the medical records of the participants. For this study, HIV status referred to a participant being (i) HIV-uninfected, which means that the pregnant women had a negative PCR test for HIV. Whereas, HIV-infected referred to the pregnant women that had a positive PCR HIV test and were receiving antiretroviral treatment.

5.3.4 Visceral adipose tissue and whole blood samples

For each participant, VAT and WB were collected. For anonymity, each participant was given a unique participant code and all samples correlating to the participant were labelled accordingly.

5.3.4.1 Visceral adipose tissue samples

Visceral adipose tissue samples were collected during the caesarean section operations. Immediately after extraction from the participant, under sterile conditions, the VAT sample was washed three times with 0.1M phosphate binder solution, dissected into smaller pieces, and placed into a sterilized cryovial with 500 µl Qiazol reagent (Qiagen, 79306). The samples were transported on ice and stored at -80°C for ribonucleic acid (RNA) isolation.

5.3.4.2 Whole blood samples

The WB samples were collected for each participant before surgery, in a fasting state, and stored in an Ethylenediaminetetraacetic acid (EDTA) collection tube. The WB samples were transported on ice and under laminar flow conditions, 500µl of WB was added into a sterilized cryovial with 500µl of Qiazol reagent. The samples were then stored at -80°C for RNA isolation.

5.3.4.3 RNA extraction with TRIzol

The AT and WB samples were thawed on ice. Under laminar flow conditions, the AT was homogenized together with the TRIzol™ reagent and centrifuged (10,000 xg, 4°C, 10 min). The supernatant was then removed for each AT sample and added to a new labelled Eppendorf tube. The WB samples were each vortexed for 10 seconds and transferred into a new labelled Eppendorf tube. Each sample was centrifuged (12,000 xg, 4°C, 15 min) and isopropanol (500 µl) was added to the aqueous phase followed by overnight incubation at −80°C. The samples were centrifuged (12,000 xg, 4°C, 20 min), supernatants were removed, and RNA pellets were washed in 75% ethanol (500 µl). Samples were centrifuged (7,400 xg, 4°C, 15 min), RNA pellets were air-dried (30 min, room temperature (RT)), and resuspended in nuclease-free water (15 µl). RNA concentration and purity were assessed with the Nanodrop2000 spectrophotometer (Thermo-Fisher Scientific). Samples with A260/A280 ratios of 1.9–2.1 were considered pure and used for all subsequent assays. RNA concentration was adjusted as required for the respective assays.

5.3.4.4 Quantitative real-time polymerase chain reaction (qRT-PCR)

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine mRNA expression of the following genes: (i) *ADIPOQ* (ii) *LEP*; (iii) *LEPR*; (iv) *FTO*; and (v) *GHRL*. The primer sequences are summarized in *Table 5.1*.

Table 5.1: Primer sequences used for qRT-PCR

Gene:	**Accession number:**	**Primer Sequence:**	**Annealing temperature (°C):**
ADIPOQ	NM_001177800.2	F: CTGTTGCTGGGAGCTGTTCTA R: TGGATCTCCTTTCTCACCCT	53.9
LEP	NM_000230.3	F: TGCCTTCCAGAAACGTGATCC R: CTCTGTGGAGTAGCCTGAAGC	59.5
LEPR	NM_001198689.2	F: ACCTCTGGTTCCCCAAAAAGG R: TTGGCACAGGCACAAGACAT	61.5
FTO	NM_001080432.3	F: ACTTGGCTCCCTTATCTGACC R: TGTGCAGTGTGAGAAAGGCTT	60.0
GHRL	NM_001134941.3	F: AGC CTC CTG CTC CTC GGC AT R: TGT GGG CGA TCA CTT GTC GGC T	62.0

ADIPOQ: Adiponectin; LEP: Leptin; LEPR: Leptin receptor; FTO: fat mass and obesity-associated; GHRL: Ghrelin

Total RNA (standardized to 1,000 ng) for each sample was reverse transcribed into complementary DNA (cDNA) using the Maxima H Minus First Strand cDNA Synthesis Kit (Thermo-Fisher Scientific, K1652). qRT-PCR was performed using the PowerUp™ SYBR™ Green Master Mix (Thermo-Fisher Scientific, A25742) and the Applied Biosystems ViiA7 Real-Time PCR System (ThermoFisher Scientific). Thermocycler conditions were as follows: initial denaturation (95°C, 8 min), followed by 40 cycles of denaturation (95°C, 15 s), annealing (*Table 1*, 40 s), and extension (72°C, 30 s). GAPDH served as the endogenous control to normalize mRNA expression. The relative change in mRNA expression was calculated using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen, 2001).

5.3.5 Upregulation and down-regulation of mRNA expression

According to Orang, Safaralizadeh and Kazemzadeh-Bavili (2014), genes are encoded by DNA, which can undergo upregulation and downregulation in terms of being transcribed to a mRNA and then translated to a protein. Upregulation indicates an increase in transcription, whereas a down-

regulation indicates a decrease in transcription. Each gene has an ATG start site that indicates where transcription should be initiated. Upstream from this transcriptional start site is what is called the promoter region. It is in this area that specific transcription factors and other factors that will help promote transcription and will bind to DNA that is to be transcribed to mRNA (Orang, Safaralizadeh and Kazemzadeh-Bavili, 2014).

5.3.6 Statistical analysis

Descriptive statistics were performed for demographic, clinical, and laboratory data. Data was captured using Microsoft Excel and continuous variables were represented as arithmetic mean ($\bar{x}$) and standard deviation (SD). Statistical analysis was performed using the statistical software packages, GraphPad Prism 5, and IBM SPSS for Windows version 27. The statistical tests included: (i) Fisher's exact test (two categories) and the χ^2 test (more than two categories) to investigate the comparison between categories; (ii) the Pearson correlation coefficient for data with normal distribution was used to identify the strength of association between variable means; (iii) the Spearman's rank-order correlation coefficient for data with non-normal distribution was used to identify the strength of association between variable means; (iv) the Mann Whitney t-test was used for comparison between two variable means; and (v) One-way ANOVA was used for the analysis of variance between the variable means. The level of significance (α) used in the statistical analysis was $p<0.05$. The raw data has been published in the NCBI gene expression omnibus (GEO) repository (GE199833).

5.3.7 Ethics approval and informed consent

The study was approved by the Biomedical Research Ethics Council (BREC) of the University of KwaZulu-Natal (UKZN) (BE269/18), KwaZulu-Natal Department of Health (KZNDOH) (HRKM261/18), and PMMH (29/RESH/2018).

5.4 Results

This study sought to investigate the effect that maternal BMI and HIV status had on the mRNA expression of *ADIPOQ*, *LEP*, *LEPR*, *FTO* and *GHRL* in pregnant black South African pregnant women. The mRNA expression of *GHRL* in the VAT and WB samples from the pregnant women was undetectable. These findings potentially indicate that in pregnancy little to none *GHRL* gene expression occurs in VAT, possibly due to the placenta and stomach being the primary sites of

GHRL gene expression in pregnancy. However, mRNA expression patterns were identified for *ADIPOQ*, *LEP*, *LEPR* and *FTO*.

This study investigated the differences in the mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in VAT compared to WB (refer to *Table 5.2*). It was identified that there were statistically significant differences for *ADIPOQ*, *LEP* and *LEPR* where mRNA expression was greater in the VAT compared to WB. The mRNA expression of *FTO* was similar in VAT and WB. This suggests that *ADIPOQ*, *LEP* and *LEPR* was more metabolically active in VAT than in comparison to WB, whereas the metabolic activity of *FTO* was similar in VAT and WB.

Table 5.2: The relative change in mRNA expression of ADIPOQ, LEP, LEPR and FTO in pregnant black South African women in VAT and WB

Group	Visceral adipose tissue	Whole blood	*p*-value
	Mean (SD)[a]	Mean (SD)[a]	
ADIPOQ	0.2305 (0.3489)	0.1739 (0.4702)	<0.0001*
LEP	0.0910 (0.0716)	0.0638 (0.0301)	0.0105*
LEPR	0.0514 (0.0678)	0.0203 (0.0133)	0.0220*
FTO	0.0042 (0.0030)	0.0046 (0.0033)	0.4039

ADIPOQ: adiponectin; FTO: fat mass and obesity-associated gene; LEP: leptin; LEPR: leptin receptor; SD: standard deviation; VAT: visceral adipose tissue; WB: whole blood
a: the relative change in mRNA expression calculated using the (2−ΔΔCt) method
**: statistically significant p <0.05*

This study investigated the differences in the mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in VAT and WB categorised according to BMI (kg/m^2) (refer to *Table 5.3*) and HIV status (refer to *Table 5.4*). It was identified that there were no significant differences between all the BMI and HIV status groups. However, there were patterns identified that could allude to BMI, HIV, and the combination of BMI and HIV which showed that there was an effect on the mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in the pregnant women.

Table 5.3: The relative change in mRNA expression of ADIPOQ, LEP, LEPR and FTO in pregnant black South African women in VAT and in WB, categorised according to BMI (kg/m^2)

Sample type	All pregnant females (n=200)	Maternal BMI (kg/m^2)		*p*-value
		≥ 25.0 (n=42)	< 25.0 (n=37)	
	Mean (SD)[a]	Mean (SD)[a]	Mean (SD)[a]	
ADIPOQ:				

VAT	0.2305 (0.3489)	0.1713 (0.1177)	0.2976 (0.4891)	0.7870
WB	0.1739 (0.4702)	0.0879 (0.0497)	0.2714 (0.6766)	0.0959
LEP:				
VAT	0.0910 (0.0716)	0.0895 (0.0729)	0.0927 (0.0710)	0.8712
WB	0.0638 (0.0301)	0.0616 (0.0258)	0.0664 (0.0346)	0.7870
LEPR:				
VAT	0.0514 (0.0678)	0.0469 (0.0712)	0.0565 (0.06435)	0.2326
WB	0.0203 (0.0133)	0.0197 (0.0111)	0.0211 (0.0197)	0.8174
FTO:				
VAT	0.0042 (0.0030)	0.0040 (0.0030)	0.0043 (0.0030)	0.6407
WB	0.0046 (0.0033)	0.0048 (0.0030)	0.0044 (0.0036)	0.2733

ADIPOQ: adiponectin; FTO: fat mass and obesity-associated gene; LEP: leptin; LEPR: leptin receptor; SD: standard deviation; VAT: visceral adipose tissue; WB: whole blood
a: the relative change in mRNA expression calculated using the (2−ΔΔCt) method.

The pregnant women with a BMI ≥25.0 kg/m^2 seemed to show an associated down-regulation in the mRNA expression of *ADIPOQ, LEP* and *LEPR* for both VAT and WB in comparison to the pregnant women with a BMI <25 kg/m^2. However, the pregnant women with a BMI ≥25.0 kg/m^2 had an associated down-regulation in the mRNA expression of FTO in VAT but there was an upregulation in WB in comparison to the pregnant women with a BMI <25 kg/m^2. Notably, there were no significant differences in mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* between the pregnant women with a BMI ≥ 25.0 kg/m^2 and the pregnant women with a BMI <25.0 kg/m^2. There were no significant differences in mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* between the HIV-infected pregnant women with a BMI ≥ 25.0 kg/m^2 and the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2 as well as no significant difference the HIV-infected pregnant women with a BMI < 25.0 kg/m^2 and the HIV-uninfected pregnant women with a BMI <25.0 kg/m^2. Likewise, there were no significant differences in mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* between the HIV-infected pregnant women with a BMI ≥ 25.0 kg/m^2 and the HIV-infected pregnant women with a BMI <25.0 kg/m^2 as well as no significant difference the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2 and the HIV-uninfected pregnant women with a BMI <25.0 kg/m^2.

Table 5.4: The relative change in mRNA expression of ADIPOQ, LEP, LEPR and FTO in pregnant black South African women in VAT and in WB, categorised according to BMI (kg/m^2)

Sample type	**Category**		***p*-value**
	Mean (SD)[a]	**Mean (SD)[a]**	

ADIPOQ			
	HIV-infected (n=30)	**HIV-uninfected (n=49)**	
VAT	0.2478 (0.4779)	0.2198 (0.2438)	0.7967
WB	0.1112 (0.0784)	0.2122 (0.0593)	0.6604
	< 25.0 HIV-infected (n=14)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.3530 (0.6918)	0.2638 (0.3247)	0.8387
WB	0.1274 (0.0895)	0.3590 (0.8503)	0.5209
	≥ 25.0 HIV-infected (n=16)	**≥ 25.0 HIV-uninfected (n=26)**	
VAT	0.1621 (0.0853)	0.0984 (0.0691)	0.9278
WB	0.0984 (0.0691)	0.0823 (0.0357)	0.9690
	≥ 25.0 HIV-infected (n=16)	**< 25.0 HIV-infected (n=14)**	
VAT	0.1621 (0.0853)	0.3530 (0.6918)	0.9834
WB	0.0984 (0.0691)	0.1274 (0.0895)	0.1190
	≥ 25.0 HIV-uninfected (n=26)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0984 (0.0691)	0.2638 (0.3247)	0.8178
WB	0.0823 (0.0357)	0.3590 (0.8503)	0.2577
LEP			
	HIV-infected (n=30)	**HIV-uninfected (n=49)**	
VAT	0.0839 (0.0679)	0.0954 (0.0741)	0.4517
WB	0.0600 (0.0247)	0.0662 (0.0330)	0.4764
	< 25.0 HIV-infected (n=14)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0957 (0.0717)	0.0910 (0.0721)	0.9750
WB	0.0557 (0.0224)	0.0730 (0.0393)	0.2340
	≥ 25.0 HIV-infected (n=16)	**≥ 25.0 HIV-uninfected (n=26)**	
VAT	0.0751 (0.0669)	0.0994 (0.0769)	0.2386
WB	0.0648 (0.0274)	0.0602 (0.0257)	0.8056
	≥ 25.0 HIV-infected (n=16)	**< 25.0 HIV-infected (n=14)**	
VAT	0.0751 (0.0669)	0.0957 (0.0717)	0.5747
WB	0.0648 (0.0274)	0.0557 (0.0224)	0.4176

	≥ 25.0 HIV-uninfected (n=26)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0994 (0.0769)	0.0910 (0.0721)	0.6667
WB	0.0602 (0.0257)	0.0730 (0.0393)	0.2662
LEPR			
	HIV-infected (n=30)	**HIV-uninfected (n=49)**	
VAT	0.0418 (0.0637)	0.0573 (0.070)	0.3713
WB	0.0190 (0.0118)	0.0212 (0.0141)	0.4337
	< 25.0 HIV-infected (n=14)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0405 (0.0539)	0.0693 (0.0144)	0.1635
WB	0.0199 (0.0144)	0.0218 (0.0163)	0.7901
	≥ 25.0 HIV-infected (n=16)	**≥ 25.0 HIV-uninfected (n=26)**	
VAT	0.0443 (0.0753)	0.0494 (0.0712)	0.9897
WB	0.0185 (0.0095)	0.0206 (0.0122)	0.5601
	≥ 25.0 HIV-infected (n=16)	**< 25.0 HIV-infected (n=14)**	
VAT	0.0443 (0.0753)	0.0405 (0.0539)	0.9834
WB	0.0185 (0.0095)	0.0199 (0.0144)	0.9834
	≥ 25.0 HIV-uninfected (n=26)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0494 (0.0712)	0.0693 (0.0144)	0.1895
WB	0.0206 (0.0122)	0.0218 (0.0163)	0.9920
FTO			
	HIV-infected (n=30)	**HIV-uninfected (n=49)**	
VAT	0.0035 (0.0024)	0.0046 (0.0032)	0.1336
WB	0.0048 (0.0030)	0.0045 (0.0029)	0.8995
	< 25.0 HIV-infected (n=14)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0037 (0.0025)	0.0047 (0.0032)	0.4245
WB	0.0042 (0.0035)	0.0045 (0.0037)	0.8387
	≥ 25.0 HIV-infected (n=16)	**≥ 25.0 HIV-uninfected (n=26)**	
VAT	0.0032 (0.0025)	0.0045 (0.0033)	0.1506
WB	0.0049 (0.0038)	0.0045 (0.0022)	0.9897

	≥ 25.0 HIV-infected (n=16)	**< 25.0 HIV-infected (n=14)**	
VAT	0.0032 (0.0025)	0.0037 (0.0025)	0.5747
WB	0.0049 (0.0038)	0.0042 (0.0035)	0.3175
	≥ 25.0 HIV-uninfected (n=26)	**< 25.0 HIV-uninfected (n=23)**	
VAT	0.0045 (0.0033)	0.0047 (0.0032)	0.8648
WB	0.0045 (0.0022)	0.0045 (0.0037)	0.4288

ADIPOQ: adiponectin; FTO: fat mass and obesity-associated gene; HIV: human immunodeficiency virus LEP: leptin; LEPR: leptin receptor; SD: standard deviation; VAT: visceral adipose tissue; WB: whole blood
a: the relative change in mRNA expression calculated using the (2−ΔΔCt) method.

The comparison of the analysis of ADIPOQ mRNA expression levels demonstrated in *Figure 5.1*, showed that the pregnant women with a BMI ≥25.0 kg/m^2 had a lower mRNA expression than compared to the pregnant women with a BMI <25.0 kg/m^2. The HIV infection showed to influence the mRNA expression, where for example in the VAT, HIV-infected pregnant women with a BMI <25.0 kg/m^2 had a lower mRNA expression than the HIV-uninfected pregnant women BMI <25 0 kg/m^2. The HIV-infected pregnant women with a BMI ≥25.0 kg/m^2 in VAT showed to have lower mRNA expression in comparison to the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2. Hence, individually overweight/obesity and HIV infection decreased the ADIPOQ mRNA expression in VAT, but the overweight and HIV infection coupled together had the greatest effect and resulted in the lowest mRNA expression pattern in the pregnant women.

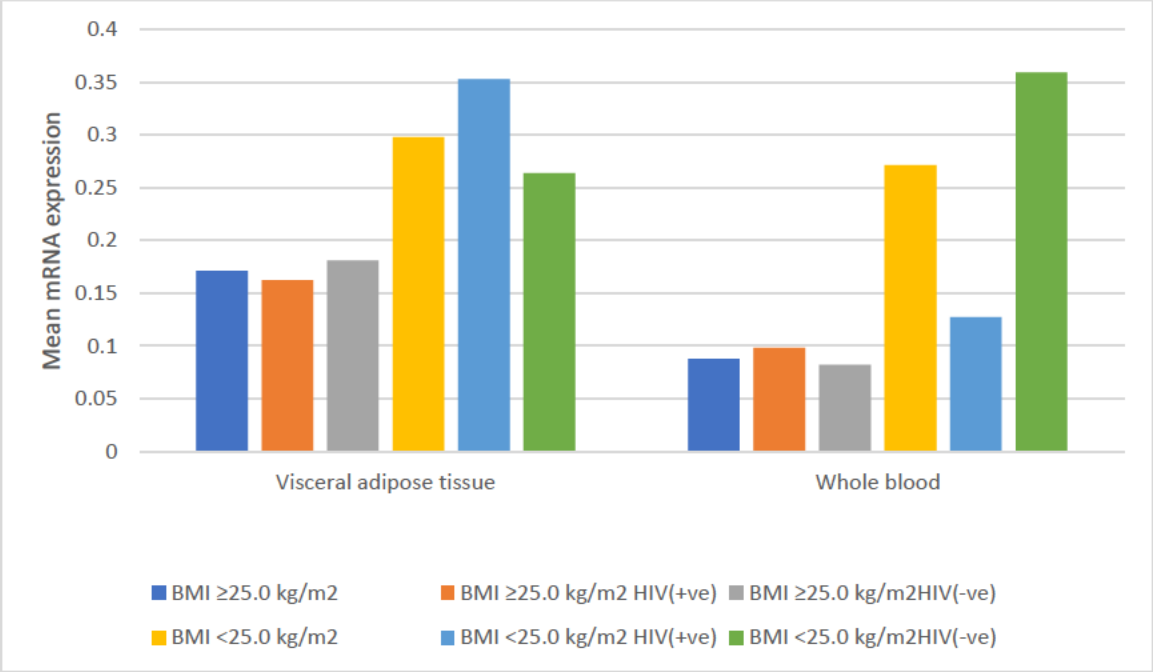

Figure 5.1: The comparison of the mean mRNA expression for ADIPOQ in pregnant black South African women in VAT and WB

The comparison of the analysis of LEP mRNA expression levels demonstrated in *Figure 5.2*, showed that the pregnant women with a BMI ≥25.0 kg/m^2 had a lower mRNA expression than compared to the pregnant women with a BMI <25.0 kg/m^2. The HIV infection showed to influence the mRNA expression, where for example in the WB, HIV-infected pregnant women with a BMI <25.0 kg/m^2 had a lower mRNA expression than the HIV-uninfected pregnant women BMI <25 0 kg/m^2. The HIV-infected pregnant women with a BMI ≥25.0 kg/m^2 in VAT showed to have lower mRNA expression in comparison to the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2. Hence, individually overweight/obesity and HIV infection decreased the LEP mRNA expression in VAT, but the overweight and HIV infection coupled together had the greatest effect and resulted in the lowest mRNA expression pattern in the pregnant women.

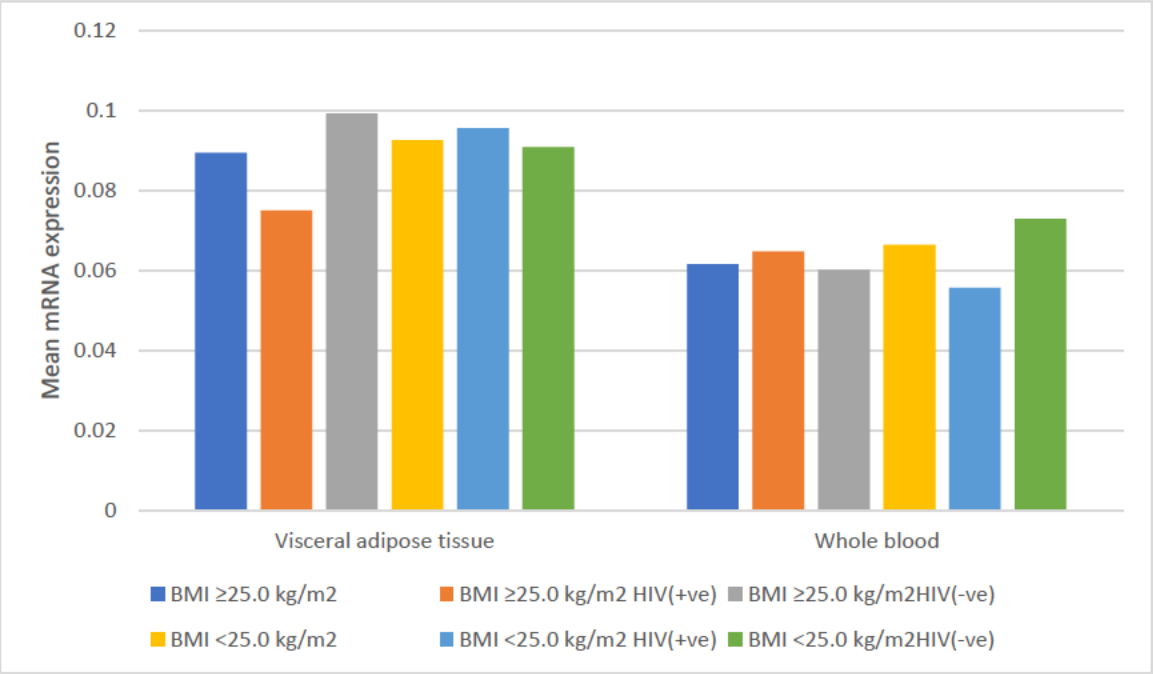

Figure 5.2: The comparison of the mean mRNA expression for LEP in pregnant black South African women in visceral adipose tissue and whole blood

The comparison of the analysis of *LEPR* mRNA expression levels demonstrated in *Figure 5.3*, showed that the pregnant women with a BMI ≥25.0 kg/m^2 had a lower mRNA expression than compared to the pregnant women with a BMI <25.0 kg/m^2. The HIV infection showed to influence the mRNA expression, where for example in the VAT, HIV-infected pregnant women with a BMI <25.0 kg/m^2 had a lower mRNA expression than the HIV-uninfected pregnant women BMI <25 0 kg/m^2. The HIV-infected pregnant women with a BMI ≥25.0 kg/m^2 in VAT showed to have lower mRNA expression in comparison to the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2. Hence, individually overweight/obesity and HIV infection decreased the *LEPR* mRNA expression in VAT, but the overweight and HIV infection coupled together had the greatest effect and resulted in the lowest mRNA expression pattern in the pregnant women.

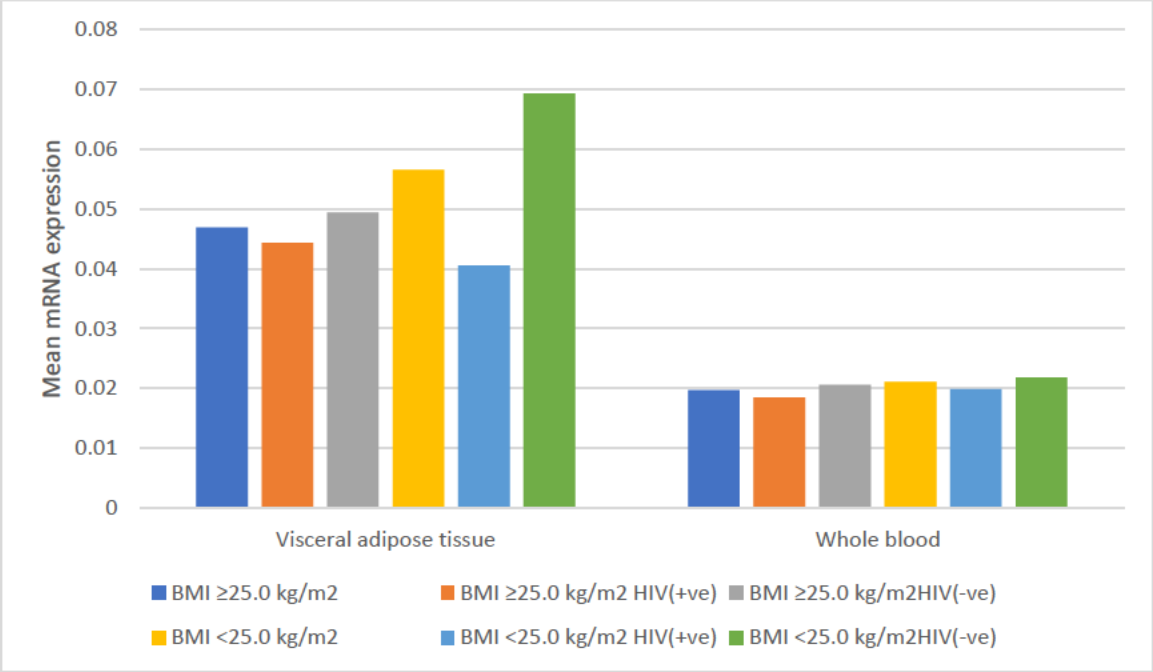

Figure 5.3: The comparison of the mean mRNA expression for LEPR in pregnant black South African women in visceral adipose tissue and whole blood

The comparison of the analysis of *FTO* mRNA expression levels demonstrated in Figure 5.4, showed that the pregnant women with a BMI ≥25.0 kg/m^2 had a lower mRNA expression than compared to the pregnant women with a BMI <25.0 kg/m^2. The HIV infection showed to influence the mRNA expression, where for example in the VAT, HIV-infected pregnant women with a BMI <25.0 kg/m^2 had a lower mRNA expression than the HIV-uninfected pregnant women BMI <25 0 kg/m^2. The HIV-infected pregnant women with a BMI ≥25.0 kg/m^2 in VAT showed to have lower mRNA expression in comparison to the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2.

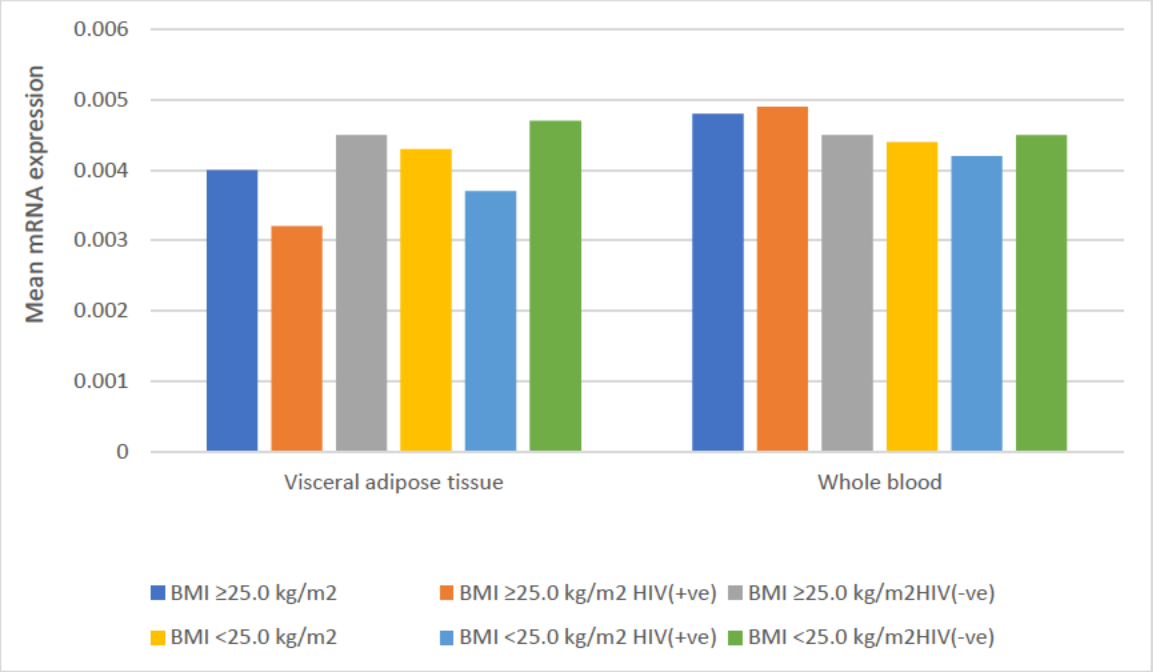

Figure 5.4: The comparison of the mean mRNA expression for FTO in pregnant black South African women in visceral adipose tissue and whole blood

Hence, individually overweight/obesity and HIV infection decreased the FTO mRNA expression in VAT, but the overweight and HIV infection coupled together had the greatest effect and resulted in the lowest mRNA expression pattern in the pregnant women.

Overall, the study findings identified that maternal BMI and HIV status in the pregnant women influenced the mRNA expression of *ADIPOQ*, *LEP*, *LEPR*, and *FTO*, as illustrated in *Figure 5.5*.

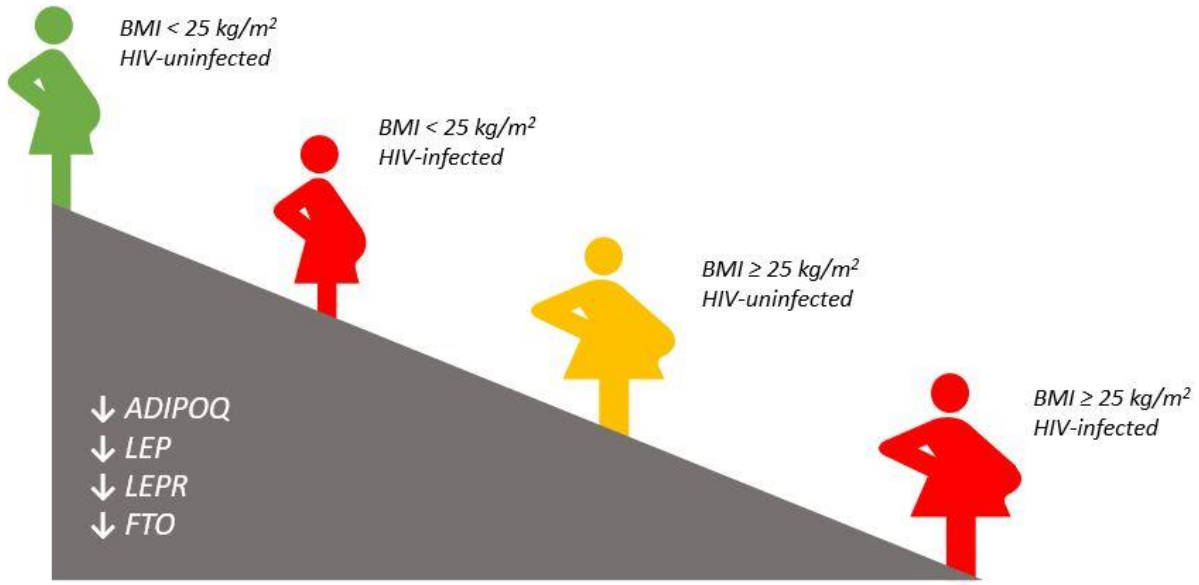

Figure 5.5: The degree of downregulation of mRNA expression of ADIPOQ, LEP, LEPR and FTO in black South African pregnant women based on BMI and HIV status

It was identified that the overall gene expression pattern in the pregnant women was a down-regulation in the mRNA expression of *ADIPOQ*, *LEP*, *LEPR*, and *FTO*, with the lowest mRNA expression identified in the HIV-infected pregnant women with a BMI ≥25.0kg/m^2.

5.5 Discussion

This study identified that maternal BMI and HIV status influenced the mRNA expression patterns in pregnant black South African women. Our findings add to the theory that the HIV infection and maternal overweight and/or obesity result in the dysregulation of metabolic processes, such as those illustrated in *Figure 5.6* **(**Orang, Safaralizadeh and Kazemzadeh-Bacili, 2014; Tessier and Ferraro, 2013; Terra, *et al*., 2010).

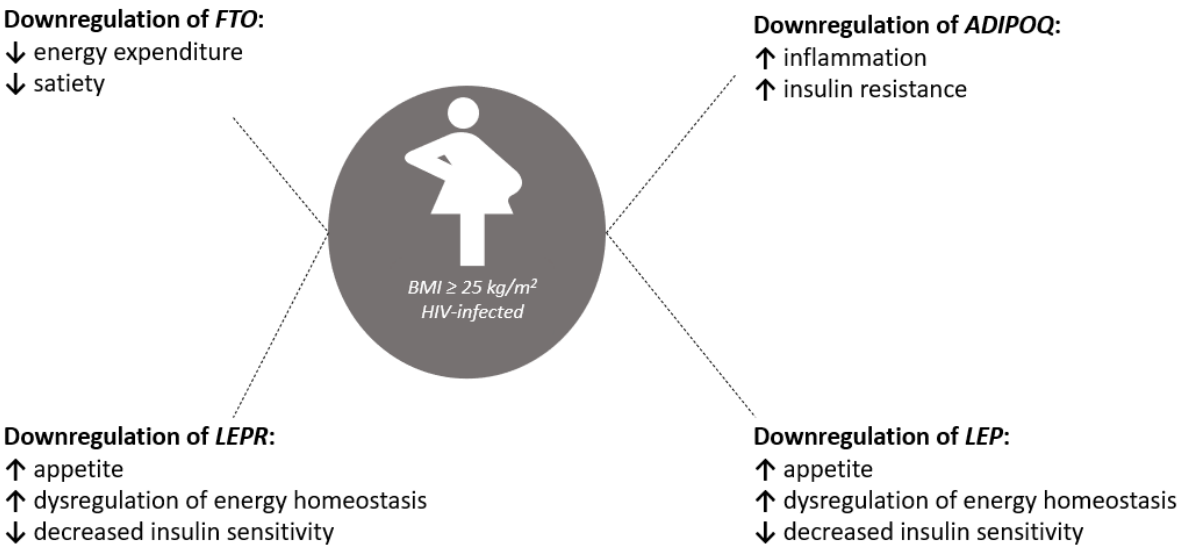

Figure 5.6: The metabolic disturbances that may result from having a BMI ≥25.0kg/m^2 and being HIV-infected during pregnancy

Therefore, the downregulation of *ADIPOQ*, *LEP*, *LEPR* and *FTO* in pregnant black South African women could be contributing factors to the metabolic disturbances that maintain and promote overweight and obesity in pregnancy within the context of the HIV infection and treatment thereof.

Focusing on the *ADIPOQ*, during pregnancy it is documented that during the first half of pregnancy *ADIPOQ* levels increase and then fall comparatively to the ratio of gestational weight gain, with the maternal adipose tissue being the primary source of *ADIPOQ* (Nogues, *et al*., 2019; Haghiac, *et al*., 2014). It has been documented that overweight and obesity in pregnancy are linked to the downregulation of *ADIPOQ* expression, with an increased *ADIPOQ* DNA methylation, with lower mRNA concentrations and hypoadiponectinemia (Haghiac, *et al*., 2014). Hypoadiponectinemia in pregnancy eventually contributes to pregnancy-induced insulin resistance which then plays a role in downregulating biological signals involved in preventing obesity (Haghiac, *et al*., 2014). Hence, our study's findings add to this picture, where the overweight and obese pregnant women displayed a downregulation pattern in the *ADIPOQ* mRNA expression in VAT and WB. In terms of the effect of the HIV infection has on *ADIPOQ* mRNA expression, this study has shown new evidence that the HIV infection, especially co-existing with overweight/obesity in pregnancy, leads to the downregulation of *ADIPOQ* mRNA expression in

the VAT and WB. The mechanism behind this could be linked to HIV infection driving the increased expression and secretion of pro-inflammatory cytokines e.g., TNFα, IL-6, and L-1β, which are responsible for causing changes to adipocyte function and a decreased expression and secretion of adipokines like *ADIPOQ* (Lagathu, *et al*., 2005). Hence, maternal overweight/obesity and HIV infection promote the downregulation in the mRNA expression of *ADIPOQ*, which may feed proinflammatory and insulin resistance pathways during pregnancy (Orang, Safaralizadeh and Kazemzadeh-Bacili, 2014).

During pregnancy *LEP* and its receptor *LEPR* play key regulatory roles in energy homeostasis and gestational weight gain (Henson, *et al*., 2000). Studies have reported that overweight and obesity in pregnancy are associated with the downregulation of *LEP* and *LEPR* which have an association with hyperleptinemia, accompanied by decreased hypothalamic *LEP* membrane-bound signalling receptors and decreased placental *LEP* membrane-bound signalling receptors leading to insulin resistance and the loss of signalling ability to satiety centres (Nogues, *et al*., 2019; Tessier and Ferraro, 2013). In consideration of the effect that the HIV infection has on pregnant women, it was identified in a South Africa study that HIV-infected pregnant women have a significantly reduced serum *LEP* levels in comparison the HIV-uninfected (Haffejee, *et al*., 2016). Therefore, maternal overweight and obesity in combination with the HIV infection, are conditions that cause the downregulation in *LEP* and *LEPR* and further promote metabolic pathways that increase appetite, encourage increased dietary intake, promote further excessive gestational weight gain, and eventually maintain a state of maternal obesity.

Little is known about the *FTO* gene expression in pregnancy within the context of HIV and maternal overweight and obesity. However, an obesity study conducted in obese women, identified that the *FTO* mRNA expression in subcutaneous adipose tissue was reduced in obese women compared to control subjects (Terra, *et al*., 2010). *FTO* has been highlighted by other studies to be involved in the regulation of *LEP/LEPR* pathways (Zhou, Hambly and McLachlan, 2017). Hence, *FTO* mRNA expression may also play a regulatory role in energy homeostasis, satiety, and appetite. In another study, *FTO* mRNA expression in subcutaneous adipose tissue was also found to be positively correlated to both circulating and mRNA expression of *ADIPOQ* (Terra, *et al*., 2010). Hence, *FTO* may have a regulatory influence on other obesogenic genes that mediate obesity in pregnancy. In our study, the HIV-infected overweight/obese pregnant women showed

to have a downregulatory pattern in *FTO* mRNA expression. Since *FTO* has a potentially regulatory effect on other obesogenic genes like *ADIPOQ*, *LEP* and *LEPR*, it could be deduced that the downregulation of *FTO* may further increase appetite, decrease satiety, and affect the energy expenditure in pregnant women, which then may encourage further gestational weight gain.

5.6 Conclusion

Pregnant black South African women presenting with overweight/obesity and being HIV-infected showed to have the worst downregulatory effect on mRNA expression of *ADIPOQ*, *LEP*, *LEPR*, and *FTO*. The downregulation of these genes may result in the dysregulation of metabolic pathways that usually control weight gain during pregnancy.

5.7 List of abbreviations

ABW: actual body weight

ADIPOQ: Adiponectin

ART: antiretroviral treatment

BMI: Body mass index

BREC: Biomedical Research Ethics Council

cDNA: Complementary deoxyribonucleic acid

CI: Confidence interval

EDTA: Ethylenediaminetetraacetic acid

FTO: Fat mass and obesity associated gene

GHRL: Ghrelin

HIV: Human immunodeficiency virus

KZNDOH: KwaZulu-Natal Department of Health

LEP: Leptin

LEPR: Leptin receptor

mRNA: Messenger ribonucleic acid

MW: Maternal weight

PMMH: Prince Mshiyeni memorial hospital

qRT-PCR: Quantitative real-time polymerase chain reaction

RNA: Ribonucleic acid

RT: Room temperature

SD: Standard deviation

SH: Standing height

UKZN: University of KwaZulu-Natal

VAT: Visceral adipose tissue

WB: Whole blood

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CHAPTER 6

6. Conclusion

6.1 Background

This cross-sectional study sought to investigate the predictive anthropometric measurements, associated factors, outcomes, and epigenetic factors involved in maternal overweight and obesity in HIV-infected and HIV-uninfected black South African pregnant women. From this study's findings conclusions and recommendations have been made about the anthropometrics, factors, maternal health outcomes and mRNA expression associated with overweight and obese pregnant women living with and without HIV.

6.2 Conclusions of the current study's findings

Overall, this study has given insight into maternal overweight and obesity in HIV-infected and HIV-uninfected pregnant women. A fundamental aspect of monitoring and assessing pregnant women is being able to determine whether the mother is nutritionally at risk or not. The most common method for determining this by using anthropometric measurements. In this study, maternal BMI was found to be statistically significantly correlated with alternative anthropometric measurements including MUAC, TSF, SSF, MAMC, WC, and frame size. Therefore, based on these findings H_{01} can be rejected because the alternative measurement methods were able to accurately predict the maternal BMI. These findings are potentially helpful for health care practitioners within the clinical setting, as it gives motivation for being able to use other methods to interpret nutritional status in pregnant women when BMI is not possible.

This study identified that the HIV-infected pregnant women had consistently lower anthropometric measurements compared to the HIV-uninfected pregnant women, however these differences were not statistically significant. Therefore, based on these findings H_{02} can be rejected. In our study, all the HIV-infected pregnant women were receiving ART throughout their pregnancy, and this might have been the reason for no significant differences between the HIV-infected and HIV-infected. Hence, the ART may have prevented wasting and catabolic processes that are usually associated with viral replication in untreated HIV-infected individuals. Despite the HIV-infected pregnant women with a BMI ≥25.0 kg/m^2 not being anthropometrically different to the HIV-uninfected pregnant women with a BMI ≥25.0 kg/m^2, our study identified that the HIV-infection

in combination with overweight/obesity in pregnancy was associated with factors, maternal health outcomes and genetic expression patterns.

In our study, we identified that there were two factors that were significantly associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women. Namely, maternal age and gestational age and for this reason H_{03} could be rejected. The gestational age was an expected association as pregnant women are known to gradually gain more weight as they progress through their pregnancy, but the maternal age was an interesting finding. Although our study found no association between maternal overweight/obesity and geriatric pregnancy, there was an association with increasing age, where older pregnant women were more likely to be overweight/obese. This age-associated weight gain might be linked to metabolic dysfunction regulated by epigenetic factors such as those mentioned in this study.

In our study, we identified that there were two maternal health outcomes that were significantly associated with overweight and obesity in both HIV-infected and HIV-uninfected pregnant black South African women. Namely, HPT disorders and anaemia and for this reason H_{04} could be rejected. The overweight/obese pregnant women were found to have a significantly decreased odds for anaemia during pregnancy. The exact reasons for this outcome were not covered in this study, but it could be due to possible dietary differences in the quantity of iron-rich foods compared to the pregnant women with a BMI <25.0kg/m^2. This study identified that overweight/obese pregnant women were more likely to have HPT disorders. This could be driven by vascular and metabolic changes that occur with excess gestational weight gain during pregnancy. Interestingly, the HIV-infected pregnant women with a BMI ≥25.0 kg/m^2 had a significantly increased odds for the HPT disorders during pregnancy in comparison to the HIV-uninfected pregnant women with a BMI, 25kg/m^2. The exact mechanisms behind this outcome have not been covered in this study but it may be due to the HIV infection and treatment thereof driving changes to maternal vascular system that lead to changes in blood pressure.

In our study, we identified that there were no significant differences between the different groups based on BMI and HIV status. However, there were downregulatory mRNA expression patterns identified for *ADIPOQ*, *LEP*, *LEPR* and *FTO* in overweight/obese pregnant black South African women, especially in the HIV-infected pregnant women with a BMI ≥25.0 kg/m^2. These patterns

indicated that BMI and HIV status may influence the metabolic activity of genes in VAT and WB, which then affect metabolic pathways. Hence, based on these findings H_{05} can be rejected.

6.3 Critique of the study

This study employed a cross-sectional study design, which is an observational study commonly used to distinguish the factors associated with diseases or outcomes (Tenny, Kerndt and Hoffman, 2021). Thus, based on the above and together with consideration of the current study's findings, various critiques were made.

6.3.1 Study strengths

For purpose of this study the following study strengths were identified:

i. A cross-sectional study design makes it possible to look at multiple risk factors at once such as exposures to HIV, physical exercise, dietary intake, smoking, drug abuse, alcohol, and socio-economic living conditions to identify those most likely associated with overweight/obesity in pregnancy (Tenny, Kerndt and Hoffman, 2021).
ii. The risk for anthropometric measurement error were decreased due to: (i) the same individual conducting all the measurements using the same techniques and equipment throughout the study; and (ii) all measurements were repeated three times and the closest two were averaged to the final measurement value (Ulijaszek and Kerr, 1999).
iii. All VAT and WB samples were collected, labelled, stored, processed, and analysed by the same individual. Therefore, allowing for consistency in the application of the study methodology.
iv. This study is a population-specific study, allowing the study findings to give a novel insight into the exposures and outcomes associated with overweight and obesity in pregnant black South African women.

6.3.2 Study limitations

For the purpose of this study the following study limitations were identified:

i. A cross-sectional study design is typically retrospective which means it can be used to establish a relationship between exposures and outcomes but cannot be used to establish causation (Wang and Chemg, 2020). Also, retrospective studies are linked to recall bias (Coughlin, 1990), which means that the pregnant women may have omitted information about exposures or were unable to recall information accurately.

ii. Although, BREC, DOH and UKZN approved that this study could be conducted at other hospital sites, logistically the main researcher could not be at more than one research site at a time.

6.3.3 Recommendations for the improvement of the study

For the purpose of improving future study designs the following recommendations were identified:

i. This study did not include teenage pregnancies (<18 years), including this group would give some insight into whether overweight and obesity is associated with teenage pregnancy.

ii. This study would benefit from including parameters like birth weight, because it will identify whether the maternal BMI may influence infant health outcomes.

iii. It would be beneficial to include other ethnic groups in the study design such as Whites, Indians, and Coloureds (mixed ancestry), because this would then give a better picture of the South African population.

iii. Larger studies be undertaken to validate the findings of this study that overweight and obese pregnant women indeed have different anthropometric measurements, risk profiles, and mRNA expression in comparison to the non-overweight/non-obese pregnant women.

6.4 Recommendations for clinical practice

Based on the findings of the current study, recommendations were made for health care professionals such as dieticians or medical doctors, who in clinical practice may treat and/or educate pregnant women. These recommendations include:

i. Surrogate anthropometric measurements such as the ones mentioned in this study, can be used as alternative measurements to BMI to assess nutritional status in pregnant women, especially when BMI is not possible.

ii. The age of pregnant women should be a considered an important assessment factor during the first antenatal visit or in pre-pregnancy planning education, because older pregnant women in our study were more likely to be overweight/obese.

iii. In this study overweight and obese pregnant women were less likely to be anaemic, therefore those that have a BMI of ≥25.0 kg/m^2 should be assessed for whether they require iron supplements or not.

iv. Pregnant women that have a BMI of ≥25.0 kg/m^2 should be routinely checked for HPT disorders, because in this study these pregnant women were more likely to have HPT disorders.

v. HIV-infected pregnant women that have a BMI of ≥25.0 kg/m^2 should be considered to have a high metabolic risk and should be routinely checked for HPT disorders, because in this study these pregnant should the lowest downregulatory pattern in the mRNA expression of *ADIPOQ*, *LEP*, *LEPR* and *FTO* as well as were more likely to develop HPT disorders.

6.5 Implications for further research

The recommendations made in this section are based on the overall results and conclusions of this study. Therefore, based on the study findings, it is recommended that:

i. The current study's methodology could be replicated in a larger sample size of pregnant black South African women. Furthermore, the results from this study could then be validated.

ii. The current study's methodology could be replicated and include pregnant women from different provinces within KwaZulu-Natal or extend to throughout South Africa.

6.6 Conclusion

In summary, this study has given insight into the predictive anthropometric measurements, associated factors, outcomes, and genetic factors involved in maternal overweight and obesity in HIV-infected and HIV-uninfected black South African pregnant women.

6.7 References

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CHAPTER 7

7. APPENDICES

7.1 Appendix 1

English questionnaire

SECTION A: QUESTIONNAIRE

1. **DEMOGRAPHICS**

Participant code:		Date:			
		Time start:		Time end:	

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
1a	What is your date of birth?	D D M M Y Y Y Y	
1b	What category does your age (years) fall under? *(please tick relevant category)*	1. 18 - 29 ☐ 2. 30 - 39 ☐ 3. 40 - 49 ☐ 4. 50 - 59 ☐ 5. 60 - 69 ☐ 6. 70 - 79 ☐ 7. ≥ 80 ☐	
2	What is your gender? *(please tick relevant category)*	1. Male ☐ 2. Female ☐	
3	What is your marital status? *(please tick relevant category)*	1. Single ☐ 2. Married ☐ 3. Divorced ☐	
4a	Are you currently employed? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 5
4b	What is your occupation, that is, what kind of work do you mainly do? *(please specify)*		

5	How many children and adults are living at home with you? *(please specify)*	1.	Children		
		2.	Adults		
		3.	Total NO.		
6	Where do you get your water from at home? *(please tick relevant category)*	1.	Tap (inside)		
		2.	Tap (outside)		
		3.	Pump		
		4.	River		
7	Where do you live? *(please tick relevant category)*	1.	Rural: live in the outskirts of town		
		2.	Urban: live in the city		

NO.	QUESTION AND FILTERS	CODING CATEGORY			SKIP
8	What is the main material your home is made of? *(please tick relevant category)*	1.	Plastic/cardboard		
		2.	Mud		
		3.	Mud and cement		
		4.	Corrugated iron/zinc		
		5.	Bare brick or cement block		
		6.	Plaster/finished		
		7.	Other		
9	How do you cook your food at home? (fuel source) *(please specify)*	1.	Electricity		
		2.	Paraffin		
		3.	Gas		
		4.	Wood		
10	What is your highest level of education completed? *(please tick relevant category)*	01.	Less than 1 year completed		
		02.	Sub A/ Grade 1		
		03.	Sub B/ Grade 2		
		04.	Standard 1/ Grade 3		
		05.	Standard 2/ Grade 4		
		06.	Standard 3/ Grade 5		
		07.	Standard 4/ Grade 6		
		08.	Standard 5/ Grade 7		
		09.	Standard 6/ Grade 8		
		10.	Standard 7/ Grade 9		

		11.	Standard 8/ Grade 10			
		12.	Standard 9/ Grade 11			
		13.	Standard 10/ Grade 12			
		14.	Further studies incomplete			
		15.	Diploma/ other post school completed			
		16.	Further degree complete			

2. HABITS AND LIFESTYLE

The next questions are about the time you spend doing different types of physical activities. This includes activities you do at home, at work, travelling from place to place and during your spare time. You are requested to answer the questions even if you don't consider yourself to be an active person.

2A. PHYSICAL ACTIVITY

Occupation-related physical activity (paid or unpaid work): When answering the following questions, think back over the past 12 months and consider (think of) a usual week:

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
11	Does your work involve mostly sitting or standing still, **or** walking for a very short periods (less than 10 minutes)? *(please tick relevant category)*	1. Mostly sitting ☐ 2. Mostly standing still ☐ 3. Mostly walking for very short periods ☐ 4. Mostly doing moderate/vigorous activity ☐ 5. None of the above ☐	→ 14 → 14 → 14
12a	Does your work involve vigorous activities (like heavy lifting, digging, or heavy construction) for **at least 10 minutes** at a time? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 13a
12b	In **a usual week**, how many days do you do vigorous activities as part of your work? *(please specify)*	Days ☐☐	
12c	On **a usual day** on which you do vigorous activities, how much time do you spend doing such work? *(please specify)*	1. Hours ☐☐ 2. Minutes ☐☐	
13a	Does your work involve moderate-intensity activities (like brisk walking or carrying light loads) for **at least 10 minutes** at a time? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 14
13b	In **a usual week**, how many days do you do moderate-intensity activities as part of your work? *(please specify)*	Days ☐☐	

13c	On **a usual day** on which you do moderate-intensity activities, how much time do you spend doing such work? *(please specify)*	1. Hours 2. Minutes	
14	How long is your usual work day? *(please specify)*	1. Hours 2. Minutes	

Travel-related physical activity: Other than activities that you're already mentioned, I would like to ask you about the way you travel to and from places (to work, to shopping, to market, to church, etc.):

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
15a	Do you walk or use a bicycle (pedal cycle) for **at least 10 minutes** at a time to get to and from places? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 16
15b	In **a usual week**, how many days do you walk or cycle for at least 10 minutes to get to and from places? *(please specify)*	Days ☐☐	
15c	On **a usual day**, how much time do you spend walking or cycling for travel? *(please tick relevant category)*	1. Hours ☐☐ 2. Minutes ☐☐	

Non-work related and leisure time physical activity: The next questions ask about activities you do in your leisure or spare time, for recreation or fitness. Do not include the physical activities you do at work or for travel already mentioned.

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
16	In your leisure or spare time do you do any vigorous or moderate-intensity physical activity lasting **more than 10 minutes** at a time? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 19
17a	In your leisure or spare time, do you do any <u>vigorous</u> activities (like running or strenuous sports, weightlifting) for **at least 10 minutes** at a time? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 18a
17b	In **a usual week**, how many days do you do <u>vigorous</u> activities as part of your leisure or spare time? *(please specify)*	Days ☐☐	
17c	How much time do you spend doing this on **a usual day**? *(please specify)*	1. Hours ☐☐ 2. Minutes ☐☐	

18a	In your leisure or spare time, do you do any moderate-intensity activities (like brisk walking, cycling, or swimming) for **at least 10 minutes** at a time? *(please tick relevant category)*	1. Yes 2. No	→ 19
18b	In **a usual week**, how many days do you do moderate-intensity activities as part of your leisure or spare time? *(please specify)*	Days	
18c	How much time do you spend doing this on **a usual day**? *(please specify)*	1. Hours 2. Minutes	

Sitting/ resting activity: Now I would like to ask you about the time spent sitting or resting, not including sleeping *in the past 7 days*. This may include time sitting at a desk, visiting friends, reading, or sitting down to watch television *during working hours and leisure spare time*.

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
19	Over the **past 7 days**, how much time did you spend sitting or reclining (lying) on **a usual day (excluding sleeping)**? *(Please specify)*	1. Hours 2. Minutes	

2B. TOBACCO USE

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
20a	Do you **currently smoke** any tobacco products, such as cigarettes, cigars, or pipes? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 23
20b	Do you currently smoke tobacco products **daily**? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 23
21a	How old were you when you **started** smoking daily? *(please tick relevant category)*	1. Years old ☐☐ 2. Do not remember/ not sure	→ 22
21b	Do you remember how long ago it was when you first **started** to smoke daily? *(please specify)*	1. Weeks ago ☐☐ 2. Months ago ☐☐ 3. Years ago ☐☐	
22	On average, how many of the following items do you smoke each day? *(please tick relevant category)* IF NONE, RECORD '00'	1. Manufactured cigarettes ☐☐ 2. Hand-rolled cigarettes ☐☐ 3. Pipes full of tobacco ☐☐ 4. Cigars/Cheroots/ cigarillos ☐☐	
23	**In the past**, did you ever smoke daily? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 25a
24a	**How old** were you when you **stopped** smoking daily? *(please specify)*	1. Years old ☐☐ 2. Do not remember/ not sure	→ 25a
24b	Do you remember how long it was when you **stopped** smoking daily? *(please specify)*	1. Weeks ago ☐☐ 2. Months ago ☐☐ 3. Years ago ☐☐	

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
25a	Do you **currently use any** smokeless tobacco, such as snuff or chewing tobacco?*(please tick relevant category)*	1. Yes 2. No	→ 27
25b	Do you currently use smokeless tobacco **daily**? *(please tick relevant category)*	1. Yes 2. No	→ 27
26	On average, how many times do you use each of the following items per day? *(please specify)* Snuff (by mouth)? Snuff (by nose)? Chewing tobacco? IF NONE, RECORD '00'.	1. Snuff (by mouth) 2. Snuff (by nose) 3. Chewing tobacco	→ 28a → 28a → 28a
27	**In the past**, did you ever use smokeless tobacco daily? *(please tick relevant category)*	1. Yes 2. No	
28a	Do you live in a house where other people smoke cigarettes regularly? *(please tick relevant category)*	1. Yes 2. No	
28b	Do you currently work in a job where other people smoke cigarettes around you? *(please tick relevant category)*	1. Yes 2. No	
28c	Have you ever worked in a job where you were regularly exposed to smoke, dust, fumes, or strong smells? *(please tick relevant category)*	1. Yes 2. No	→ 29a
28d	How long did you work in that job? *(please specify)* IF LESS THAN 1 YEAR, WRITE '00'	Years	
29a	Do you use recreational drugs?	1. Yes 2. No	→ 30a
29b	What type of drugs?	1. Dagga 2. Whoonga 3. Tik	

		4.	Others	

2C. ALCOHOL USE

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
30a	Have you ever consumed a drink that contains alcohol such as beer, wine, spirits, or sorghum beer? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 31a
30b	Was within the past 12 months? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 31a
31a	Do you drink home brewed alcohol? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	→ 35a
31b	What is it made from? *(please specify)*	☐	
32	In the **past 12 months, how frequently** have you had at least one drink? *(please tick relevant category)* READ ANSWER CATEGORIES TO RESPONDENT.	1. 5 or more days a week ☐ 2. 1-4 days per week ☐ 3. 1-3 days a month ☐ 4. Less than once a month ☐	
33a	When you drink alcohol, **on average**, how many drinks do you have during one day? *(please specify)*	1. Drinks ☐☐ 2. Don't know	
33b	During the **past 7 days**, how many standard drinks of any alcoholic drink did you have each day? *(please specify)* RECORD FOR EACH DAY IF NONE, RECORD '00'.	1. Monday ☐☐ 2. Tuesday ☐☐ 3. Wednesday ☐☐ 4. Thursday ☐☐ 5. Friday ☐☐ 6. Saturday ☐☐ 7. Sunday ☐☐	
34a	Have you ever felt that you should cut down on drinking? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	
34b	Have people annoyed you by criticizing your drinking? *(please tick relevant category)*	1. Yes ☐ 2. No ☐	

34c	Have you ever felt bad or guilty about your drinking? *(please tick relevant category)*	1.	Yes		
		2.	No		
34d	Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover? *(please tick relevant category)*	1.	Yes		
		2.	No		

2D. DIETARY INTAKE

Now, I would like to ask you some questions about the foods that you eat. There are no right or wrong answers so please feel free to give us your information as it is.

35. Which of the following do you **usually** eat? *(please tick relevant category)*

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
35a	Chicken/Poultry	1. With skin 2. Without skin 3. None	
35b	Red meat	1. Fatty meat 2. Lean meat 3. None	
35c	Spread: (Butter/ Margarine)	1. Butter 2. Hard margarine (brick) 3. Soft margarine (tub) 4. None	
35d	Milk/Milk products in powder form	1. Full cream 2. 2% or low fat 3. Skim/ Fat free 4. Blends 5. None	

36. How often do you **usually** eat the following? *(please tick relevant category)*

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
36a	Fried foods, e.g. chips, fish, potatoes, doughnuts, eggs	1. Occasionally/ Never 2. Weekly (at least once a week) 3. Daily	
36b	Chips, e.g. packet of 'Simba' chips or salty snacks	1. Occasionally/ Never 2. Weekly (at least once a week) 3. Daily	
36c	Processed meat e.g., polony, vienna, meat pies, sausage rolls	1. Occasionally/ Never 2. Weekly (at least once a week) 3. Daily	
36d	Sugar sweetened beverages such as: Juice concentrates, soft drinks, fruit drinks, sports and energy drinks, vitamin water drinks, flavoured iced tea, lemonade, flavoured milk, etc.	1. Occasionally/ Never 2. Weekly (at least once a week) 3. Daily	
36e	Sugar-containing foods such as: Biscuits, cake, desserts, chocolates, sweets, sweetened yoghurt; snack/energy/protein bars e.g. jungle oat bar; sweetened breakfast cereals e.g. Cocoa pops, Frosties, Fruit loops, Pronutro, etc.	1. Occasionally/ Never 2. Weekly (at least once a week) 3. Daily	
37a	Do you usually add table sugar to your serving **food** e.g. porridge, breakfast cereals? *(please tick relevant category)*	1. Yes 2. No	→ 38
37b	How many teaspoons do you **usually** add? *(please specify)*	Teaspoons	
38	Do you usually add a sugar alternative such as, honey, jam, or syrup to your serving food? *(please tick relevant category)*	1. Never 2. Sometimes 3. Always	

NO.	QUESTION AND FILTERS	CODING CATEGORY	SKIP
39a	Do you usually add table sugar to your **drink** e.g. tea, coffee? *(please tick relevant category)*	1. Yes 2. No	→ 40
39b	How many teaspoons do you **usually** add? *(please specify)*	Teaspoons	
40	Do you usually add/ use products containing artificial sweeteners? *(please tick relevant category)*	1. Yes 2. No	
41	Do you usually eat your food very salty, lightly salted or not salted? *(please tick relevant category)*	1. Very salted 2. Lightly salted 3. Not salted 4. Don't know	
42	Do you usually add salt or Aromat/Fonder to your serving of food? *(please tick relevant category)* IF YES, ASK: Before or after tasting food?	1. No, I never add salt/ aromat 2. Yes, but I taste first and then add 3. Yes, even before having tasted food 4. Don't know	
43	Do you eat salty snacks more often than three times per week (such as chips, niknaks, salted peanuts, salty biscuits, biltong, dried sausage, dried fish)? *(please tick relevant category)*	1. Yes 2. No	
END OF QUESTIONNAIRE THANK YOU			

SECTION B: FOOD FREQUENCY QUESTIONNAIRE

Participant code:		Date:	

We are interested in how often people eat certain kinds of foods. Now think about your food intake.

During the **past 7 days (1 week)**, did you eat any of the following?

If YES, ask how often. If NO, circle never. Do not read answer categories to respondent.

NO.	FOOD ITEM	NEVER	NOT DAILY		EVERY DAY			CODE
			1-3 TIMES PER WEEK	4-6 TIMES PER WEEK	1 TIMES A DAY	2 TIMES A DAY	3+ TIMES A DAY	
A1	Red meat (any type)	0	1	2	3	4	5	
B1	Chicken (any type)	0	1	2	3	4	5	
C1	Fish (tinned/fresh)	0	1	2	3	4	5	
D1	Organ meat e.g. liver, tripe	0	1	2	3	4	5	
E1	Eggs (any type)	0	1	2	3	4	5	
F1	Milk/ Maas/ Yoghurt/ Amahewu, to drink or on cereals	0	1	2	3	4	5	
G1	Milk in tea/coffee	0	1	2	3	4	5	
H1	Cheese (except cottage cheese)	0	1	2	3	4	5	
I1	Legumes, e.g. baked beans, lentils, green peas	0	1	2	3	4	5	
J1	Peanuts and nuts	0	1	2	3	4	5	
K1	Bread or rolls (Brown/ wholewheat/ White)	0	1	2	3	4	5	
L1	Steamed bread (uJeqe)	0	1	2	3	4	5	
M1	Breakfast cereal (instant, not cooked) e.g. cornflakes	0	1	2	3	4	5	

N1	Oat-porridge	0	1	2	3	4	5	
O1	Maize meal (supermarket/ home-grown)	0	1	2	3	4	5	
P1	Sorghum (maltabella)	0	1	2	3	4	5	
Q1	Samp	0	1	2	3	4	5	
R1	Rice (any type)	0	1	2	3	4	5	
S1	Pasta	0	1	2	3	4	5	
T1	Margarine (soft tub/brick)	0	1	2	3	4	5	
U1	Oil (sunflower, canola, olive)	0	1	2	3	4	5	
V1	Mayonnaise	0	1	2	3	4	5	
W1	Broccoli, cauliflower, brussel sprouts	0	1	2	3	4	5	
X1	Onions	0	1	2	3	4	5	
Y1	Cabbage	0	1	2	3	4	5	
Z1	Spinach and/or morogo/ imifino	0	1	2	3	4	5	
A2	Carrots	0	1	2	3	4	5	
B2	Tomato (raw/cooked)	0	1	2	3	4	5	
C2	Green beans	0	1	2	3	4	5	
D2	Green/yellow/red pepper	0	1	2	3	4	5	
E2	Mixed vegetables	0	1	2	3	4	5	
F2	Gem Squash	0	1	2	3	4	5	
G2	Pumpkin/ butternut	0	1	2	3	4	5	
H2	Sweet potato	0	1	2	3	4	5	
I2	Amadumbe	0	1	2	3	4	5	

J2	Potato (any preparation)	0	1	2	3	4	5	
K2	Citrus fruit, e.g. orange, grape fruit, lemons	0	1	2	3	4	5	
L2	Fruit juice (100%)	0	1	2	3	4	5	
M2	Bananas	0	1	2	3	4	5	
N2	Mangoes	0	1	2	3	4	5	
O2	Berries	0	1	2	3	4	5	
P2	Grapes (white/red)	0	1	2	3	4	5	
Q2	Apples	0	1	2	3	4	5	
R2	Pears	0	1	2	3	4	5	
S2	Avocado	0	1	2	3	4	5	
T2	Garlic	0	1	2	3	4	5	
U2	Ginger	0	1	2	3	4	5	
V2	Chilli	0	1	2	3	4	5	

SECTION C: MEDICAL INFORMATION

Participant code:		Date:	

NO.	CATEGORY	SUBJECT INFORMATION			
		TOPIC			CODE
A	**Admission Diagnosis** *(please specify)*				
B	**Co-morbidities** IF NONE, RECORD '00'.	1.	Diabetes Mellitus (type 1, 2 or Gestational)		
		2.	CVD	Hypertension	
				Hyperlipidaemia	
				Hx of CVA/ MI	
		3.	Arthritis		
		4.	Cancer		
		5.	COPD		
		6.	Other		
C	**RVD status** *(please tick relevant category)*	1.	Positive not on HAART		
		2.	Positive on HAART		
		3.	Negative		
		4.	Unknown		
		5.	Does not want to disclose		
D	**Drugs** IF NONE, RECORD '00'.	1.	Insulin		
		2.	Statins		
		3.	Anti-hypertensive		
		4.	Hormone Replacement Therapy (HRT)		
		5.	Recreational drugs		
		6.	Anti-pyretic e.g. ibuprofen, aspirin, paracetamol		

SECTION D: BIOCHEMISTRY

Participant code:		Date:	

INDICATOR	ABBREVIATION	DATE	VALUE IF NONE AVALIABLE, RECORD '0000'			
Albumin (g/L)	Alb					
Total Protein (g/L)	TP					
Urea (mmol/L)	BUN					
Creatinine (umol/l)	Cr					
C-reactive protein (mg/dL)	CRP					
Haemoglobin (g/dl)	Hb					
Total Cholesterol (mmol/l)	TC					
Triglycerides (mmol/l)	TG					
High density lipoprotein (mmol/l)	HDL					
Low density lipoprotein (mmol/l)	LDL					
Absolute CD4 (cells/ul)	CD4					
Viral Load (copies/ml)	VL					
Glycosylated Hb (%)	HbA1c					
Thyroid Function (thyroid stimulating hormone) (mIU/L)	TSH					
Serum cortisol (ug/dL)	-					
Surgical Notes:						

SECTION E: ANTHROPOMETRIC ASSESSMENT

Participant code:		Date:	

TYPE:	UNIT OF MEASUREMENT	MEASUREMENT			
		1	2	3	Final (average)
Height	Centimetres (cm)				
Actual Body Weight	Kilograms (kg)				
Weight History	Pre-pregnancy weight:				
Body Mass Index (BMI)	Kg/m^2				
MUAC (left)	Centimetres (cm)				
MUAC (right)	Centimetres (cm)				
Wrist circumference (right)	Centimetres (cm)				
Tricep skin fold	Millimetres (mm)				
Sub-scapular skin fold	Millimetres (mm)				
INTERPRETATION/ NOTES:					

7.2 Appendix 2

isiZulu Questionnaire

INGXENYE A: IMIBUZO

1. **IMININGWANE YOMUNTU**

nombolo yobambe qhaza:		Usuku:			
			Isikhathi sokuqala:		Isikhathi sokuqeda

NO.	IMIBUZO	ISIGABA SOKUKHETHA INIMBOLO	YEQA
1a	Lunini usuku lwakho lokuzalwa?	D D M M Y Y Y Y	
1b	Iminyaka yakho ingena kusiphi isigaba? *(khetha kuleminyaka)*	1. 18 - 29 2. 30 - 39 3. 40 - 49 4. 50 - 59 5. 60 - 69 6. 70 - 79 7. ≥ 80	
2	Yini ubulili bakho? *(Khetha)*	1. Owesilisa 2. Owesifazane	
3	Sithini isimo sakho somshado? *(Khetha)*	1. ungawedwana 2. Ushadile 3. Uhlukanisile	
4a	Engabe ungumsebenzi? *(Khetha)*	1. Yebo 2. Cha	→ 5

4b	Yini umsebenzi wakho, yini oyenza embebenzini wakho?*(cacisa)*		
5	Bangaki abantwana nabantu abadala abahlala endlini yakho? *(cacisa)*	1. Izingane 2. Abadala 3. Sebebonke	
6	Niwathola kuphi amanzi asekhaya? *(khetha)*	1. Umpompi ongaphakathi 2. Umpompi ongaphandle 3. Isigayo 4. Umfula	
NO.	**IMIBUZO**	**ISIGABA SOKUKHETHA INIMBOLO**	**YEQA**
7	Uhlala uphi? (*khetha)*	1. Emakhaya ngaphandle kwedoloba 2. Edolobheni	
8	Wakhiwe ngani umuzi wakini? *(khetha)*	1. Ipulangu/uplastiki 2. Udaka 3. Udaka no simende 4. Uthayela 5. Izitina ezinga valive ngosimende 6. Izitina zika simende ezivalwe ngo simende 7. Okunye	
9	Nisebenzisa ini uma nipheka ? *(cacisa)*	1. Ugesi 2. upharafini 3. Isigubhe segesi 4. izinkuni	

10	Athini amabanga akho emfundo yakho yokugcina? *(khetha)*	01.	Ngaphansi konyaka		
		02.	Ufestiya/Ibanga 1		
		03.	Usekhendiya/Ibanga 2		
		04.	Standadi 1/ Ibanga 3		
		05.	Standadi 2/ Ibanga 4		
		06.	Standadi 3/ Ibanga 5		
		07.	Standadi 4/ Ibanga 6		
		08.	Standadi 5/ Ibanga 7		
		09.	Standadi 6/ Ibanga8		
		10.	Standadi 7/ Ibanga 9		
		11.	Standadi 8/ Ibanga10		
		12.	Standadi 9/ Ibanga 11		
		13.	Standadi10/ Ibanga 12		
		14.	Izifundo zemfundo ephakeme aziphelele		
		15.	Idiploma noma isitifiketi emva kwamatikula etsheni		
		16.	Idigri nokungaphezulu		

2. IMIKHUBA NENDLELA YOKUPHILA

Imibuzo elandelayo imayelana nesikhathi esisetshenziswayo ekwenzeni imisebenzi evocavoca umzimba. Lokhu kuhlanganisa imisebenzi oyenza ekhaya, emsebenzini, ukuhamba izindawo ngezindayo kanye nangesikhathi sakho esingenamsebenzi. Uyacelwa ukuba uphendule imibuzo noma ungesuye umuntu omatasatasa

2A. Umsebenzi obandakanya umzimba

Umsebenzi obandakanya umzimba (ohlangene nomsebenzi wakho owuqashelwe) (kungaba okhokhayo noma ongakhokhi): Uma uphendula lemibuzo elandelayo, phendula ngokucabangela ezinyangeni eziyi shuminambili ezedlule. Uphinde ucabangele ukuthi wenza njani evikini.

NO.	IMIBUZO	ISIGABA SOKUKHETHA INAMBA	YEQA
11	Ngabe umsebenzi wakho ubandakanya ukuhlala kakhulu, noma ukuma kakhulu? **Okanye** ukuhamba isikhathi esincane. (ngaphansi kwemizuzu eyishumi)? *(khetha)*	1. Ukuhlala kakhulu 2. Ukuma endaweni eyodwa kakhulu 3. Ukuhamba izinkathi ezincane 4. Ukwenza umbenzi okahle noma ogqilazayo 5. Akukho engikukhethayo	→ 14 → 14 → 14
12a	Ngabe umsebenzi wakho uyagqilaza (njengokuphakamisa izinto ezisindayo, ukugubha kanye nokusebenza ezinkontilakeni kanzima) imizuzu eyishumi okungenani? *(khetha)*	1. Yebo 2. Cha	→ 13a
12b	**Ngevik**i, uwenza kangaki umsebenzi ogqilazayo, njengomsebenzi wakho wasemsebenzini? *(cacisa)*	Izinsuku	
12c	**Ngosuku** owenza ngalo umsebenzi ogqilazayo,uwenza isikhathi esingakanai? *(cacisa)*	1. Amahora 2. Imizuzu	
13a	Ngabe umsebenz wakho ubandakanya umsebenzi onzima no nzima kakhulu (<u>njengoku gxala okukhulu nokuphakamisa izinto ezisindayo</u>) **imizuzu eyishumi** okungenani *(khetha)*	1. Yebo 2. Cha	→ 14

13b	**Ngeviki**, uwenza kangaki umsebenzi onzima kuya konzima kakhulu *(cacisa)*	Izinsuku					
13c	**Ngosuku** owenza ngalo imisbenzi enzima, uwenza isikhathi esingakanani? *(cacisa)*	1.	Amahora				
		2.	Imizuzu				
14	Uthatha isikhathi eingakanani umsebenzi wakho ngelanga? *(cacisa)*	1.	Amahora				
		2.	Imizuzu				

Umsebenzi wamabanga amade obandakanya ukusebenza komzimba: Ngaphandle kwalomsebenzi osuwushilo, sicela ukubuza mayelana nendlela oyisebenzisayo uma uhamba amabanga amadenyana. (Uma uya emsebenzini, uya ezitolo, emakethe, esontweni kanjalo.

NO.	IMIBUZO	KHETHA INAMBA	YEQA
15a	Ingabe usebenzisa ibhayisikili uma uhamba izindawo ngezindawo imizuzu eyishumi okungenani? *(khetha)*	1. Yebo 2. Cha	→ 16
15b	**Ngeviki**, uhamba izinsuku ezingaki noma ulisebenzisa kangaki ibhayisikili uma uya ezindaweni, imizuzu eyishumi okungenanai ? *(Cacisa)*	Izinsuku	
15c	**Ngosuku**, Singakanani isikhathi osisebenzisa endleleni uma uhamba noma ugibele ibhayisikili? *(khetha)*	1. Amahora 2. Imizuzu	

Ukusebenza komzimba okungahlangene nomsebenzi owuqashelwe: Imibuzo elandelayo ibuza mayelana nokwenzayo ngesikhathi sakho esiseceleni. Sibuza mayelana nezemidlalo kanye nokujima. Uyacelwa ukuti ungahlanganisi ukusebenza komzimba okumayelana nomsebenzi owuqashelwe

NO.	IMIBUZO	KHETHA INAMBA	YEQA
16	Ngesikhathi sakho esiseceleni, ngabe uba matasatasa ngokusezingeni noma kanzima noma, okungenani imizuzu eyishumi? *(khetha)*	1. Yebo 2. Cha	→ 19
17a	Ngesikathi sakho esiseceleni ngabe uba matasa ngokugqilazayo (njengokugijima, ezemidlalo ezicindezelayo, ukuphakamisa izinsimbi) okungenani imizuzu eyishumi? *(khetha)*	1. Yebo 2. Cha	→ 18a
17b	**Ngeviki**, zingaki iizinsuku ozivocavoca ngazo kanzima usebenzisa isikhathi sakho esiseceleni? *(cacisa)*	Izinsuku	
17c	Ngosuku, singakanani isikhathi osisebenzisela ukuzivocavova, ngaso lesisikhathi esiseceleni? *(khetha)*	1. Amahora 2. Imizuzu	

18a	Ngesikhathi sakho esiseceleni, uyawenza umsebenzi wokuzivocavoca onzima (njengokugxala kakhulu, ukushova ibhayisikili, noma ukubhukuda. Okungenani imizuzu esishumi? *(khetha)*	1. Yebo 2. Cha	→ 19
18b	**Ngeviki**,zingaki izinsuku ozisebenzisayo uma wenza umsebenz wokuzi vocavoca unzima ngalesisikhathi sakho esisieceleni? *(cacisa)*	Izinsuku	
18c	**Ngosuku,** singakanani isikhathi osisebenzisela lokhu kuzivocavoca? *(cacisa)*	1. Amahora 2. Imizuzu	

Ngesikhathi sokungcebeleka nesokuhlala: Uyacelwa ukuba uphendule ngesikathi osisebenzisela ukuphumula noma uzihlalele, asikubali ukulala (ezinsukwini eziyisikhombisa ezendlule). Lokhu kungahlanganisa isikhathi osisebenzisa uhleli edeskini, uvakashele abangani, ufunda, ubuka umabona kude ngesikhathi sokusebenza noma lesi esiseceleni.

NO.	IMIBUZO	KHETHA INAMBA	YEQA
19	**ezinsukwini eziyisikhombisa ezendlule**, singakanani isikhathi osisebenzise uhleli noma ucambalele ngosuku **(Ngaphandle kokulala)**? *(cacisa)*	1. Amahora 2. Imizuzu	

2B. UKUSETSHENZISWA KO GWAYI

NO.	IMIBUZO	KHETHA INAMBA	SKIP
20a	Kulenkathi yamanje, ngabe uyabhema ugwayi? *(khetha)*	1. Yebo 2. Cha	→ 23
20b	Kulenkathi yamanje, ngabe uwubhema njalo ugwayi? *(khetha)*	1. Yebo 2. Cha	→ 23
21a	Wawungakanani mhlamane uqala ukubhema njalo? *(khetha)*	1. Iminyaka 2. Angisakhumbuli	→ 22
21b	Usakhumbula ukuth sekuyisikhathi esingakanani selokhu waqala ukubhema njalo? *(cacisa)*	1. Emavikini endlule 2. Ezinyangeni ezindlule 3. Eminyakeni endlule	
22	Ngokuphelele, ubhema ogwayi abangaki ngelanga kulaba *(khetha)* Uma kungekho, faka u"00"	1. iluzi 2. Ugwayi ogoqwayo 3. Ugwayi wepipi 4. Igudu	
23	**Enkathini endlule,** usake wabhema njalo? *(khetha)*	1. Yebo 2. Cha	→ 25a
24a	**Wawuneminyaka** emingaki mhlamane uyeka ukubhema? *(cacisa)*	1. Iminyaka 2. Angisakhumbuli kahle	→ 25a
24b	Usakhumbula ukuth kwakuyisikhathi esingakanani mhlamane uyeka ukubhema njalo?	1. Emavikini endlule	

	(cacisa)	2.	Ezinyangeni ezendlule				
		3.	Eminyakeni eyendlule				

NO.	IMIBUZO	KHETHA INAMBA	YEQA
25a	Ngabe uyawubhema ugwayi ongena ntuthu, onjenge sinenfu kanye nohlafunwayo?*(khetha)*	1. Yebo 2. Cha	→ 27
25b	Ngabe ubhema ugwayi ongenantuthu njalo nje? *(khetha)*	1. Yebo 2. Cha	→ 27
26	Ngokuphelele? Lungaki loluhlobo lukagwayi olusebenziyayo ngosuku *(cacisa)* Isinemfu(Ngomlomo)? Inisenemfu(ngekhala? Ugwayi ohlafunwayo? Uma ungekho faka '00'.	1. Isinemfu (ngomlomo) 2. Isinemfu (ngekhala) 3. Ugwayi ohlafunywano	→ 28a → 28a → 28a
27	**Enkathini eyandlula**, usake wabhema ugwayi ongena nthuthu? *(khetha)*	1. Yebo 2. Cha	
28a	Ngabe uhlala endlini enabantu abahlala bebhema ugwayi? *(khetha)*	1. Yebo 2. Cha	
28b	Ngabe emsebenzini wakho uhlala nabantu ababhema ugwayi ebukhoneni bakho? *(khetha)*	1. Yebo 2. Cha	
28c	Usake wasebenza endaweni lapho kwakunesi mokwe, izintuli, intuthu kanye nephunga elinamandla *(khetha)*	1. Yebo 2. Cha	→ 29a
28d	Kunesikhathi esingakanani waqala ukusebenza kulowo msebenzi? *(cacisa)* Uma kungaphansi konyaka bhala '00'	Iminyaka	
29a	Uyazisebenzisa izidakamizwa zenjongo yokuzijabulisa?	1. Yebo 2. Cha	→ 30a

29b	Yiluphi lolo hlobo lwezidakamizwa?	1.	Insangu				
		2.	iwunga				
		3.	Tik				
		4.	Ozinye				

2C. UKUPHUZWA KUKATSHWALA

NO.	IMIBUZO	KHETHA INAMBA	YEQA
30a	Usake waphuza utshwala obunjenge bhiya, iwayini, umqombothi noma ugologo? *(khetha)*	1. Yebo 2. Cha	→ 31a
30b	Ngabe lokho kwenzeka ezinyangeni eziyishumi ezendlule? *(khetha)*	1. Yebo 2. Cha	→ 31a
31a	Uyawuphuza utshwala owenziwe ekhaya? *(khetha)*	1. Yebo 2. Cha	→ 35a
31b	Wenziwe ngani? *(cacisa)*		
32	Ezinyangeni eziyi 12 ezendlule**, ubuphuza kangakanani** *(cacisa)*	1. Izinsuku eziyi 5 ngeviki 2. 1-4 wezinsuku ngeviki 3. 1-3 wezinsuku ngenyanga 4. Kanye ngenyanga	
33a	Uma uphuza utshwala, **ngokuphelele**, uphuza amabhodlela amangaki ngelanga? *(khetha)*	1. amabhodlela 2. Angazi	
33b	Ezinsukwini eziyi-7 ezendlule, ubuphuza kangakanani ngosuku *(cacisa)* Bhala ngosuku Uma ungekho faka '00'.	1. Msombuluko 2. Lwesibili 3. Lwesithathu 4. Lwesine 5. Lwesihlanu 6. Mgqibelo 7. Sonto	

34a	Uke wazizwa ngathi kumele wehlise kancane eziphuzweni? *(khetha)*	1.	Yebo		
		2.	Cha		
34b	Ngabe uke wacasuka uma abantu bekugxeka indlela ophuza ngayo? *(khetha)*	1.	Yebo		
		2.	Cha		
34c	Ngabe usake waphathwa unembeza ngikuhuza kwakho? *(Khetha)*	1.	Yebo		
		2.	Cha		
34d	Ngabe usake waphuza utshwala ekuseni ungakadli ukuze uzoqeda ibhabhalazi? *(khetha)*	1.	Yebo		
		2.	Cha		

2D. KWEZOKUDLA

Uzobuzwa imibuz mayelana nokudal okudlayo. Sicela uphendule ngokukhululeka ngoba ayikho impendulo esigigxekayo nesithi ilungile, sithatha zonke izimpendulo.

35. Kulokhu okulandelayo, yikuphi **ojwayele** ukukudla? *(khetha)*

NO.	IMIBUZO	KHETHA INAMBA	YEQA
35a	Inyama yenkukhu	1. enesikhumba 2. engenasikhumba 3. Cha akukho	
35b	Inyama ebomvu	1. Enamafutha 2. Engenamafutha 3. Akukho	
35c	Okokugcoba isinkwa	1. Ibhotela 2. Imajarini (yesitina) 3. imajarina (esesitsheni) 4. Akukho	
35d	Ubisi kanye nobisi oluyi mpuphu	1. Oluno khilimu 2. 2% noma olunamafutha amancane 3. olungenamafutha 4. Uyahlanganisa 5. Cha akukho	

36. Ujwayele ukukudla kangakanani lokhu okulandelayo? *(khetha)*

NO.	IMIBUZO	KHETHA INAMBA	YEQA
36a	Ukudla okuthosiwe, e.g. amashibsi, ufish, amazambane, amagwinya, amaqanda	1. Gqwagqwa/angikaze 2. Okungenani kanye ngeviki 3. Usuku nosuku	
36b	Ama ships ngenama snekisi, simba chips	1. Gqwagqwa/angikaze 2. Okungenani kanye ngeviki 3. Usuku nosuku	
36c	Inyama esetshenziwe e.g. pholoni, viyenna, ama soseji	1. Gqwagqwa/angikaze 2. Okungenani kanye ngeviki 3. Usuku nosuku	
36d	<u>Iziphuzo ezinoskuhleka</u> njenge: Jusi, udilinki, udikinki wezemidlalo, lemonade,	1. Gqwagqwa/angikaze 2. Okungenani kanye ngeviki 3. Usuku nosuku	
36e	<u>Ukudla okunoshukela</u> njenge: amakhekhe, uswidi, ushokoledi, yogathi; Kanye nokudla kwasekuseni okunjenge phalishi, oats.	1. Gqwagqwa/angikaze 2. Okungenani kanye ngeviki 3. Usuku nosuku	
37a	Ngabe uyengeza ushukela ekuseni uma udla iphalishi, oats kanye necereals? *(khetha)*	1. Yebo 2. Cha	→ 38
37b	Wengenza ngezipuni ezincane ezingaki (teaspoon)? *(cacisa)*	Izipuni ezinace	

38	Ngabe kuyenzeka wengeze ngezinto ezinjengoju, ujamu esikhundleni sokufaka ushukela? *(khetha)*	1.	angikaze				
		2.	Kwesinye isikhathi				
		3.	Njalo nje				

NO.	IMIBUZO	KETHA INAMBA	YEQA
39a	Ngabe uyawufaka ushukela etiyeni noma ekhofini lakho *(khetha)*	1. Yebo 2. Cha	→ 40
39b	Ufaka izipuni ezincane ezingaki ? *(khetha)*	Teaspoons (izipuni ezincane)	
40	Ngabe udla izidlo ezikhiqizwa sezinoshukela wazo? *(khetha)*	1. Yebo 2. Cha	
41	Ngabe ukudla kwakho ukudla kunetswayi eliningi, itswayi elikahle noma okungenatswayi? *(khetha)*	1. Itswayi eliningi 2. Itswayi elincane 3. Kungena tswayi 4. angazi	
42	Ngabe ujwayele ukwengeza itswayi noma I aromethi ngaphambi kokuthi udle? *(khetha)* Uma uthi yebo, ngabe ukwenz alokho ngaphambi kokuzwa ukudla kwakho?	1. Cha angengezi itswayi 2. Yebo, kodwa ngiqala ngokuzwa kuqala 3. Yebo noma ngingakakuzwa ukudla 4. angazi	
43	Ngabe ujwayele ukudla amasinekisi ano tswayi (njengama chips, niknaks, amakinati anetswayi. Amakhekhe anetswayi, umqebu, amasoseji omile, inhlanzi eyomile)? *(khetha)*	1. Yebo 2. Cha	

UKUPHELA KWEMIBUZO

SIYABONGA

INGXEYE B: IMIBUZO EMAYErLANA NEMVAMISA ODLA NGAYO

Inamba yobambe iqhaza:		Usuku:	

Sinentshisekelo yokwazi ngemvamisa abantu abakudla ngayo ukudla kwabo. Manje cabanga indlela odla ngayo.

Ezinsukwini **eziyi 7 ezedlule (Iviki elilodwa)**, kukhona owakudla kulokhu okulandelayo?

Uma uthi YEBO, phendula nokuth kangakani, uma uthi CHA, kekelezela iqanda elingaphasi kuka "ANGILOKOTHI".

NO.	UKUDLA	ANGILOKOTHI	HHAYI NJALO		NJALO			IKHODI
			KA 1-3 NGEVIKI	KA 4-6 nGEVIKI	KANYE NGOSUKU	KA 2 NGOUKU	KA 3+ NGOSUKU	
A1	Inyama ebomvu (noma iyiphi)	0	1	2	3	4	5	
B1	Inyama yenkukhu (noma iyiphi)	0	1	2	3	4	5	
C1	Inhlanzi esethinini	0	1	2	3	4	5	
D1	Inyama eyisitho somzimba,njesibingi, izinso	0	1	2	3	4	5	
E1	Amaqanda (noma yiliphi)	0	1	2	3	4	5	
F1	Uyaphuza noma uyadla Ubisi/ Yoghathi/ amasi	0	1	2	3	4	5	
G1	Ubisi etiyeni/ekhofini	0	1	2	3	4	5	
H1	ushizi(ngaphandle kuka cothegi shizi)	0	1	2	3	4	5	
I1	Obhontshisi	0	1	2	3	4	5	
J1	amakinati	0	1	2	3	4	5	
K1	Isinkwa esimhklophe noma esinsundu	0	1	2	3	4	5	

L1	Ibhulakfesi (ngabe eliphekiwe noma ngelisheshayo	0	1	2	3	4	5	
M1	Ipalishi le oths	0	1	2	3	4	5	
N1	Imajarina ethambile	0	1	2	3	4	5	
O1	i-Bhroccoli, i-cauliflower, i-brussel sprouts	0	1	2	3	4	5	
P1	Spinashi nemi fino	0	1	2	3	4	5	
Q1	Izaqhatha	0	1	2	3	4	5	
R1	Utamatisi (oluhlaza/ophekiwe)	0	1	2	3	4	5	
S1	Upease oluhlaza	0	1	2	3	4	5	
T1	Ubhontshisi oluhlaza	0	1	2	3	4	5	
U1	Izitshalo ezihlangene	0	1	2	3	4	5	
V1	Ithanga	0	1	2	3	4	5	
W1	Ubatata	0	1	2	3	4	5	
X1	Izambane (noma lenziwe kanjani)	0	1	2	3	4	5	
Y1	Izithelo ezimuncwana, e.g. iwolinthsi, amagrebhisi	0	1	2	3	4	5	
Z1	Unjuice owenziwe nge wolintshi noma ugwava (onoshukela noma ongenawo)	0	1	2	3	4	5	
A2	Ubhanana	0	1	2	3	4	5	
B2	Umango	0	1	2	3	4	5	
C2	Ama-apula nama ganandoda	0	1	2	3	4	5	
D2	ukotapheya	0	1	2	3	4	5	

7.3 Appendix 3

BREC ethics approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

08 August 2018

Ms CR Lahner (210514170)
School of Laboratory Medicine and Medical Sciences
College of Health Sciences
lahner@ukzn.ac.za

Protocol: A genetic and epigenetic evaluation of obesity in a Black South African Audit.
Degree: PhD **BREC Ref No: BE269/18**

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 12 April 2018.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 18 July and 01 August 2018 to BREC letter dated 28 May 2018 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 08 August 2018. Please ensure that site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from **08 August 2018.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on **11 September 2018.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

Prof D Wassenaar
Deputy Chair: Biomedical Research Ethics Committee

cc postgraduate administrator: dudhrajhp@ukzn.ac.za
Supervisor: chuturr@ukzn.ac.za
Co investigators: Singhb3@ukzn.ac.za Nagiah.savania@gmail.com

Biomedical Research Ethics Committee
Professor V Rambiritch (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

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7.4 Appendix 4

BREC Ethics approval recertification

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

28 June 2019

Ms CR Lahner (210514170)
School of Laboratory Medicine and Medical Sciences
College of Health Sciences
lahner@ukzn.ac.za

Dear Ms Lahner

Protocol: A genetic and epigenetic evaluation of obesity in a Black South African Audit.
Degree: PhD
BREC Ref No: BE269/18

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	08 August 2019
Expiration of Ethical Approval:	07 August 2020

I wish to advise you that your application for Recertification received on 10 June 2019 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

Please add full BREC details to participant information sheet. See BREC template.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 July 2019.

Yours sincerely

[redacted]

Prof D Wassenaar
Acting Chair: Biomedical Research Ethics Committeec:

cc postgraduate administrator: [redacted] Supervisor: [redacted] Co investigators: [redacted]

7.5 Appendix 5

KZN DOH ethics approval

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

330 Langalibalele street,
Private Bag X9051 PMB, 3200
Tel: 033 395 2805/3189/3123 Fax: 033 394 3782
Email: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management (HKRM)

Reference: HRKM261 /18
KZ_201807_002

26 July 2018

Dear Ms C Lahner
(UKZN)

Subject: Approval of a Research Proposal

1. The research proposal titled **'A genetic and epigenetic evaluation of obesity in a Black South African adult population'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

   The proposal is hereby **approved** for research to be undertaken at Prince Mshiyeni Memorial & King Edward VIII Hospitals.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facilities before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Health Research Committee
Date: 30/07/18

Fighting Disease, Fighting Poverty, Giving Hope

7.6 Appendix 6

PMMH ethics approval

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE: Senior Medical Manager

Mangosuthu Highway, Private Bag X 07
MOBENI
Tel: 031 907 8317/8304 Fax: 031 906 1044 Email:myint.aung@kznhealth.gov.za
www.kznhealth.gov.za

Prince Mshiyeni Memorial
Hospital

Enquiry: Dr M AUNG
Ref No: 29/RESH/2018
Date: 02/10/2018

TO: Christen Lahner

RE: LETTER OF APPROVAL TO CONDUCT RESEARCH AT PMMH

Dear Researcher;

I have pleasure to inform you that PMMH has granted to conduct research on **"A genetic and epigenetic evaluation of obesity in a Black South African Adult population"** in our institution.

Please note the following:

1. Please ensure this office is informed before you commence your research.
2. The institution will not provide any resources for this research.
3. You will be expected to provide feedback on you finding to the institution.

With kind regard

[redacted]

MYINT AUNG
Senior Medical Manager & specialist in Family Medicine
MBBS, DO(SA), PGDip in HIV (Natal), M.Med.Fam.Med (natal), PhD
Tel: 031 9078317
Fax: 031 906 1044
myint.aung@kznhealth.gov.za

7.7 Appendix 7

Informed consent form for storage of human biological material for research purposes

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

INFORMED CONSENT FORM FOR STORAGE OF HUMAN BIOLOGICAL MATERIAL FOR RESEARCH PURPOSES[1]
BIOMEDICAL RESEARCH ETHICS COMMITTEE, UNIVERSITY OF KWAZULU- NATAL

A genetic and epigenetic evaluation of obesity in a Black South African Adult population

To Whom it may concern,

You are invited to participate in a research project that will be investigating the relationship between genetics and obesity. Should you agree to be included in the study, the research process will involve the extraction of biological material including blood and adipose tissue.
I, Christen Lahner (PhD student, University of KwaZulu-Natal), am seeking your permission to store either residual (left over/unused) and/or additional (extra tube/s) of your biological material/s.

USE AND STORAGE
The purpose of storage is to preserve the biological material, which will be analysed for its genetic material. In addition, the biological material/s may be stored to confirm test results/ for additional testing/ for future review related or unrelated to the current research.
The biological material will be stored in -80 degree conditions and will be labelled using a barcode to maintain anonymity at all times. The storage facility is located at the department of Medical Biochemistry, George Campbell Building, 3rd floor, Howard Campus, University of KwaZulu-Natal, South Africa. The biological material will be stored for about 3 years until the completion of the current study, but may be used for post-doctoral research. The biological samples will only be taken once: a) blood, 2 x 5ml tubes; and b) a trucut biopsy of adipose tissue taken during your elective surgery procedure.

BENEFITS
At present there are no known direct benefits that may be generated from the study. The information obtained from stored samples may benefit others with similar conditions. The samples may be used for teaching and education, for public health surveillance, research, to generate new knowledge, for publications, presentations and/or academic qualifications. The information obtained from the analysis of the biological material, will help the scientific community better understand the pathogenesis of obesity and may help in the development of prevention strategies. The biological samples will not be sold for profit.

[1] *Human biological material is a quantity of tissue or other biologically derived material used for diagnosis, analysis, education and research. It can include everything from sub-cellular structures, polar bodies, blastomeres, genetic material (DNA, RNA) to cells, tissue (bone, muscle, connective tissue, and skin), organs (e.g .liver, bladder, heart, kidney), blood, gametes (sperm and ova), embryos, foetal tissue, and waste (urine, faeces, sweat, hair, and nail clippings, shed epithelial cells and placenta)[DOH,NIH,NCI,ICMR & HTA(UK]*

RISKS
There are no foreseen risks involved in the participation of this study.

CONFIDENTIALITY
All personal information and biological material will be stored and used confidentially and no names will be used in the analysis of any results as you will be allocated a subject code.

PARTICIPANTS RIGHTS
Your participation in this study is entirely voluntary and You may withdraw at any time without it affecting any treatment or care that You would usually be entitled to. Refusal to store sample/s will not affect Your participation in the study. The biological material will be used to isolate genetic material (RNA/DNA), which will be analysed for its potential link with obesity. You have the right to choose the conditions under which your biological material may be used, over and above those that have already been specified.

You have the right to redraw permission at anytime and are welcome to contact the researchers, should you have any further queries.

RESEARCHER CONTACT DETAILS

	Email addresses
Researcher: Christen Lahner	lahnerchristen@gmail.com or lahner@ukzn.ac.za
Supervisor: Prof. Anil Chuturgoon	CHUTUR@ukzn.ac.za

This study has been ethically reviewed and is aligned with the protocols and procedures governed and approved by BREC (BE269/18) at the University of KwaZulu-Natal and National Department of Health (KZ_201807_002).

CERTIFICATE OF CONSENT

In the light of the information that I have received, and having had the opportunity to ask questions that have been answered, and if any of the biological material [blood and/or adipose tissue] I _____ have provided for this research project [A genetic and epigenetic evaluation of obesity in a Black South African Adult population] is unused or leftover or additional samples have been provided, I agree to participate in the research study and consent to the following:

	Yes	No
The samples [blood and/or adipose tissue] to be disposed of lawfully, immediately	☐	☐
The sample/s [blood and/or adipose tissue] to be disposed of lawfully after 5 years.	☐	☐
The sample/s [[blood and/or adipose tissue] returned to me for burial/cremation.	☐	☐
The sample/s [blood and/or adipose tissue] to be stored for 5 years	☐	☐
The sample/s [blood and/or adipose tissue] to be stored indefinitely	☐	☐
The samples [blood and/or adipose tissue] to be exported under BREC oversight	☐	☐

AND if the sample is to be stored I consent to the following:

The sample/s collected during this study may be stored at department of Medical Biochemistry, Howard Campus, University of KwaZulu-Natal	☐	☐
The sample/s [blood and/or adipose tissue] to be stored and used in future research for the specific purposes of this study [genetic and epigenetic evaluation of obesity] approved by BREC	☐	☐
The sample/s [blood and/or adipose tissue] to be stored and used in future research of any type which has been approved by BREC.	☐	☐
The sample/s [blood and/or adipose tissue] to be used for teaching, quality assurances, public health surveillance, clinical audit, publications and presentations approved by BREC	☐	☐
The sample/s [blood and/or adipose tissue] to be used by researchers for the development of commercial products without any financial benefit to me	☐	☐
The samples to be used by secondary *bona fides* researchers approved by BREC	☐	☐
The samples may be exported under BREC's oversight and approval	☐	☐
AND		
I want my identity to be removed from my sample/s [blood and/or adipose tissue]	☐	☐
I want my identity to be kept with my samples [blood and/or adipose tissue]	☐	☐

AND	**Yes**	**No**
I am willing to be re-contacted by the researcher/s about possible future use of my sample/s in the future	☐	☐
I do not want to be re-contacted to provide more sample/s in the future or take part in future studies.	☐	☐

I declare:

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily to have my samples stored in the manner and for the purpose indicated above. I have been informed of my right to withdraw my consent to the storage and/or use of my samples at any time and without giving any reason and without prejudice to myself or my treatment.

I have been informed that I will be given information from the research team concerning the progress and general results of the research studies upon my explicit request. I have also been informed that they will not communicate any individual results to me.

*Name of Participant*_____

Signature of Participant _____

Date _____
Day/month/year

If illiterate:

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

*Print name of witness*_____ ***Thumb print of participant***

Signature of witness _____

Date _____ *Time*_____
Day/month/year

STATEMENT BY THE RESEARCHER/PERSON TAKING CONSENT

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the goals, objectives and the storage and use of the biological material.

I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed consent form has been provided to the participant.

*Name of Researcher/person taking the consent*_____

*Signature of Researcher /person taking the consent*_____

Date _____ *Time*_____

Day/month/year

7.8 Appendix 8

English BREC informed consent form

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

INFORMED CONSENT FORM

Date: 11 June 2018

To Whom it may concern,

I am a doctorate student studying towards my PhD in Medical Biochemistry at the University of KwaZulu-Natal (UKZN), Howard Campus. My contact details as well as that of my supervisor at the Department of Medical Biochemistry are indicated below, should you wish to contact us.

You are hereby invited to participate in a study where the genetics and epigenetics in obesity within a Black South African population will be investigated.

STUDY INFORMATION

Study aims: To investigate whether there are genetic variables that may be associated with being overweight in Black South African males and females. Also, to determine whether environmental factors such as diet, exercise and smoking may influence how genes behave.

Procedures: The study will involve the collection of basic information about demographics and habits and lifestyle via a questionnaire. Basic anthropometric measurements such as weight and height will be taken by the researcher. Blood and tissue samples will be collected via the surgeon during your elective surgical procedure, stored and analyzed for it's genetic material. This genetic material will be analyzed in a laboratory and tested for links with influencing factors, for example hormones such as Leptin or Adiponectin, and whether there are any single nucleotide polymorphism's that will predispose you to obesity.

Duration: The duration of questionnaire and measurements should not take longer than 30 minutes.

Risk: There are no foreseen risks involved in the participation of this study.

Participation: Your participation in this study is voluntary and you have the right to withdraw from participating at any stage without giving a reason and any negative consequences.

Confidentiality: All personal information and tissue samples will be stored and used confidentially and no names will be used in the analysis of any results as you will be allocated a subject code.

Findings: The information collected will be used by the researcher for the write up of a PhD dissertation and publishing the results in peer reviewed scientific journals.

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (approval number: BE269/18).

RESEARCHER CONTACT DETAILS

	Email addresses
Researcher: Christen Lahner	lahnerchristen@gmail.com or lahner@ukzn.ac.za
Supervisor: Prof. Anil Chuturgoon	CHUTUR@ukzn.ac.za

If you are willing to participate in the study, please consent by signing the informed consent form attached.

CONSENT FORM TO PARTICIPATE IN THE STUDY

I, _____ have been informed about the study entitled A g**enetics and epigenetic evaluation of obesity in a Black South African Adult population** by Christen Lahner.

I declare that the purpose of the study and methods used to collect study data have been explained to me by the researcher/fieldworkers.

I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

I have been informed about any available compensation or medical treatment if injury occurs to me as a result of study-related procedures.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher.

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

_____ _____
Signature of Participant **Date**

_____ _____
Signature of Witness **Date**
(Where applicable)

_____ _____
Signature of Translator **Date**
(Where applicable)

7.9 Appendix 9

isiZulu BREC informed consent form

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

IFOMU LOKUVUMA ULWAZI

Usuku : 11 KuNhlangulana 2018

Othintekayo

Ngingumfundi ofundela iziqu zobu Dokotela kwi Medical Biochemistry enyuvesi yaKwaZulu-Natal (Howard college). .

Ngingumfundi ofundela iziqu zobu Dokotela kwi Medical Biochemistry enyuvesi yaKwaZulu-Natal (Howard college). Imininingwane yokuxhumana nami kanye nomqeqeshi wami osemnyangweni we Medical Biochemistry iqoshiwe ekugcineni, ningasithinta uma nifisa.

Uyamenywa ukuba uzibandakanye kulolucwaningo olumayelana nofuzo kanye ne-epigenetics kubantu besifasazane abamnyama, abakhuluphele baseNingizimu Africa.

ULWAZI LOCWANINGO

Inhloso yalolucwaningo: Ukuphenya ukuba lukhona yini ufuzo oluhambisana nokukhuluphala ngokweqile kubantu besilisa nabesifazane abamnyama base Bingizimu Africa. Futhi, nokubheka ukuba iknona yini inking yemvelo enjengo kudla, ukuvocavoca, ukubhema engaba nomthelela ekuziphatheni kwizakhi zofuzo lwabo.

Inqubo: lolucwaningo lubandakanya ukuqoqwa kolwazi oluyisisekelo mayelana nenani Labantu bendawo, imikhuba yabo Kanye nendlela yokuphila kwabo ngemibuzo. Umcwaningi uzoqoqa izilinganiso eziyisisekelo zomzimba wabo zobude nesisindo ngokubakala. Igazi nezicubu zomzimba zizothathwa kudokotela ohlinzayo ngokulandela inqubo mngomo. Zizibe seziyagcinwa, bese ziyahlaziywa kuze kuze kutholakale izakha fuzo, Lezi zakha fuzo zizohlaziywa elabhoratoory zihlolwe kuze kutholakale izici ezithonya lokhu kukhuluphala, ezinjenge hormonesafana ne Leptin noma i-Adiponectin. Zophinda zihlolwe ukuthi zingama single nucleotides polymorphisms eziba nomthelela wokukhuluphala.

Isikhathi : Isikhathi semibuzo nikukalwa komzimba angeke seqe emizuzwini eyi 30.

Ubungozi: Abukho ubungozi obubonakalayo ekuzibandakanyeni kulolu cwaningo.
.

Ukuzibandakanya: Ukuzibandakanya kwakho kungukuzithandela kanti futhi unelungelo lokuhoxa noma nini ngaphandle kokuchaza isizathu, nokwehlelwa okubi.

Ukufihleka: Yonke imininingwane yabantu Kanye nezicubu zabo kuzogcinwa kahle ngokufihlekile, futhi awekho namagma azosetshenziswa ekuhlaziyweni kwemiphumela njengalokhu kuzobe kusetshenziswa izinombolo.

Okutholiwe: Ulwazi oluqoqiwe luzosetshenziswa ngumseshi ukuze azobhala i-dissertation yakhe yobudokodela, bese iyashicilelswa ngabanye ososayensi abalingana naye.

Lolucwaningo selubuyekezelwe, lwebuye lwavunywa ngabe UKZN Biomedical research Ethics Committee (approval number: BE269/18).

IMININGWANE YOKUXHUMANA KAMCWANINGI

	Email addresses
Umcwaningi: Christen Lahner	lahnerchristen@gmail.com or lahner@ukzn.ac.za
Umqeqeshi: Prof. Anil Chuturgoon	CHUTUR@ukzn.ac.za

Uma ufisa ukuzibandakanya kulolucwaningo, cela uvume ngokusayina iformu lokuvuma elifakiwe
IFOMU LOKUVUMA UKUZIBANDAKANYA KULOLUCWANINGO.

Mina, _____ ngazizisiwe mayelana nalolucwaningo olunesihloko esithi- **genetics and epigenetic evaluation of obesity in a Black South African Adult population** by Christen Lahner

Ngysho ukuthi injngo yalolucwaning Kanye nenqubo ezosetshenzizswa ukuqoqa ulwazi ichaziwe ngunwaningi noma abasebenzi base abasendaweni.

Ngiphiwe ithuba lokubuza mayelana nalоluncwzning futhi ngaphiwa nezimpendulo ezingigculisayo

Ngiyasho ukuthi ukuzibandakanya kwami kulolucwaningo kungukuthanda kwami ngokuphelele futhi ngingahoxa noma inini makuthinteka ukwelashwa kwami noma ukunakekelwa kwami.

Ngaziziwe ngesinxephezelo esitholakalayo noma usizo lokulashwa uma ngingase ngilimakle ngenxa yezinqubo zaloluncwaningo.

Ngiyaqonda ukuthi uma nginemibuzo kabanzi ngalolucwaningo ngizoxhumana nomcwaningi.

Uma nginemibuz noma ukukhathazeka mayelana namalungelo ami ngokuzibandakanya kulolucwaningo, noma uma ngikhathazekile nganoma yisiphi isici salolu cwaningo noma umcwaningi ngizoxhumanan nabo.

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
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Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

_____ _____
Isignesha yomhlanganyeli **Date**

_____ _____
Isihnesha kafakazi **Date**
(lapho kusebenza khona)

_____ _____
Isignesha yomhumushi **Date**
(lapho kusebenza khona

7.10 Appendix 10

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Obstetrics and Gynaecology Research

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Maternal overweight and obesity and its associated factors and outcomes in human immunodeficiency virus (HIV)-infected and HIV-uninfected black South African pregnant women

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7.11 Appendix 11

Supplementary Figure 7.1: The comparison of dietary intake patterns in pregnant females between cases and controls