

**THE EFFECT OF ADIPOKINES  
IN HIV ASSOCIATED PRE-ECLAMPSIA**

(C-peptide, ghrelin, gastric inhibitory polypeptide, glucagon like peptide -1,  
glucagon, insulin plasminogen activator inhibitor-1 and visfatin)

by

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## PREFACE

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Optics & Imaging Centre, Doris Duke Medical Research Institute, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professors T. Naicker and J Moodley.



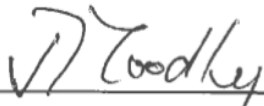
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## DECLARATION

I, **Colisile Mathonsi** declare that:

- (i) The research reported in this dissertation, except where otherwise indicated is my original work.
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Signed:  Date: 10 March 2016

## **DEDICATION**

To my mother who has always encouraged me to work hard and to always strive to improve myself, to be the better version of myself. This is also dedicated to everyone who has made a difference in my life.

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## LIST OF ABBREVIATIONS

A-chain	Alpha chain
ACOG	American College of Obstetricians and Gynecologists
ADMA	Asymmetric DiMethylArginine
AIDS	Acquired ImmunoDeficiency Syndrome
AMP	Adenosine MonoPhosphate
ANOVA	Analysis of Variance
ART	Anti-retroviral Treatment
ARV	Antiretroviral therapy
B-chain	Beta-chain
BMI	Body Mass Index
BP	Blood pressure
C-peptide	Connecting peptide
C-section	Caesarean sections
cAMP	Adenosine MonoPhosphate
CD4	Cluster of Differentiation 4
CRP	C-reactive protein
DIC	Disseminated Intravascular Coagulation
EGF	Epidermal growth factor
ELISA	Enzyme-Linked ImmunoSorbent Assay

EOPE	Early Onset Pre-Eclampsia
G-protein	Guanine nucleotide-binding protein
GA	Gestational Age
GIP	Gastric Inhibitory Polypeptide
GLP-1	Glucagon-Like Peptide (GLP)-1
GHRH	Growth Hormone Releasing Hormone
GHSs	Growth Hormone Secretagogues
GHS-R	Growth Hormone Secretagogues Receptor
IQR	InterQuartile Range
IUFD	IntraUterine Fetal Death
HAART	Highly Active Anti-Retroviral Therapy
HCV	Hepatitis C Virus
HELLP	Hemolysis, Elevated liver enzymes, and Low platelets
HIV	Human Immunodeficiency virus
HSD	Tukey honest Significant Difference
KZN	KwaZulu-Natal
LOPE	Late Onset Pre-Eclampsia
MDG	Millennium Development Goal
MMP	Matrix Metalloproteases
mRNA	RiboNucleic Acid

NAmPRTase	Nicotinamide phosphoribosyltransferase
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
PAI-1	Plasminogen Activator Inhibitor
PBEF1	Pre-B-cell colony-Enhancing factor 1
PE	Pre-Eclampsia
PI	Protease Inhibitors
PMMH	Prince Mshiyeni Memorial Hospital
PMTCT	Prevention of mother-to-child transmission
RPM	Revolutions Per Minute
SERPINE 1	Serpin Peptidase Inhibitor, Clade E type 1
SSI	Staten Serum Institut
TB	Tuberculosis
TGF	Transforming Growth Factor
TH1	T helper lymphocytes type 1
TNF- $\alpha$	Tumor Necrosis factor Alpha
tPA	tissue Plasminogen Activator
UN	United Nations
UNICEF	United Nations Children's Fund
uPA	urokinase Plasminogen Activator

USA

United State of America

WHO

World Health Organisation

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## ABSTRACT

**Introduction:** South Africa has a maternal ratio of 300 deaths/100,000 live births. Non-pregnancy related infections (mainly deaths in HIV infected pregnant women complicated by tuberculosis and pneumonia) accounts for 34.7% of maternal deaths followed by obstetric haemorrhage and hypertension accounts (15.8% and 14.8% respectively). Moreover, 61% of women South Africa is overweight or obese (almost double the global rate of 30%). In pregnancy, endocrine and metabolic maternal adaptations include increase in body weight, however, the impact of adipokines in HIV associated pre-eclampsia remains unknown. The aim of the study was to examine the levels of adipokines viz., C-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon like peptide 1 (GLP), plasminogen activator inhibitory (PAI) 1, visfatin, glucagon and insulin in HIV associated pre-eclampsia.

**Materials and Methods:** Following institutional and regulatory approval, participants (n=301) were recruited from RK Khan Hospital and divided into groups non-pregnant (n=90), normotensive (n=121), early (n=32; EOPE) and late onset (n=58; LOPE) pre-eclampsia. The pregnant cohort was stratified according to their HIV status. Maternal clinical demographics, indications and mode of delivery were recorded. Serum was used to quantify the adipokines levels using the multiplex ELISA technique. Absorbance was read spectrophotometrically at 450 nm. Graph Pad Prism (version 6) was used to analyse all data.

**Result:** C-peptide did not differ according to HIV status. With regards pregnancy type, there was a significant difference in c-peptide between the non-pregnant *versus* normotensive pregnant ( $p<0.01$ ) and the normotensive *versus* LOPE groups ( $p<0.01$ ) being elevated both the pre-eclamptic groups (EOPE +LOPE). Ghrelin did not differ across study groups ( $p>0.05$ ), by HIV status ( $p>0.05$ ). When considering HIV status, GIP varied between positive and negative groups ( $p<0.001$ ). Additionally, there was a significant difference in GIP between the non-pregnant *versus* normotensive pregnant ( $p<0.01$ ); normotensive pregnant *versus* EOPE ( $p<0.05$ ) and the normotensive pregnant *versus* the LOPE group ( $p<0.01$ ). GIP was elevated in the HIV positive EOPE group. Moreover, a significant difference in GLP-1 was noted across the study groups

( $p < 0.05$ ) and between non-pregnant versus normotensive groups ( $p < 0.01$ ). When considering HIV status, HIV positive groups differed from negative study groups ( $p < 0.05$ ). Additionally, the Mann Whitney U test showed a significant difference between the non-pregnant and the normotensive group ( $p < 0.01$ ).

Glucagon-like peptide-1 was significantly different across the study groups, with its levels elevated in the pre-eclamptic groups compared to the normotensive pregnant group ( $p < 0.05$ ), additionally, there was a difference between non-pregnant versus normotensive pregnant groups ( $p < 0.01$ ). Glucagon did not differ across the study groups ( $p > 0.05$ ), however, was significantly different between the non-pregnant and normotensive group ( $p < 0.05$ ). HIV status did not affect glucagon levels ( $p > 0.05$ ). A significant difference between HIV positive non-pregnant and HIV negative non-pregnant was noted ( $p < 0.05$ ).

Insulin was not significantly different across the study groups ( $p > 0.05$ ) and by HIV status ( $p > 0.05$ ). However, a significant difference between the non-pregnant *versus* normotensive group ( $p < 0.05$ ) was noted. PAI-1 did not differ across the study groups ( $p > 0.05$ ) and between the groups ( $p > 0.05$ ). PAI-1 did not differ according to HIV status ( $p > 0.05$ ). A significant difference in visfatin across the study groups ( $p < 0.05$ ) and between the non-pregnant *versus* normotensive pregnant group ( $p < 0.05$ ) and the late onset pre-eclamptic *versus* the non-pregnant group ( $p < 0.01$ ) was observed. There was no effect of HIV status on the level of visfatin across the study groups ( $p < 0.05$ ). There was a significant difference between the HIV positive *versus* negative non-pregnant groups ( $p < 0.05$ ), furthermore, we have observed low levels of visfatin in the HIV positive pre-eclamptic groups.

**Discussion and conclusion:** This study demonstrates elevated c-peptide, GIP, GLP-1, Insulin, PAI-1 and Visfatin in the pre-eclamptic groups compared to normotensive pregnant groups. These adipokines play a role in glucose homeostasis and have been reported to play a role in development of insulin resistance which is a high risk factor for developing pre-eclampsia. Several studies have reported that adipose tissue derived hormones, play a crucial role in the pathogenesis or as risk factors of pre-eclampsia development. Additionally, it is reported that adipokines are elevated in people with higher BMI (obese and overweight) which in turn predisposes one for developing pre-eclampsia. In terms of HIV status, we have observed that many of the adipokines were elevated in the HIV positive compared to the HIV negative group. This correlates with studies which reported that HIV plays a role in dysregulation of adipokines. In conclusion, our study is the first to examine adipokine dysregulation in the triad of HIV infection, pre-eclampsia and obesity. Furthermore, we have established that adipokines: C-peptide, GIP, GLP-1, PAI-1 and visfatin were significantly dysregulated hence they may have predictor test value in diagnosing pre-eclampsia development.

## CHAPTER 1

# INTRODUCTION

## 1.1 Maternal Deaths

Maternal deaths is defined as the death of a woman during pregnancy or 72 days after delivery due to causes related to or aggravated by the pregnancy. The World Health Organisation (WHO) reports that every day, approximately 800 women die worldwide during, or after child delivery. Additionally, it is reported that women in sub-Saharan Africa are more likely to die from maternal deaths compared to other population areas in the world. In 2013, more than half of all maternal deaths occurred in Sub-Saharan Africa, and about a quarter occurred in South Asia. In 2013, 99% of 289,000 global maternal deaths occurred in developing countries with more than half occurring in sub-Saharan Africa and about a quarter in South Asia (WHO. 2014 “Maternal Mortality” Fact sheet 348). Additionally, it is also reported that women aged between 15–49 years in Sub-Saharan Africa are faced with a 2.6% chance of dying in childbirth. In Chad and Somalia, lifetime risk is still more than 5%, meaning that more than one woman in twenty will on average die in childbirth.

The Millennium Development Goals (MDGs) set a target for the global reduction of maternal mortality ratio by three quarters, by the year 2015 (Post-2015 Development Agenda: Goals, Targets and Indicators, 2015). Despite immense progress being achieved improving maternal health in low and medium income countries still remains a major health challenge. Maternal deaths in South Africa has decreased by 50% since 2000, however this is still lower than expected target of 35:100 000 maternal deaths (Saving Mothers report of 2011-2013: Sixth report on confidential enquiries into maternal deaths in South Africa, 2015).

### 1.1.1 Causes of maternal deaths

In South Africa, the top 5 causes of maternal deaths are non-pregnant related, obstetrics haemorrhage, medical disorders, hypertension in pregnancy and sepsis. The latest report indicates that in the period of 2011-2013, the top 5 causes of maternal deaths were non-pregnancy related infections (NPRI) which attributed 34.7% of maternal deaths mainly due to HIV infection complicated by Tuberculosis (TB) and pneumonia. Secondly was obstetric haemorrhage which causes 15.8% of maternal deaths. This is followed by complications of hypertension in pregnancy, medical and surgical disorders and sepsis causing 14.8%, 11.4% and 9.5% maternal deaths respectively (Saving mothers report of 2011-2013: Sixth report on confidential enquiries into maternal deaths in South Africa, 2015). The thesis focuses on is the conditions of pre-eclampsia, HIV and obesity and their impact on adipokines.

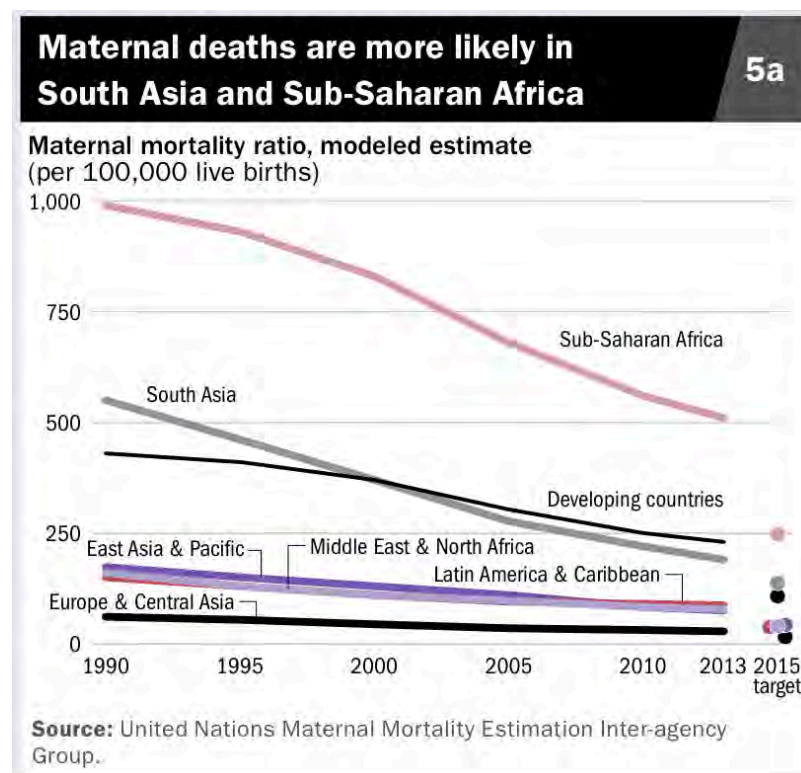


Figure 1.1: Graphic illustration of maternal deaths global and regional epidemics (Source: <http://blogs.worldbank.org/opendata/mdg5-despite-progress-improving-maternal-health-still-challenge>)

## 1.2 Pre-eclampsia

### 1.2.1 Definition and clinical characteristics

Pre-eclampsia is a hypertensive disorder exclusive to pregnancy, occurring after the 20<sup>th</sup> week of gestation and is one of the top 5 causes of maternal and fetal morbidity and mortality (Walsh, 2007). It is known as a multisystem disorder characterized by an increase in blood pressure and protein in the urine (>300mg/dl) (Sibai *et al.*, 2005).

Pre-eclampsia is the most common medical condition related to pregnancy, reportedly increasing the risk of a mother to develop convulsions, liver and kidney failure all of which may lead to maternal deaths (Walsh, 2007; Williams and Pipkin, 2011). The pathogenesis of pre-eclampsia is a multi-factorial process that includes the interaction of genetic and environmental factors. The high blood pressure is a result of peripheral vasoconstriction and decreased arterial compliance (Powe *et al.*, 2011). According to Moodley (2010) both systolic and diastolic blood pressure are important in pregnancy induced hypertension. The proteinuria is caused by glomerular endotheliosis, due to the swelling of the endothelial cells of the glomerulus and the loss of endothelial fenestrations that contributes to a decreased glomerular filtration rate (Powe *et al.*, 2011).

Some researchers believe that immunological factors play a role in the development of pre-eclampsia by initiating an imbalanced cascade of events between angiogenic and anti-angiogenic factors (Kalumba *et al.*, 2013). Pre-eclampsia is a primigravid condition, ie., affecting women who are pregnant for the first time, ie., young women who are between the ages of 18-24 years.

The impact of the disease is felt more severely in developing countries (Hall *et al.*, 2014) where unlike other more prevalent causes of maternal mortality (such as haemorrhage and sepsis), access to professional medical care is difficult and medical interventions may be ineffective due to late

presentation. The problem is confounded by the continued mystery of the aetiology and the unpredictable nature of the disease.

In South Africa and other developing countries, pre-eclampsia is associated with high maternal and neonatal mortality and morbidity (Hall *et al.*, 2014; Kalumba *et al.*, 2013; Young *et al.*, 2010). There is no cure for pre-eclampsia, the current resolution is delivery of the placenta. Early delivery of the fetus is usually recommended for women who have severe pre-eclampsia. Both mother and child have a risk of developing serious cardiovascular and metabolic complications (Sibai *et al.*, 2005). Other complications associated with pre-eclampsia include eclampsia, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, acute renal failure, hepatic enzymes derangement, abruptio placenta, disseminated intravascular coagulation (DIC) and maternal death. Perinatal complications include preterm delivery, low birth weight, intrauterine fetal death (IUFD) and neonatal deaths.

### ***1.2.2 Signs & Symptoms***

The fact that pre-eclampsia has a variety of aetiologies and its presentation may vary, makes diagnosis difficult (Young *et al.*, 2010). Pre-eclampsia may present itself as a mild to severe disease, whilst in some women it may lack the cardinal signs and symptoms (Trogstad *et al.*, 2011; Young *et al.*, 2010). Pre-eclampsia has no resilient diagnostic test, and the key features that have been used to diagnose this condition are the inception of hypertension and proteinuria in pregnancy (Moodley, 2010; Young *et al.*, 2010) Previously, oedema used to be used as a diagnostic triad for pre-eclampsia, but was found to be problematic due to the fact that oedema is not only specific to pre-eclamptic patients, as normotensive women also present with oedema during pregnancy (Young *et al.*, 2010). Currently the clinical signs used for the diagnosis of pre-eclampsia are hypertension, proteinuria, glomerular endotheliosis, HELLP (Young *et al.*, 2010). Hypertension and proteinuria are at a diagnostic level in pregnancy when they both present as new onset systolic



blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg and  $\geq 300$  mg/24hours of protein in a 24 hour urine specimen (Moodley, 2010; Young *et al.*, 2010)

Pre-eclampsia has a wide clinical spectrum and in most circumstances presents as a mild disease with few clinical signs (Trogstad *et al.*, 2011; Young *et al.*, 2010). In severe cases of pre-eclampsia women may develop headaches or visual changes, pain in the upper abdomen arising from acute liver injury, pulmonary oedema, acute renal failure, haemolysis and/or thrombocytopenia (Young *et al.*, 2010). In severe pre-eclampsia, convulsions may occur, the condition is then referred to as eclampsia with resultant severe complications even postpartum.

### **1.2.3 Risk Factors**

Despite years of intensive research on pre-eclampsia, its risk factors and aetiology are still not yet fully understood (Powe *et al.*, 2011; Trogstad *et al.*, 2011). Several medical conditions and preferences of certain lifestyles are associated with increased risk of pre-eclampsia development. August and Sibai (2013) state that the magnitude of each risk factor depends upon genetic factors, disease recurrence, multiple gestation etc. (Trogstad *et al.*, 2011). The age at which a female conceives is a major factor that determines manifestation of the disease, most studies have shown that pre-eclampsia predominates in young women, first pregnancies and advanced maternal age (Young *et al.*, 2010).

Pre-existing hypertension disorders, metabolic disorders, diabetes mellitus, obesity and renal diseases are medical conditions associated with an increased risk of pre-eclampsia development (Trogstad *et al.*, 2011; Young *et al.*, 2010).

Obstetric conditions such as increased placental mass, mole development within the uterus and a multi-fetal pregnancy increases ones risk (Trogstad *et al.*, 2011; Young *et al.*, 2010). England and associates demonstrated that smoking is a protective mechanism against pre-eclampsia

development (England *et al.*, 2002). Yet this fact can be intensively debated as smoking may indirectly increase the risk of the woman acquiring predisposing disorders of pre-eclampsia like cardiovascular diseases later in life. Studies have shown that neonatal mortality rate of babies born to pre-eclamptic mothers is 5 times higher than that of babies born to mothers with normal pregnancy.

### **1.2.3 Epidemiology**

#### **1.2.3.1 Globally**

Globally, pre-eclampsia affects 7-10% of pregnant women and is a leading cause of maternal and perinatal mortality and morbidity (Kalumba *et al.*, 2013). Although a reduction in maternal death has been reported since the Millenium Development Goals (MDGs) in high income countries, there is still a high rate of maternal deaths in low to middle income countries. Pre-eclampsia is more prevalent in developing countries due to limitations of access to health care, which results in an estimated >60 000 maternal deaths per annum (Young *et al.*, 2010). In developed countries, access to health care and patient management is better; therefore maternal deaths due to pre-eclampsia are low.



Figure 1.2: Graphic illustration of the global and regional statistics of maternal deaths due to pre-eclampsia (Source: The Lancet Global health, June 2014).

### 1.2.3.2 Pre-eclampsia in sub-Saharan Africa

In developing countries, the incidence of pre-eclampsia is high posing as a serious threat to the health of pregnant women. According to the World Health Organization (WHO), in 2010, approximately 287,000 women died in pregnancy and childbirth ([http://lifeofafricanmothers.org/maternal health](http://lifeofafricanmothers.org/maternal%20health)). It is reported that more than half of women affected by pre-eclampsia have poor access to health care, hence they are not diagnosed early enough to receive good management of the condition (Moodley, 2010). In general, sub-Saharan African countries are under-resourced and provision of health care facilities is poor. Moreover, unavailability of some medication and a lack of standard protocol of management of pre-eclampsia contribute to the high maternal deaths (Moodley, 2010).

In South Africa, hypertension is the second direct cause of maternal death (14%), among pregnant women with eighty three percent been attributed to pre-eclampsia (Govender *et al.*, 2013).

Moreover, in South Africa, maternal deaths due to pre-eclampsia are more common among younger than older women (Saving Mother's Report; 2012). Teenage pregnancy is a major problem in African countries providing a plausible reason why pre-eclampsia is common among younger women.

In KwaZulu-Natal, pre-eclampsia is a common condition in the obstetric care unit. Sixteen percent of all hypertensive patients at the maternity unit of King Edward VIII and 12% Prince Mshiyeni Memorial Hospital (PMMH) suffer from hypertension in pregnancy.

#### ***1.2.4 Pathogenesis***

In recent years the pathogenesis of this condition has been better explored (Athukorala et al., 2010). The disease originates in the placenta (Powe et al., 2011; Trogstad et al., 2011; Young et al., 2010).

Pre-eclampsia pathogenesis includes abnormal placentation, cardiovascular problems, maladaptation to pregnancy, genetic and immune mechanisms, an enhanced systemic inflammatory response, and nutritional, hormonal and angiogenic factors (Appay and Sauce, 2008). The maternal systemic inflammatory response, endothelial cell dysfunction and increased coagulation is secondary to abnormal placental function (Moodley, 2010). Extravillous cytotrophoblast migration and invasion of the decidual myometrial junction is limited in pre-eclampsia (Naicker *et al.*, 2003). Moodley (2013), proposed that pre-eclampsia is a two stage disorder; viz., inadequate vascular remodelling of the spiral arterioles in the placental bed, hence reduction of blood flow to the placenta with resultant hypoxic environment and inadequate oxygen and nutrient supply to the fetus. This is followed by a cascade of events with resultant oxidative stress, widespread damage to the endothelium and consequential maternal signs and symptoms of pre-eclampsia.

The key purpose of the placenta is the transfer of nutrients and vital gases to the developing fetus, and also the elimination of waste through the mother's blood supply. The placenta of women with

severe pre-eclampsia are associated with infarcts, inflammation, and haemorrhage/blood clot (Powe *et al.*, 2011).

In normal pregnancy cytotrophoblasts arising from the tips of the anchoring villi through the decidua into the myometrium and invade the maternal spiral arterioles. This invasion results in the cytotrophoblast adopting a vascular phenotype and the muscular tunica media is replaced by a fibrinoid type material (Lam *et al.*, 2005). The spiral arteries are remodelled into large-capacitance, low-resistance vessels, a feature referred to as a physiological conversion. The placenta releases high levels of anti-angiogenic factors and low levels of pro-angiogenic factors, thus contradicting the process of angiogenesis and disrupting the maternal endothelium (Young *et al.*, 2010). The reason for the modification from the usual angiogenic stability into an anti-angiogenic state is not known (Young *et al.*, 2010). This shift however, is said to be the main cause of the maternal hypertensive syndrome, proteinuria as well as other clinical signs of pre-eclampsia and eclampsia (Young *et al.*, 2010).

In pre-eclampsia however, there is an abnormal trophoblast cell migration due to the fact that cytotrophoblasts fail to adopt an invasive phenotype. This results in shallow invasion of the spiral arteries and the myometrial arteries remain as small-caliber, resistance vessels, which then leads to poor blood flow to meet the growing demands of the fetus.

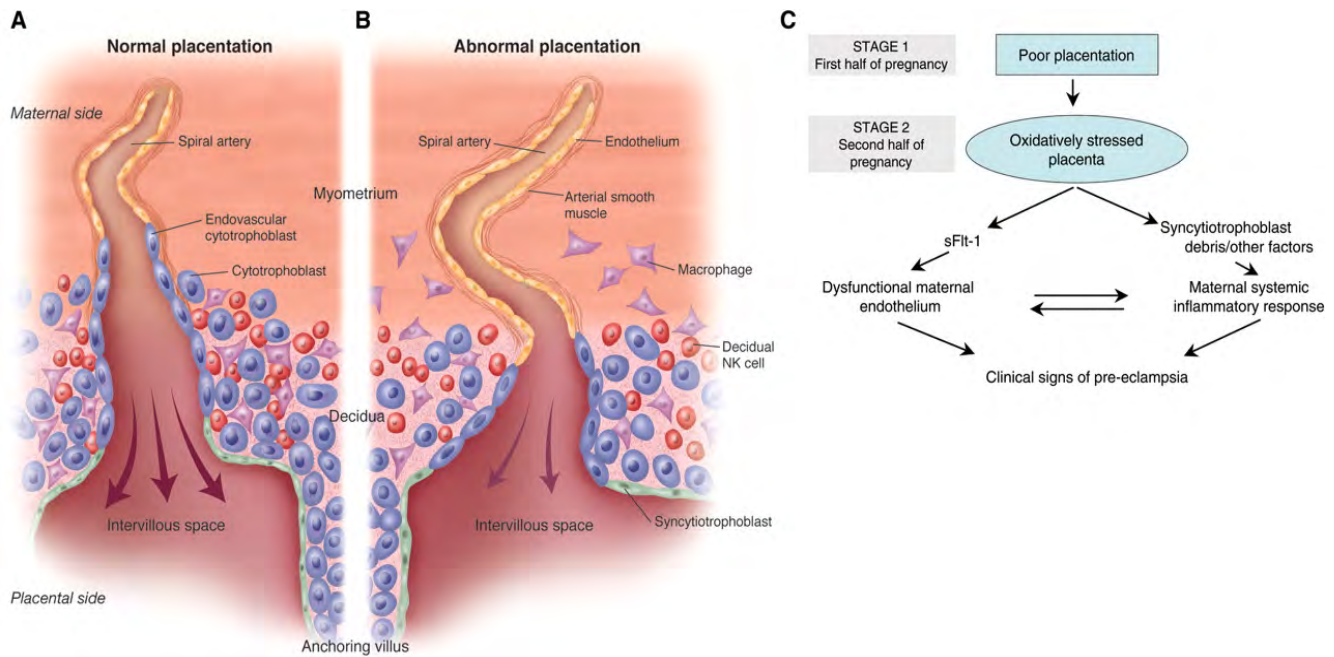


Figure 1.3: Trophoblast invasion/migration in normal pregnancy *versus* abnormal pregnancy in the case of pre-eclampsia, image adapted from ([www.sciencemag.org/latest](http://www.sciencemag.org/latest) advances in understanding pre-eclampsia).

### 1.2.4 Management

Currently, the management of pre-eclampsia is timely diagnosis, proper management and early delivery, to prevent complications (Sibai *et al.*, 2005; Trogstad *et al.*, 2011; Young *et al.*, 2010). A decision of induced premature delivery is mostly used in developed countries to protect the health of the mother, this method has shown to have adverse results in the survival of the neonate and may result in low birth weight, morbidity and/or mortality (Powe *et al.*, 2011; Young *et al.*, 2010). Close monitoring of the fetus using ultrasound examination and fetal heart rate testing is crucial (Young *et al.*, 2010).

## 1.3 Human Immunodeficiency Virus

### 1.3.1 Definition and clinical characteristics

Human immunodeficiency virus (HIV) infection is an immune-dysfunction pandemic that has affected and infected millions of people worldwide. It was discovered in 1981 and has since been a major threat to human health globally but it more common in African countries. HIV-1 accounts for 95% of infections worldwide and characterized by a depletion of CD4+ T cells (Appay and Sauce, 2008). Cells that express CD4+ include T helper cells, macrophages and dendritic cells, and are important in the effective functioning of the immune system. T helper cells are especially essential for activating B cells to produce antibodies. The production of antibodies is the hallmark for the execution of the whole immune system, failure of the T helper cells to activate B cells leaves the body to be vulnerable to opportunistic diseases such as tuberculosis (TB) and eczema.

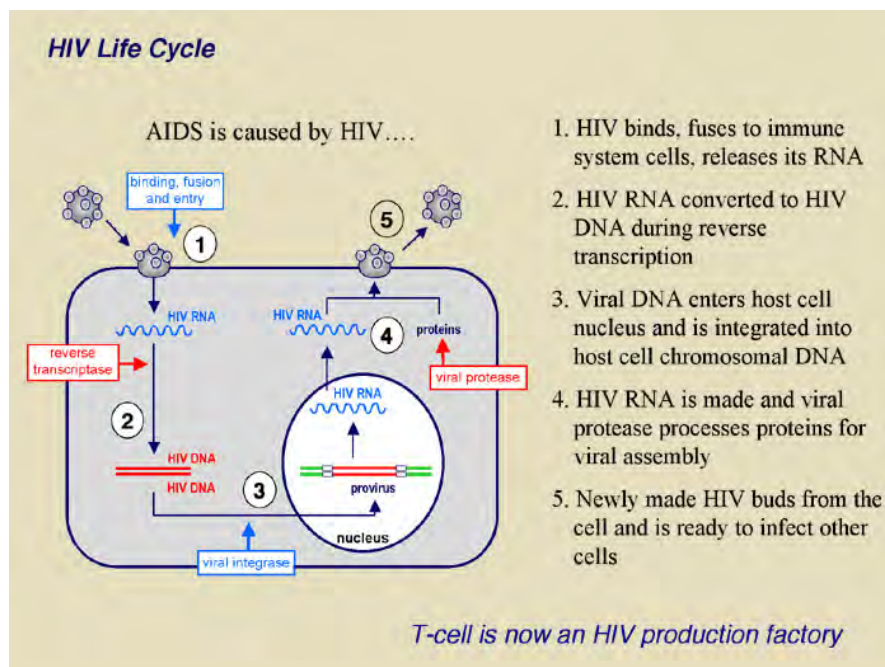


Figure 1.4: Figure demonstrating the mechanism of HIV-1 and host cell interaction (Source: Sheehy *et al.*, 2002).

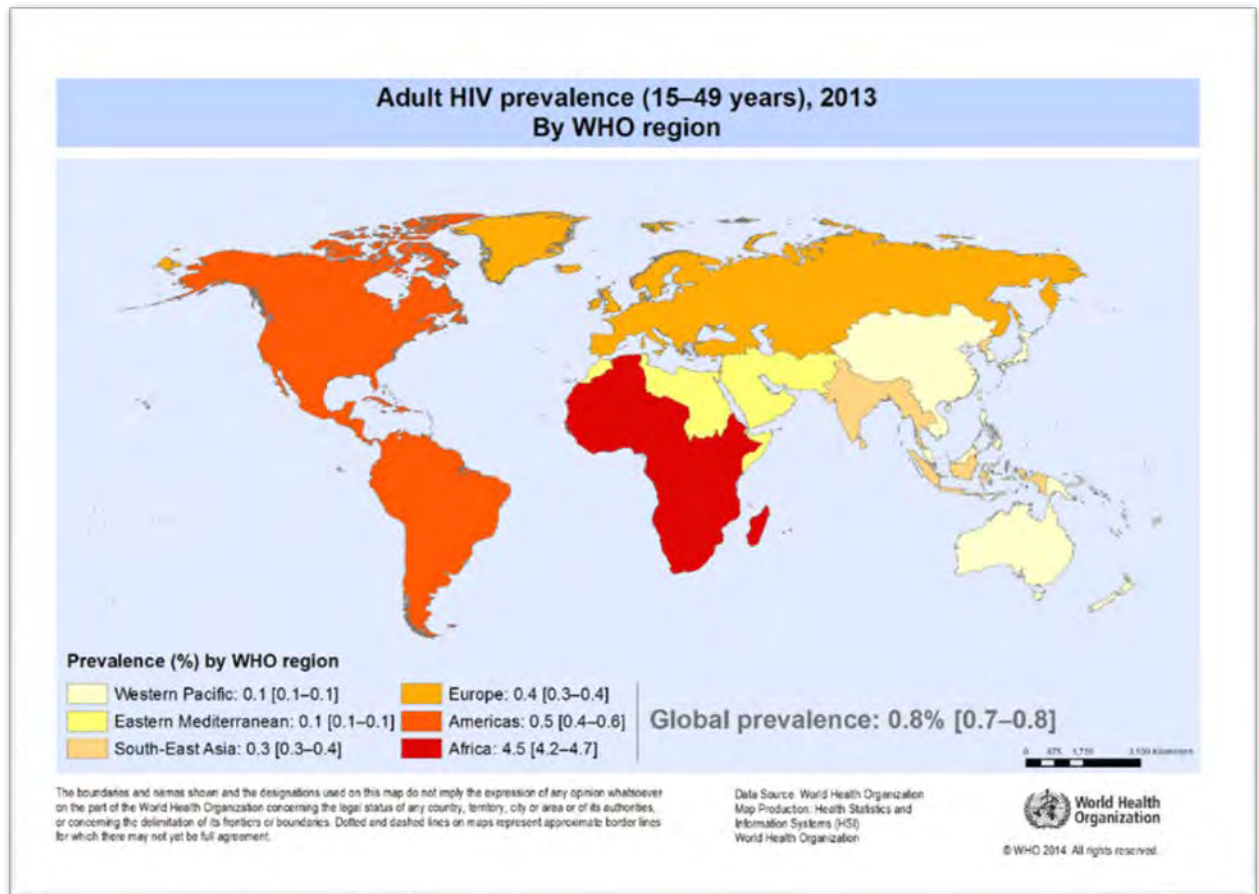


Figure 1.5: Graph showing the burden of HIV prevalence and incidences globally and regionally (Source: <http://www.who.int/gho/hiv/en/>).

### 1.4 HIV and Pregnancy

According to the South African Saving Mother’s report (2012), HIV is the most common underlying condition associated with maternal deaths (39%). HIV/AIDS may influence maternal mortality in several ways. HIV can influence maternal death in HIV positive women by making them more susceptible to direct or obstetric causes of maternal mortality, such as post-partum haemorrhage, puerperal sepsis and complications of caesarean section. It is plausible to suggest that the hyperimmune reactivity in pre-eclampsia may be neutralised by the immunocompromised state of HIV infection. Kalumba and associates suggests that HIV provides some sort of immunity



against pre-eclampsia, thus HIV positive women are at a lower risk of developing pre-eclampsia than the general population (Kalumba *et al.*, 2013) .

HIV and pre-eclampsia, are common conditions in developing countries especially Sub-Saharan African countries. Pre-eclampsia was more common in HIV negative pregnant women (Hall *et al.*, 2014; Kalumba *et al.*, 2013). These findings support the idea that pre-eclampsia which has a major proliferative aseptic inflammation cannot progress enough on immunocompromised ground. Nevertheless, recent studies have shown no difference on the prevalence of pre-eclampsia in HIV negative and HIV positive women. A study by Hall and associates (Hall *et al.*, 2014) show that women on anti-retroviral therapy were at lower risk of developing pre-eclampsia. In contrast, a study by Boyajian and associates indicated that HIV positive woman developed a more severe form of pre-eclampsia (Boyajian *et al.*, 2012). To date, there has been no agreement on the link of HIV infection regarding pre-eclampsia progression. Although the aetiology of pre-eclampsia is not fully understood, hyper-reactivity of the immune system is presumed to be involved in the progression of pre-eclampsia (Appay and Sauce, 2008). Therefore, in the case of HIV infection the associated immune deficiency may neutralize the immune hyper reactivity of pre-eclampsia (Hall *et al.*, 2014).

Based on a retrospective study done by Kalumba *et al.*, (2013), in South Africa, approximately 30% of antenatal patients are infected with HIV. This study is relevant in South Africa, because both HIV and pre-eclampsia are the most common indirect and direct causes of maternal deaths respectively (Govender *et al.*, 2013; Kalumba *et al.*, 2013) hence South Africa represents an ideal site for a study involving HIV and pre-eclampsia. Moreover, the province of KwaZulu-Natal represents the epicentre of the global HIV pandemic and our province has a 12% incidence of pre-eclampsia.

## 1.5 Adipokines

Adipokines are cytokines or secretory protein hormones that are produced by adipose tissue. It may also be referred to as adipocytokines (Haugen, 2005). They include c-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon like peptide (GLP)-1, glucagon, insulin, plasminogen activator inhibitor (PAI)-1, resistin, adiponectin, leptin and visfatin. Adipokines are involved in lipid metabolism, insulin sensitivity, the alternative complement system, vascular haemostasis, blood pressure regulation and angiogenesis, as well as the regulation of energy balance (Haugen, 2005). They are implicated in the regulation of satiety, appetite, physical activity and energy expenditure. Adipokines also influence metabolic disease, cardiovascular disease and insulin resistance (El-Shafe *et al.*, 2014). Adipokines are also produced by other organs, for example, during pregnancy adipokines are produced by the placenta, because the body has to adjust in order to meet the demands of the both mother and child.

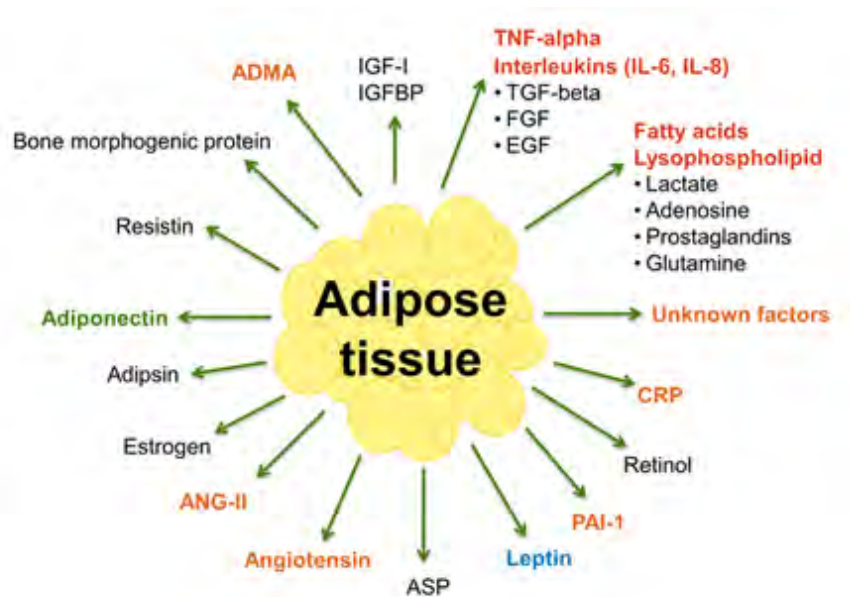


Figure 1.6: The adipose tissue and the cytokines/protein hormones that are produced by it. These cytokines play various roles in the body, some like TNF $\alpha$  plays an essential in the activation of the immune system which contributes to antigen/pathogen clearance. Some, like glucagon-like peptide-1, visfatin play a crucial role in the homeostasis of glucose metabolism. Image adapted from ([www.medscape.org](http://www.medscape.org))

### ***1.5.1 Adipokines & Pregnancy***

During pregnancy, the placenta produces these adipokines. Adipocyte-secreted factors play a key role in the development of pathogenesis of pre-eclampsia (Fasshauer *et al.*, 2008). During pregnancy, women experience an increase in weight, body fat mass, and insulin resistance. Leptin is responsible for regulating food intake and body weight and is elevated in pre-eclamptic women. Moreover women who are overweight/obese have an increased risk of difficult pregnancy outcomes (Athukorala *et al.*, 2010)

### ***1.5.2 Adipokines, HIV and pre-eclampsia***

In HIV infection, adipose tissue and the resultant adipokines are dysregulated due to lipoatrophy. Exacerbating this condition is the fact that patients on long term anti-HIV therapy experience lipoatrophy (Lake and Currier, 2013). Lipohypertrophy is a result of adipose tissue perturbations, including increased fibrosis without inflammation, increased small adipocyte numbers, and decreased vascularity. The increase in lipid production increases the risk of cardiovascular disease development among HIV positive patients on ART treatment with resultant death.

The mechanism by which metabolic perturbations of adipose occur is not yet understood. This study compares C-peptide, Ghrelin, gastric inhibitory polypeptide (GIP), glucagon like peptide-1 (GLP-1), Glucagon, Insulin, plasminogen activator inhibitor-1 (Pal-1) and Visfatin levels in non-pregnant, normotensive pregnant and pre-eclamptic immunocompromised women.

### ***1.5.3 C-peptide***

C-peptide is a short protein that is reportedly formed during the biosynthesis of insulin. C-peptide is a 30-35 amino acid sequence molecule. The signal sequence of C-peptide is cleaved from the N-terminus by a signal peptidase, following cleavage it leaves pro-insulin, the A-chain and B-chain are left bound together by disulphide bonds that constitute the insulin molecule (Kunt *et al.*, 1999).

The main function of c-peptide is to connect insulin's A-chain to its B-chain in the pro-insulin molecule (Kunt *et al.*, 1999). C-peptide has a half-life of 30 minutes which is really high compared to insulin which has a half-life of 5 minutes (Valensise *et al.*, 2002). Together with insulin, C-peptide is stored in secretory granules of the pancreatic beta cells and are released into the portal circulation.

Since c-peptide is responsible for activating insulin, it is plausible to suggest that it may be used as a marker to measure insulin activity (Valensise *et al.*, 2002). Moreover, c-peptide together with regular insulin therapy, may be beneficial in the prevention and treatment of diabetes and microvascular complications.

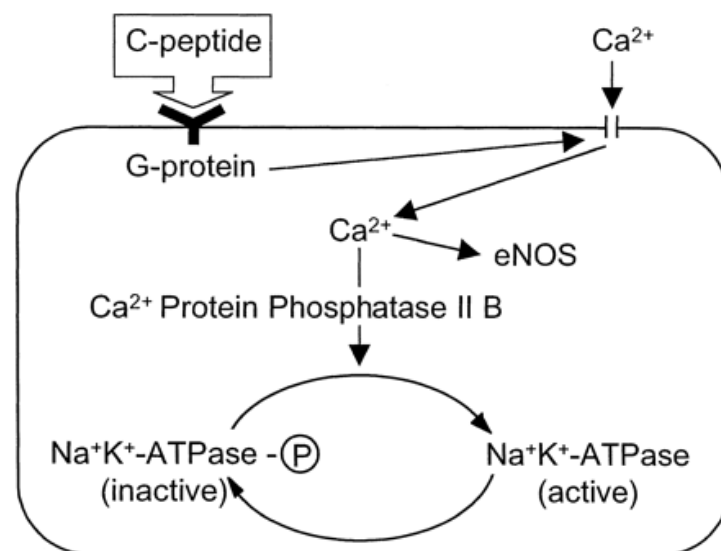


Figure 1.7: Mechanism mechanism of action of c-peptide. C-peptide binds to a cell membrane receptor which is coupled to a G-protein. This leads to the activation of calcium channels or cause the release of calcium from intracellular stores resulting in increased intracellular calcium and activation of calcium dependent protein phosphate IIB which turn, converts inactive phosphorylated sodium, potassium AT-pase to its active dephosphorylated form (Kunt *et al.*, 1999).

#### **1.5.4 Ghrelin**

Ghrelin is a hunger-stimulating peptide (also known as appetite-regulating hormone) made up of 28-amino acids, in which Ser 3 is modified by a fatty acid n-octanoic acid (Kojima and Kangawa, 2005). It consists of a bulky hydrophilic side-chain group which plays an essential role in its activity. Ghrelin is produced mainly by the cells lining the fundus of the human stomach and epsilon cells of the pancreas. Its receptors are expressed in a wide variety of tissues, including the pancreas, thyroid, pituitary, stomach, heart, thymus, intestine, hypothalamus, hippocampus and gonads.

Ghrelin is well-known for its role in stimulating the release of growth hormone hence also known as growth hormone secretagogues (GHSs) (Kojima and Kangawa, 2005). This occurs via an alternative pathway from that of growth hormone releasing hormone (GHRH), suggesting that it can be used in treatment of growth hormone deficiency. Ghrelin acts through the growth hormone secretagogues receptor (GHS-R) via G protein coupled receptors located in diverse areas of the body, hence have diverse biological functions (Kojima and Kangawa, 2005). Falasca *et al.*, (2006) found that ghrelin initiates the pathogenesis of metabolic disorders in HIV-infected patients by inducing metabolic abnormalities. Furthermore, studies have shown that the levels of ghrelin are reduced in lipodystrophy among HIV positive patients, resulting in fat redistribution (Koutkia, 2004).

Antiretroviral therapy (ARV) treatment contributes to low ghrelin levels among HIV positive participants. Ghrelin is considered to be the counterpart of the hormone leptin because its levels are high before meals and decreases right after a meal, indicating that it transmits a hunger signal from the periphery to the central nervous system, hence is sometimes referred to as orexigenic hormone. Based on Wang *et al.*, (2013), this elevation regulates various physiological processes such as increasing sympathetic activity and mitochondrial superoxide synthesis, inducing expansion of Th1 cells secreting pro-inflammatory cytokines and activating expression of matrix metalloproteases

(MMPs) and tissue MMP inhibitors, thus modulating vascular structure. These alterations contribute to endothelial and placental dysfunction which can eventually result in pre-eclampsia development.

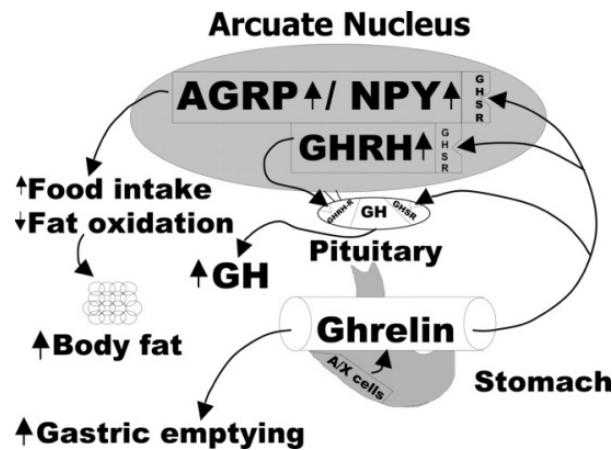


Figure 1.8: The mechanism of action of ghrelin. Adapted from (St-Pierre *et al.*, 2003)

### 1.5.5 Gastric inhibitory polypeptide (GIP)

GIP is an incretin hormone that is also known as the glucose-dependent insulinotropic peptide. It is produced by cells which are found in the mucosa of the duodenum and the jejunum of the gastrointestinal tract and its receptors are found in the beta cells of the pancreas (Kieffer, 2004). Receptors for GIP are expressed on pancreatic beta cells, where binding of GIP results in the activation of adenylyl cycles leading to increase in cyclic AMP levels and resultant stimulation of insulin release, which increases the uptake of glucose by the liver, brain and kidney cells, eventuating in the production of glycogen and fat production

In cases where the receptors of GIP are disrupted, glucose intolerance occurs (Kieffer, 2004), probably because when GIP is not evoking increased in cAMP, the production of insulin is low due to reduced incretin effect. Furthermore, low insulin production is associated with high blood sugar levels. Several studies have implicated GIP or GIP receptor disruption in the pathogenesis of type 2

diabetes (Kieffer, 2004). Notably, some studies have reported that GIP alone does not account for the full incretin effect. There is a paucity of data correlating GIP to HIV associated pre-eclampsia development.

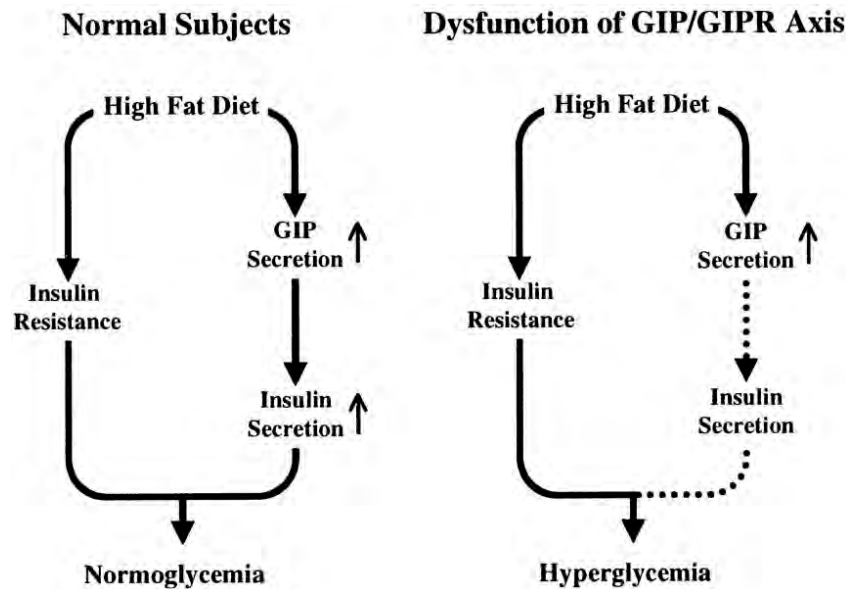


Figure 1.9: Mechanism of action of GIP in normal and abnormal conditions (www.pnas.org)

### 1.5.6 Glucagon-like peptide 1 (GLP-1)

In the early 1980's, during the cloning of anglerfish pre-proglucagons, the scientist observed that the molecule being cloned, in addition to glucagon, contained a glucagon-related peptide which appeared to have strong homology to the sequence of GIP (Kieffer, 2004). Additionally, the similar resemblance to GIP homology sequence suggested that the molecule might be an intestinal incretin hormone. According to Kieffer (2004), the notion that the molecule is an incretin hormone was highly welcomed by the scientific community since other investigators have observed similar pre-proglucagons mRNAs were expressed in the anglerfish pancreas and intestine. Furthermore, mammalian pre-proglucagons were subsequently cloned and were termed glucagon-like peptides. In 1987, it was demonstrated that that GLP-1, just like GIP, is a potent insulinotropic hormone (Marathe *et al.*, 2013). According to Kieffer (2004), GLP-1 is produced by intestinal L cells during

post translational processing of pro-glucagon. Following that, the molecule was name glucagon-like peptide (GLP)-1 (Kieffer, 2004).

Its release is stimulated in response to feeding, suggesting that it plays a role in the control of blood glucose level. Receptors of GLP-1 are also found in the hypothalamus, brain and in areas without a blood-brain barrier. GIP-1 has several physiological functions which include; increasing insulin secretion from the pancreas in a glucose-dependent manner, decreasing glucagon secretion from the pancreas by engaging specific G protein-coupled receptor and increasing insulin-sensitivity in both alpha cells, and beta cells and decreasing food intake by increasing satiety in brain (MacDonald *et al.*, 2002). GLP-1 is found in milk and according to Schueler *et al.*, (2013). Additionally, breast milk fed babies regulate their weight better than formula fed babies, hence less likely to be overweight, this suggest that GLP-1 has some protective effect against obesity later in life.

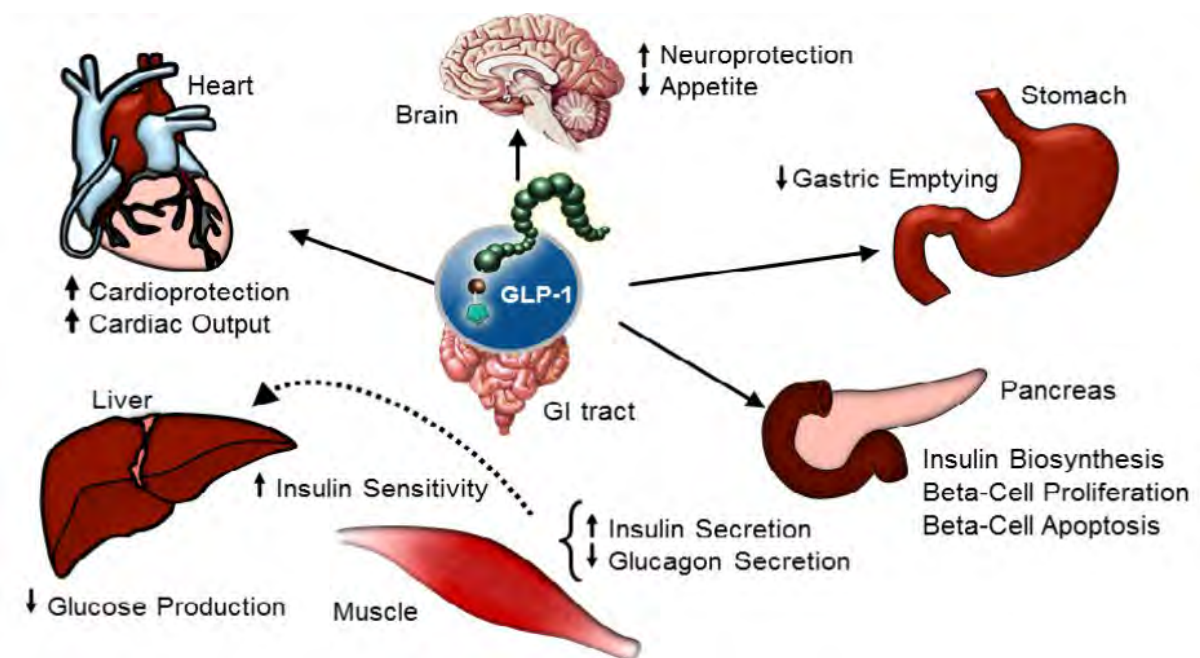


Figure 1.10: An illustration of the mechanism of action of GLP-1. GLP-1 increase the secretion of insulin secretion in pancreatic beta cells (Drucker & Nauck, 2006).



### 1.5.7 Insulin

Insulin is a peptide hormone that was named by Sir Edward Schafer, additionally, he described the actions of insulin. In 1921, it was discovered by Banting and Best, the details of insulin discovery are highlighted/outlined in further by Karamitsos (2011). It plays a crucial role in the regulation of metabolism of carbohydrates and fats by increase the uptake of glucose by the cells, glycogenesis, lipogenesis and inhibition of production of glucose by the liver (Sonksen and Sonksen, 2000). The human insulin protein is composed of 51 amino acids, and has a molecular mass of 5808 Da. It is a dimer of an A-chain and a B-chain, which are linked together by disulfide bonds (Kunt *et al.*, 1999). Insulin's structure varies slightly between species of animals. Insulin from animal sources differs somewhat in "strength" (in carbohydrate metabolism control effects) from that in humans because of those variations. Porcine insulin is especially close to the human version.

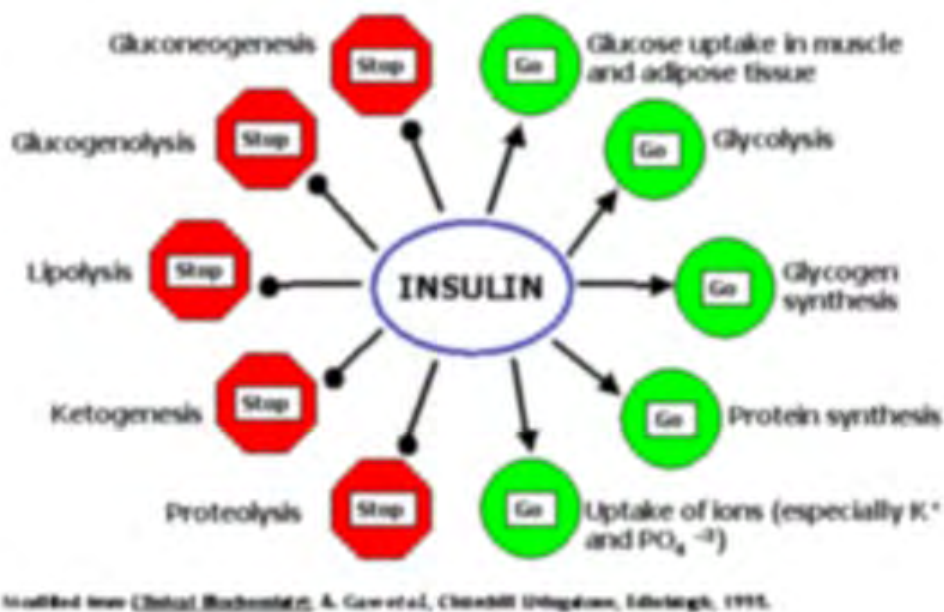


Figure 1.11: Schematic diagram showing the actions of insulin once it is released by the pancreatic cells.

#### 1.5.7.1 Insulin secretion and receptors

Insulin is secreted by the Islets of Langerhans of pancreas beta cells, it is normally released during food ingestion and when blood glucose levels are rising, except in the case of diabetes mellitus and metabolic syndrome. It comprises of two polypeptide chains namely; A- and B- chains, which are linked together by disulfide bonds. According to Kunt *et al.*, (1999), insulin is first produced as a single polypeptide called pre-proinsulin which contains a 24-residue signal peptide which is responsible for directing the nascent polypeptide chain to the rough endoplasmic reticulum (RER). The pro-insulin molecule is cleaved in the secretory granules into equimolar amounts of insulin and c-peptide (Kunt *et al.*, 1999).

When control of insulin levels fails, diabetes mellitus can result. As a consequence, insulin is used medically to treat some forms of diabetes mellitus. Patients with type 1 diabetes depend on external insulin (most commonly injected subcutaneously) for their survival because the hormone is no longer produced internally. Patients with type 2 diabetes are often insulin resistant and, because of such resistance, may suffer from a "relative" insulin deficiency. Some patients with type 2 diabetes may eventually require insulin if dietary modifications or other medications fail to control blood glucose levels adequately. Over 40% of those with Type 2 diabetes require insulin as part of their diabetes management plan.

Previous studies have shown a connection between the increased frequency of type 2 diabetes in women with a history of pre-eclampsia. Carr and associates reports that the degree of insulin sensitivity determines glucose metabolism (Carr *et al.*, 2011). A decrease in insulin sensitivity is associated with high levels of blood glucose and this subjects pregnant women to a greater risk of developing pre-eclampsia. Long exposure to antiretroviral therapy (ART) specifically protease inhibitors is associated with insulin resistance among HIV infected patients (Schueler *et al.*, 2013); (van Zoelen *et al.*, 2013). Factors such as obesity and physical inactivity, immune dysregulation and inflammation contribute to the increased prevalence of insulin resistance and diabetes in HIV infected patients (Hall *et al.*, 2014; Young *et al.*, 2010).

### ***1.5.8 Glucagon***

Glucagon is a 29-amino acid polypeptide produced by the alpha cells of the pancreas. Its primary structure in humans is: NH<sub>2</sub>-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-COOH. It is an insulin antagonist, increasing blood glucose unlike insulin which decreases blood glucose levels (Reece and Campbell, 2002). Glucagon was initially discovered by Kimball and Murlin in 1920 but was only officially documented in 1923. In tandem with insulin it regulates blood glucose level, *ie.*, insulin release is stimulated by high glucose levels (e.g. after eating) whilst glucagon release is stimulated by low blood glucose levels (e.g. during starvation). Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level.

Glucagon elevates the concentration of glucose in the blood by promoting gluconeogenesis and glycogenolysis. It accomplishes this by binding to glucagon receptors and stimulating liver cells to convert glycogen into glucose with subsequent release into the bloodstream, in a process known as glycogenolysis. When glucose stores in the liver run out, glucagon levels turn-off glycolysis in the liver and then encourages the liver and kidney to produce additional glucose through a process known as gluconeogenesis. Additionally, glucagon is also responsible for regulating the rate of glucose production through the breakdown of fats (Liljenquist *et al.*, 1974). Elevated levels of glucagon are associated with conditions such as pancreatic tumours and glucagonoma.

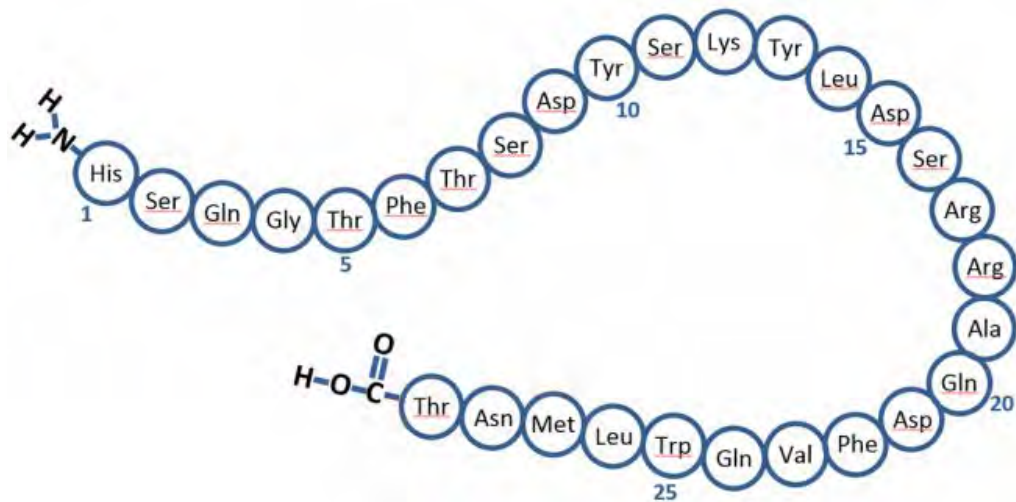


Figure 1.12: Amino acid sequence of glucagon ([www.diapedia.org](http://www.diapedia.org)). Glucagon is a 29-amino acid polypeptide. Its primary structure in humans is: NH<sub>2</sub>-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-COOH.

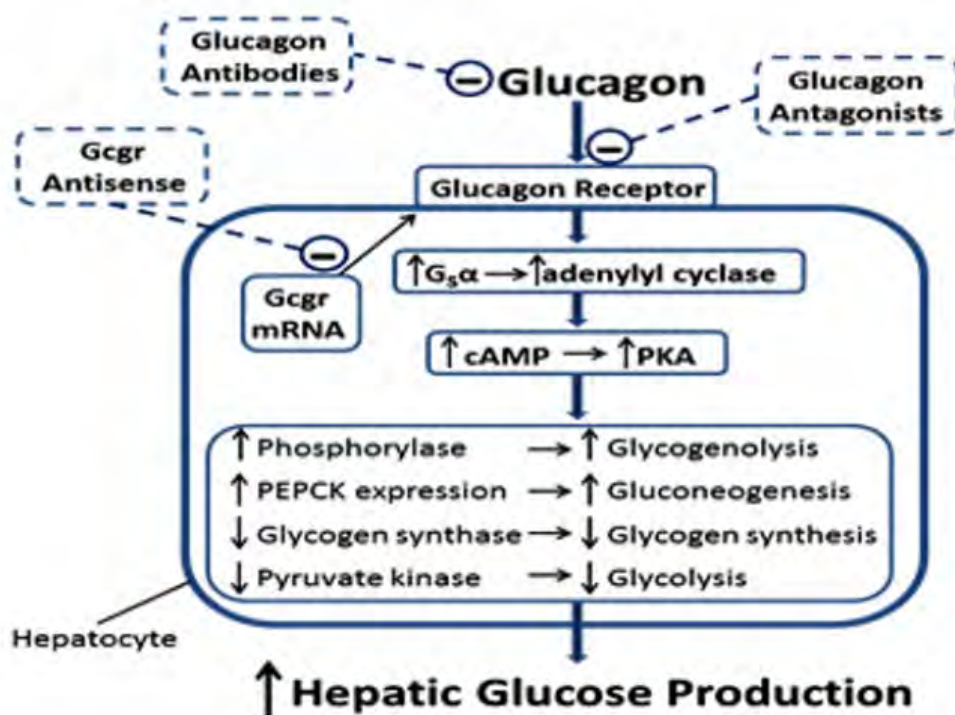


Figure 1.13 Schematic outline of the mechanism of action of peptide hormone, glucagon. Adapted from ([www.dipedia.org](http://www.dipedia.org)).

### ***1.5.9 Plasminogen activator inhibitor (PAI)-1***

#### *1.5.9.1 Background information*

PAI-1 is a serine protease inhibitor protein that is encoded by serine protease inhibitor protein SERPINE 1 gene in humans and is also known as endothelial plasminogen activator inhibitor-1 or SERPINE 1. The SERPINE1 gene is reportedly located on chromosome 7. Knudsen and colleagues reported that PAI-1 is the main inhibitor of the tissue plasminogen activator, which prevents the natural degradation of thrombin (Knudsen *et al.*, 2014). Receptors of PAI-1 are expressed in several tissues such as the liver, lung, heart, kidney, platelets and adipose tissue (Samad & Loskutoff, 1996). Its regulators include cytokines, growth factors, hormones, endotoxins, glucocorticoids, angiotensin, p53 and fatty acids (Sandberg *et al.*, 1997; Loskutoff & Samad, 1998).

PAI-1 is also a principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA), which are the activators of plasminogen and hence fibrolysis. Fibrolysis is known as the physiological breakdown of blood clots. It also plays a crucial role in the extracellular matrix remodelling. It is synthesized by the endothelial cells and is present in plasma and in blood platelets. It is also known as endothelial plasminogen activator inhibitor and is said to be associated with decrease fibrinolytic activity of euglobulins.

#### *1.5.9.2 Synthesis and secretion*

PAI-1 is one of the earliest inflammation response genes, stimulated by cytokines, growth factors and endotoxins. It is mainly produced by the endothelium, but it is also secreted by other tissue types such as adipose tissue. PAI-1 can convert to a stable latent form automatically. Its deficiency is associated with abnormal bleeding, which has been observed in patients that are homozygous for the PAI-1 gene (Fay *et al.*, 1997). Another function of PAI-1 is that it inhibits the activity of the

matrix metalloproteinases, which reportedly play a crucial role in the invasion of malignant cells across the basal lamina.

In obesity, PAI-1 levels are reportedly elevated which leads to metabolic syndrome. Risk of thrombosis is high in obesity and in metabolic syndromes. Additionally, angiotensin 2 increases the synthesis of PAI-1, resulting in accelerated risk of atherosclerosis development. Furthermore, studies have shown/ reported that increased levels of PAI-1 are associated with arterial hypertension.

#### *1.5.9.3 Over-expression of PAI-1*

The expression of PAI-1 is regulated by EGF and TGF-beta, it is reported that alteration in the gene expression of PAI-1 and its elevation, are associated with fibrosis and metabolic syndromes. Thus elevated levels of PAI-1 are associated with increased risk of developing conditions such as thrombosis and atherosclerosis. Additionally, previous studies have demonstrated PAI-1 as a potential biomarker of endothelial dysfunction, inflammation and coagulation which are associated with atherosclerosis, pre-eclampsia and cardiovascular diseases.

According to Knudsen and associates (2014), elevated levels of PAI-1 are associated with high levels of first time myocardial infarction in HIV-1 infected individuals. They report that HIV is associated with high chronic state of inflammation, immune activation via TNF-alpha. Individuals who are HIV positive and or on ART have even higher markers of endothelial dysfunction, however the mechanistic effect of ART on endothelial dysfunction markers still requires clarity. Some suggest that, regardless of whether a person receives anti-HIV treatment or not, the mere fact that HIV is associated with inflammation and activation of immune cells will lead to increase in markers of endothelial dysfunction elucidating to the fact that infection alone can increase the levels of PAI-1. Nonetheless, there seem to be an association between HIV positive individuals on ART and the development of first time myocardial infarction (Herrari *et al.*, 2003). Another plausible

suggestion is that HIV infected individuals only have elevated PAI-1 levels after years of chronic inflammation state in HIV-1 infected individuals. Furthermore, Gudinchet *et al.*, (1988), reported that PAI-1 is associated with multiple sclerosis in the nervous system. During pregnancy profound alterations of the coagulation and the fibrinolytic system occur and elevated levels of PAI-1 during pregnancy are associated with miscarriage.

#### *1.5.9.4 PAI-1 gene polymorphism and adverse pregnancy outcomes*

There is reportedly a common deletion polymorphism that results in a sequence of 4G instead of 5G in the promoter region of the gene associated with small increase in the risk of venous thromboembolism (Said *et al.*, 2012). Some studies have reported that this polymorphism is associated with adverse pregnancy outcomes, however, the topic remains controversial. In the study by Said and colleagues, women were genotyped for the 4G/5G polymorphism and no association was found (Said *et al.*, 2012).

Notably, it is reported that the polymorphism does not alter the gene expression of PAI-1, however, it alters the expression which is reportedly a high risk for thrombosis which is associated with high risk of venous thromboembolism and coronary artery disease which can significantly affect pregnancy outcome.

During pregnancy, profound changes take place in the hemostatic system and the level of PAI-1 increases (Kruithof *et al.*, 1987). In general, normal pregnancy is said to be associated with a reduction in fibrinolysis, which is due to an increase in fibrinolytic inhibitors particularly PAI-1 which is responsible for inhibiting plasminogen, this results in the reduction in the activation and production of plasminogen to plasmin which is associated with thromboembolism (Kruithof *et al.*, 1987). Fibrinolytic activity during pregnancy decreases, due to increase levels of PAI-1 derived from the placenta and from an increase of fibrinogen factor VII and VIII and the Von Willebrand's

factor. PAI-1 is associated with the development of severe pre-eclampsia and consequentially with fetal growth restriction, placental abruption, stillbirth and neonatal deaths.

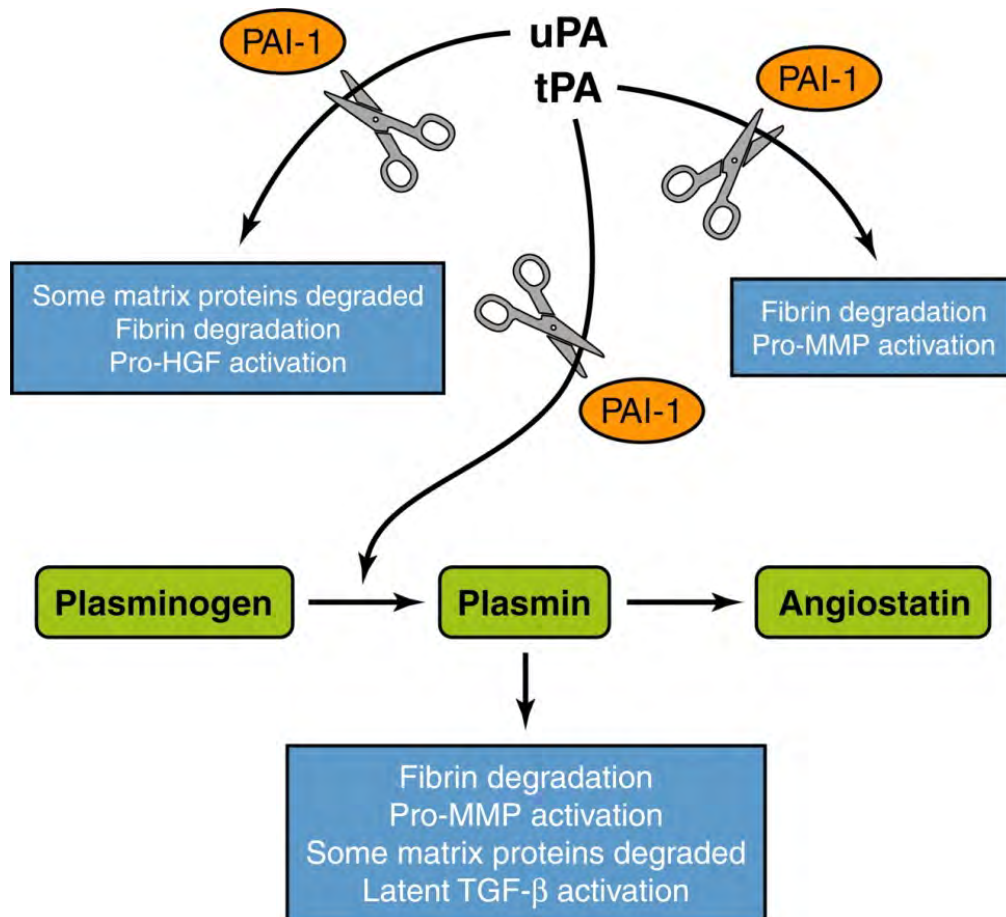


Figure 1.14: PAI-1 mechanism of action in the cell migration pathway. (Czekay *et al.*, 2011).

### 1.5.10 Visfatin

Visfatin is an enzyme/cytokine produced by adipose tissue. It is also known as nicotinamidephosphor-ribosyltransferase (NAmPRTase or Nampt) or as pre-B-cell colony-enhancing factor 1 (PBEF1), additionally, It is also implicated in nicotinate and nicotinamide metabolism (Zulfikaroglu *et al.*, 2010). NAmPRTase catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide, one step in the biosynthesis of nicotinamide adenine dinucleotide. Visfatin is involved in the regulation of glucose



homeostasis because it has insulin-mimicking effect through the activation of an insulin receptor (Zulfikaroglu *et al.*, 2010). The protein is an adipokine that is localized to the bloodstream and has various functions, including the promotion of vascular smooth muscle cell maturation and inhibition of neutrophil apoptosis. Visfatin is expressed in the brain, lung, kidney, spleen, testes etc, but it reportedly expressed more in visceral adipose tissue than any other organ and elevated levels of this protein have been reported correlate with obesity (Fasshauer *et al.*, 2007).

It is also responsible for the promotion of B cell maturation and is linked with the pathogenesis of type 2 diabetes through its role in the pathophysiology of insulin resistance, especially among obese individuals (EI-Shafey *et al.*, 2014). Off note, diabetes type 2 is more prevalent in obese individuals who are allied to high expression of adipose tissue derived cytokines/factors, it is therefore plausible to suggest that individuals with higher BMI will express high levels of visfatin hence higher have a higher risk of becoming insulin resistance and developing diabetes type 2 (Fasshauer *et al.*, 2007).Visfatin dysregulation in patients with insulin resistance may mediate predisposition to pre-eclampsia development. Visfatin levels are elevated in pregnancies especially in the third trimester.

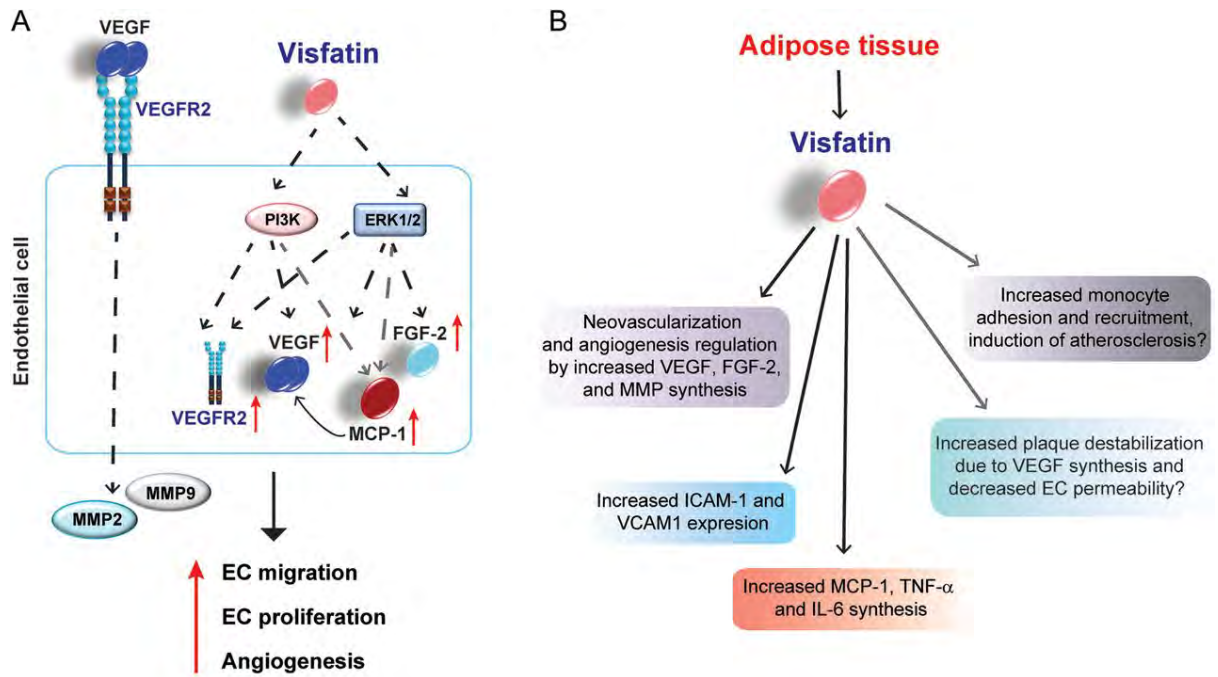


Figure 1.15: Schematic outline the mechanism of action of Visfatin (Cardiovascres.oxfordjournals.org)

## **1.11 Aims, objectives and null hypothesis**

### ***1.11.1 Aims***

To compare the level of adipokines viz., C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in non-pregnant, normotensive pregnant and pre-eclamptic women infected and uninfected with HIV.

### ***1.11.2 Objectives***

- To determine the maternal serum levels of adipokines (C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin) across all study groups.
- To compare maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in normotensive pregnant compared to the non-pregnant women.
- To compare maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in HIV positive women matched with HIV negative normotensive pregnant women.
- To compare maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in HIV associated pre-eclampsia compared to HIV negative pre-eclamptic patients.
- To compare maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in patients with early onset and compare to patients with late onset pre-eclampsia.
- To compare maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in pre-eclamptic participants versus normotensive participants
- To compare maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin in pre-eclamptic participants versus non-pregnant participants

### ***1.11.3 Null Hypothesis***

The maternal serum levels of C-peptide, Ghrelin, GIP, GLP-1, Glucagon, Insulin, Pal-1 and Visfatin will remain unchanged across all groups irrespective of HIV status and type of pregnancy (normotensive & pre-eclamptic) compared to the non-pregnant state.

## CHAPTER 2

## **MATERIALS AND METHODS**

### **2.1 Study design**

This is a retrospective experimental study that utilises blood archived from a previous study (BE256/12). The blood was obtained from pregnant Black South African women attending the RK Khan Hospital (KwaZulu-Natal, South Africa). Following approval by the institutional Biomedical Research Ethics Committee (Ethics reference no: BE256/12; appendix 1), Department of Health (appendix 2) and the medical superintendent (appendix 3) the study commenced. Informed patient consent was obtained from all women (appendix 4).

#### ***2.1.1 Recruitment of participants***

Pregnant women were recruited at their first antenatal visit at the Obstetric Unit, RK Khan Hospital. Only primiparous women over 18 years of age were enrolled in the study, after signing informed written consent forms. The non-pregnant group was recruited from the family planning clinic, RK Khan Hospital.

Women were first screened and counselled before being offered entry into the study. Patient demographics (ethnicity, age, blood pressure, gestational age, maternal weight, body mass index (BMI) at first visit, fasting glucose tolerance test, fasting insulin levels, HIV status, CD4 counts and ARV / PMTCT use as appropriate) was collated on a formal patient data sheet (appendix 5).

### **2.2 Study population**

The study population was divided into two main study groups, the pregnant and non-pregnant (Figure 2.1). The pregnant group was subdivided into the pre-eclamptic and the normotensive groups. The pre-eclamptic group was further subdivided into early onset and late onset pre-

eclamptic groups. These subgroups underwent stratification into HIV positive and HIV negative groups.

Non pregnant participants were eligible for entry provided they were between 18 – 45y, did not have hypertension, were not being treated for a chronic illness including endocrinopathies or cancer and were more than six weeks post partum.

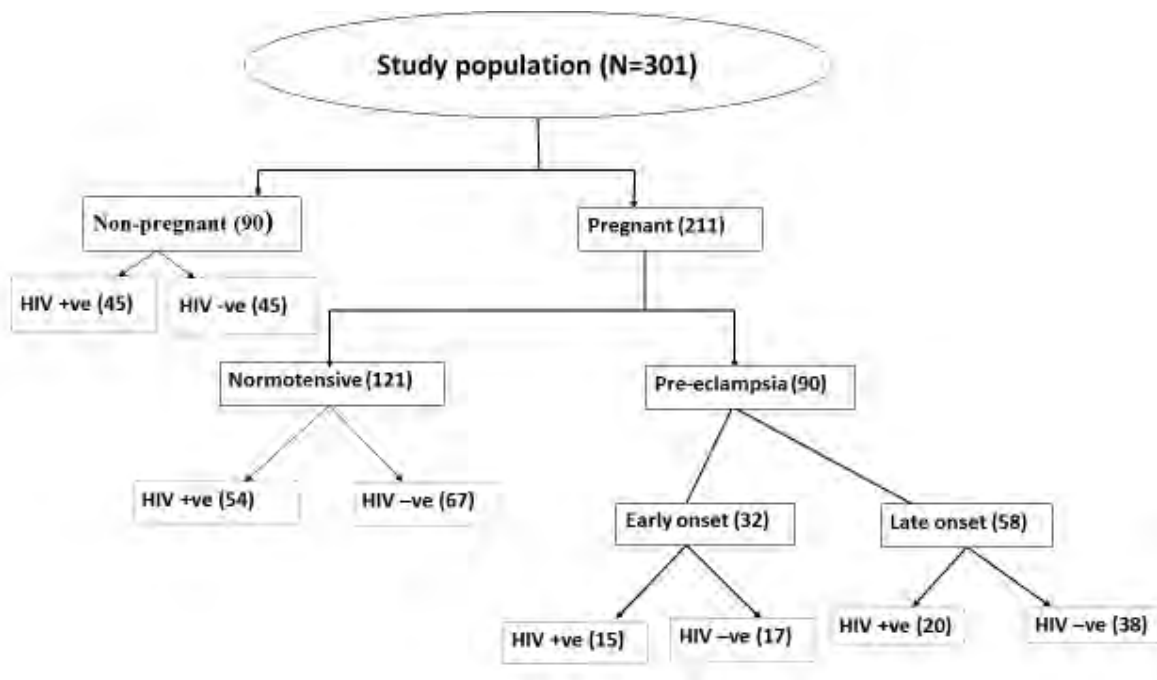


Figure 2.1: Schematic outline of study population of our study.

**2.2.1 Inclusion criteria**

Inclusion criteria for pre-eclampsia included the new onset of hypertension (BP  $\geq$  140/90 mmHg) and proteinuria after 20 weeks of gestation (proteinuria of at least 1+ on dipstick). HIV positive participants were sub categorized according to their CD4 count (i.e.  $\geq$  200 or  $<$  200) within each cohort.

### ***2.2.2 Exclusion criteria***

Women who had chronic hypertension, were non-black African, had gestational diabetic, chronic diabetic, unknown HIV status, chorioamnionitis, polycystic ovarian syndrome, thyroid disorders, chronic renal disease, cardiac failure, connective tissue disease / antiphospholipid syndrome and abruptio placentae were excluded from the study.

### **2.3 Sample collection and preparation**

Blood samples were collected from the women by an obstetrician and gynecologist at the Obstetric Unit, RK Khan Hospital and delivered to the Optics and Imaging Centre, Doris Duke Medical Research Centre, University of KwaZulu-Natal within 2 hours of collection.

The samples were then centrifuged at 3000 revolutions per minute (rpm) for 10 min. Following centrifugation, plasma, serum and buffy coats were aliquoted and stored in cryovials tubes at -80 °C. Upon completion of sample collection, these samples were then transferred to the Staten Serum Institut (SSI) in Copenhagen, Denmark in a 4 °C ice box in a period of 48 hours. Transfer of samples to Copenhagen were in accordance with the international standards (appendix 6). At the Staten Serum Institut, samples were stored in a cold room until analyses

## **2.4 Method**

### ***2.4.1 Principles of multiplex analysis***

A commercially available 10 plex diabetes kit was used to determine the levels of adipokines in all study groups. The 10 plex diabetes kit was used to perform a luminex technique that works in a



flowmetric manner and is based on microspheres/beads that (5,6 $\mu$ m in diameter) act as a solid support for the luminex reactions. Bio-plex assays are referred to as bead-based multiplex assays which allows the measurement of diabetes biomarkers in various matrices including serum, plasma and culture supernatant ([www.bio-rad.com/bio-plex](http://www.bio-rad.com/bio-plex)). Circulating proteins are used as biomarkers for Bio-plex and are involved in the regulation of digestion and glucose metabolism. The multiplexing feature makes it possible to quantify the levels of multiple diabetes markers in a single well of a 96-well microplate in just 4 hours, using as little as 12.5 $\mu$ l of serum or plasma samples, or 50  $\mu$ l of tissue culture supernatant ([www.bio-rad.com/bio-plex](http://www.bio-rad.com/bio-plex)). The principles of the Bioplex diabetes assays are otherwise the same as with ELISA, and it can be used for direct, indirect, competitive and sandwich immunoassays. This study used the sandwich immunoassay form of ELISA which involved coupling captured antibodies with the beads, incubating the beads with the samples, bionylated detection antibodies and adding a substrate (fluorophore) to detect the reactions.

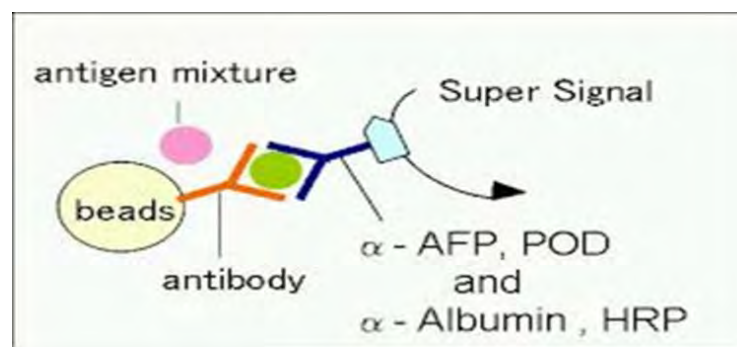


Figure 2.0.2: Schematic representation of multiplex enzyme linked immunosorbent assay ([www.pssbio.com/multiplexELISA](http://www.pssbio.com/multiplexELISA)).

#### 2.4.2 Multiplex method

The first step in the bio-plex assay preparation involved planning the plate layout which was followed by preparing the standards. The standards were prepared in a 1:4 dilution. For sample preparation, 20 $\mu$ l of the sample and 80 $\mu$ l of the assay diluent were mixed together to make a total

volume of 100µl (1:4 dilution ratio). The 20x coupled beads were vortexed for 30 seconds and diluted to 1x (288µl of beads and 5,472µl of assay buffer) in bioplex assay buffer. The diluted standards, samples, beads and controls were then equilibrated at room temperature for 20 min before use.

The 96-well filter plate was pre-wetted with 100µl bioplex assay buffer. The diluted beads were vortexed for 10-20 seconds prior to adding 50µl of the beads to each well of the assay plate. The assay plate was then washed three times with 100µl of the bioplex wash buffer. The diluted standards, samples and controls were vortexed before adding 50µl to each well (changing the pipet tip after every volume transfer) of the assay plate. This was followed by covering the plate with a sealing tape and then incubating it on a shaker at 850+/- 50 rmp at room temperature for 1 hour. While the samples were incubating, the 20x detection antibody was vortexed for 30 sec and rapidly-spun prior to pipetting in order to collect the entire required volume at the bottom of the vial. The 20x detection antibody was diluted to 1x by mixing 300µl of 20x detection antibody with 2700µl of detection antibody diluent to make a sufficient volume (3000µl) of detection antibody.

After incubating the samples, the sealing tape was removed slowly and the plate was placed onto a magnetic plate and thereafter supernatant was discarded by inversion. Thereafter, the plate was rinsed 3 times with 100µl of the Bioplex wash buffer. Detection antibody (25µl) was then added gently to each well and the plate was incubated for 30 min on a shaker at 850+/- 50 rmp at room temperature.

While the detection antibody was incubating with 10 minutes left to incubation time, a quick-spin centrifugation of the streptavidin-PE (100x) was performed prior to pipetting in order to collect the entire volume at the bottom of the vial. A sufficient volume of streptavidin-PE (1x) was prepared by mixing 60µl of 100x streptavidin-PE and 5940µl assay buffer and then covered with aluminium

foil in order to protect from sunlight. After the detection antibody incubation, the sealing tape was slowly removed, the magnetic plate attached, and supernatant discarded. The plate was then washed 3 times with 100µl of the Bioplex wash buffer. This was followed by addition of 50µl streptavidin-PE (1x) to each well, the plate was then covered with foil and incubated for 10 minutes. After the streptavidin-PE incubation, the sealing tape was removed before reading the plate using a Bioplex MAGPIX instrument which was prepared 30 minutes prior to use.

The results obtained were exported to excel to be analysed. Measurements and data analysis of all assays were performed on the Bioplex system in combination with Bioplex manager software version 4.1.1 using 5-parametric curve fitting (Bio-Rad).

## **2.5 Data analysis**

Graph Pad Prism (version 6) was used to analyse the data. To assess the normality distribution of data, the Shapiro-Wilk test was performed. To analyse non-normal data, we used non-parametric tests (Kruskal Wallis H test and Mann-Whitney U test) and to analyse normal distributed data, we used parametric tests (Analysis of variance test (ANOVA) and independent samples t-test). A *p* value of <.05 was used to measure the statistical significance of the dependent variable.

## CHAPTER 3

## **RESULTS**

### **3.1 Demographic and clinical data of patients**

The study population consisted of 30% non-pregnant, 40% normotensive pregnant and 30% pre-eclamptic women, equally stratified for HIV status. With regards HIV status, the study population comprised 45% HIV positive and 55% HIV negative women. The pre-eclamptic group was stratified into early and late onset pre-eclampsia. The patient demographics are displayed in Table 1 below as mean and standard deviations

Table 3.1: Clinical demographics of adipokines across the study groups (stratified by HIV status) of pregnant and non-pregnant women. Clinical demographics values are shown as <sup>a</sup>median and interquartile range and as <sup>b</sup>mean and standard deviations.

	Non-pregnant n=90	Normotensive n=121	EOPE n=32	LOPE n=58
Age (years) <sup>b</sup>	33.10±7.5	25.59±5.63	28.19±6.26	25.28±5.9
Weight (kg) <sup>a</sup>	75.25 (64.85- 88.0)	75.00 (65-82.75)	69 (63.13-83.15)	78.25(69.75-97.70)
Systolic Blood Pressure (mmHg) <sup>a</sup>	119 (112-127)	121 (110-128)	168 (161.3-178.8)	161 (151-171.5)
Diastolic Blood Pressure (mmHg) <sup>a</sup>	74.50 (65-80)	73 (68-80)	104 (95.25-114.8)	101 (94.50-108)
CD4 (cells/mm <sup>3</sup> ) <sup>b</sup>	466.95±442.70	425.02±197.79	348.20±145.98	427.44±6.79
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	30.40 (25.97- 34.78)	29.50 (26.15-33.33)	28.59 (25.42-35.40)	31.61 (27.10-36.88)
Gestational age (weeks) <sup>a</sup>	-	39 (38-40)	31 (27.75-35.25)	37 (34-38)
Baby weight (kg) <sup>a</sup>	-	3.2 (2.99-3.57)	1.4 (1.01-1.920)	2.87 (2.4-3.12)
Placental weight (g) <sup>b</sup>	-	621.13±94.73	461.36±201.71	624.36±158.80

Table 3.2: Patient clinical demographics across the study population stratified by HIV status. Clinical demographics values are shown as <sup>a</sup>median and interquartile range and as <sup>b</sup>mean and standard deviations.

	NON-PREGNANT		NORMOTENSIVE		EARLY ONSET		LATE ONSET	
	HIV <sup>-ve</sup> n=45	HIV <sup>+ve</sup> n=45	HIV <sup>-ve</sup> n=54	HIV <sup>+ve</sup> n=67	HIV <sup>-ve</sup> n=15	HIV <sup>+ve</sup> n=17	HIV <sup>-ve</sup> n=21	HIV <sup>+ve</sup> n=37
Age (years) <sup>b</sup>	31.42±8.604	34.76±5.748	24.16±5.236	27.35±5.637	28.94±7.420	27.33±4.731	24.51±5.695	26.78±6.385
Weight (Kg) <sup>a</sup>	75.50 (65.75-88.50)	73 (64-86.25)	76.00 (68-86.90)	70.60 (64.75-80)	68.50 (62.70-82.10)	69 (63.5-89.70)	76.80 (69.75-96.25)	84.10 (68.50-101.2)
Systolic pressure (mmHg) <sup>a</sup>	120 (111.5-127)	117 (112-127)	122 (111.5-128)	119.5 (110-125.3)	166 (160.5-179.5)	171 (163-178)	164.5 (156.5-175)	156 (149.5-168)
Diastolic pressure (mmHg) <sup>a</sup>	75 (64-78)	74 (63-81.50)	75 (66-82)	72.50 (69-78)	104 (93.50-113.5)	106 (97-116)	102.5 (97.75-109.3)	99.50 (92.25-106.5)
CD4 (cells/mm <sup>3</sup> ) <sup>b</sup>	---	467±442.7	---	425±197.8	---	348.2±146	----	427.4±173.8
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	31.02 (26.73-36.28)	29.40 (25.86-34.36)	31.51 (27-34.64)	28.54 (25.84-31.53)	29.52 (26.14-32.29)	26.29 (23.61-35.73)	29.66 (26.99-36.88)	34.04 (27.02)
Gestational age (weeks) <sup>a</sup>	----	----	39 (38-40)	39 (38-40)	31 (29-35)	31 (27-36)	38 (34-39)	36 (34-38)
Baby weight (kg) <sup>a</sup>	----	---	3.250 (3-3.655)	3.2 (2.97-3.47)	1.51 (1.193-2.423)	1.33 (0.93-1.8)	3 (5.545-3.250)	2.6 (2.24-3.050)
Placental weight (g) <sup>b</sup>	---	---	628±98.24	612.5±90.35	492.3±236.2	416.7±138.9	666.3±164.5	557.3±127.1

Table 3.3: Analysis of clinical demographics across study groups. Statistical test used; <sup>a</sup>Mann Whitney U test (U and p value) and <sup>b</sup>independent t-test (t and p value). Values highlighted in red indicate significant difference.

	Normotensive Vs Non-pregnant	Normotensive Vs Early onset PE	Normotensive Vs Late onset PE	Non-pregnant Vs Early onset PE	Non-pregnant Vs Late onset PE	Early onset Vs Late onset PE
Age (years) <sup>b</sup>	8.337 (p<0.001)	2.271 (p<0.05)	0.3216 (p>0.05)	3.326 (p>0.05)	6.491 (p<0.001)	6.491 (p<0.001)
Weight (kg) <sup>a</sup>	5187 (p>0.05)	1770 (p>0.05)	2861 (p<0.05)	1293 (p>0.05)	2257 (p>0.05)	700.5 (p>0.05)
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	5240 (p>0.05)	1867 (p>0.05)	2992 (p>0.05)	1354 (p>0.05)	2341 (p>0.05)	788 (p>0.05)
Systolic BP (mmHg) <sup>a</sup>	5156 (p>0.05)	20 (p<0.001)	70.50 (p<0.001)	19 (p<0.001)	51 (p<0.001)	705.5 (p>0.05)
Diastolic BP (mmHg) <sup>a</sup>	5231 (p>0.05)	130 (p<0.001)	271 (p<0.001)	91 (p<0.001)	180.5 (p<0.001)	819 (p>0.05)
CD4 (cells/mm <sup>3</sup> ) <sup>b</sup>	0.6197 (p>0.05)	1.397 (p>0.05)	0.04627 (p>0.05)	1.015 (p>0.05)	0.3658 (p>0.05)	1.401 (p>0.05)
GA (weeks) <sup>a</sup>	---	292.5 (p<0.001)	1136 (p<0.001)	---	---	286.5 (p<0.001)
Baby weight (Kg) <sup>a</sup>	---	125.5 (p<0.001)	1826 (p<0.001)	---	---	228 (p<0.001)
Placental weight (g) <sup>b</sup>	---	5.822 (p>0.05)	0.1526 (p>0.05)	---	---	3.488 (p<0.001)



Table 3.4: Analysis of patient clinical data in study groups stratified by HIV status. Statistical test used; <sup>a</sup>Mann Whitney U test (U and p value) and <sup>b</sup>independent t-test (t and p value). Values highlighted in red indicate significant difference.

	Non-pregnant	Normotensive	Early onset PE	Late Onset PE
Age (years) <sup>b</sup>	2.175 (p<0.05)	3.217 (p<0.01)	0.719 (p>0.05)	1.315 (p>0.05)
Weight (Kg) <sup>a</sup>	948.5 (p>0.05)	1367 (p<0.05)	119.5 (p>0.05)	352.5 (p>0.05)
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	904.5 (p>0.05)	1369 (p<0.05)	104 (p>0.05)	330 (p>0.05)
Systolic blood pressure (mmHg) <sup>a</sup>	940 (p>0.05)	1485(p>0.05)	124 (p>0.05)	294.5 (p>0.05)
Diastolic blood pressure (mmHg) <sup>a</sup>	960.5 (p>0.05)	1633 (p>0.05)	108 (p>0.05)	297 (p>0.05)
Gestational age (weeks) <sup>a</sup>	---	1561 (p>0.05)	77 (p>0.05)	198 (p>0.05)
Baby weight (kg) <sup>a</sup>	---	1481 (p>0.05)	87.50 (p>0.05)	260.5 (p>0.05)
Placenta weight (g) <sup>b</sup>	---	0.876 (p>0.05)	0.859 (p>0.05)	2.185 (p<0.05)

### 3.1.1 Maternal age

The mean age± standard deviation was 33.10 ± 7.47 years, 25.59 ± 5.63 years, 28.19 ± 6.30 years and 25.28 ± 5.98 years in the non-pregnant, normotensive pregnant, early-onset pre-eclamptic and the late-onset pre-eclamptic groups respectively. Maternal age ranged from 18-45 years across all the study groups.

The Shapiro-Wilks test revealed that the distribution of maternal age was normally distributed hence a parametric one-way analysis of variance (ANOVA) test was used to analyse maternal age across study groups. Based on HIV status, there was a significant difference in maternal age across the study

categories ( $F(3.292) = 28.23, p < 0.001$ ; Figure 3.1). A multiple comparison Tukey Honest significant difference (HSD) test indicated that the non-pregnant, normotensive and late-onset group were older in the HIV positive group compared to their corresponding HIV negative group (Figure 3.2). In contrast, the early onset pre-eclamptic HIV positive group were younger than their corresponding HIV negative group.

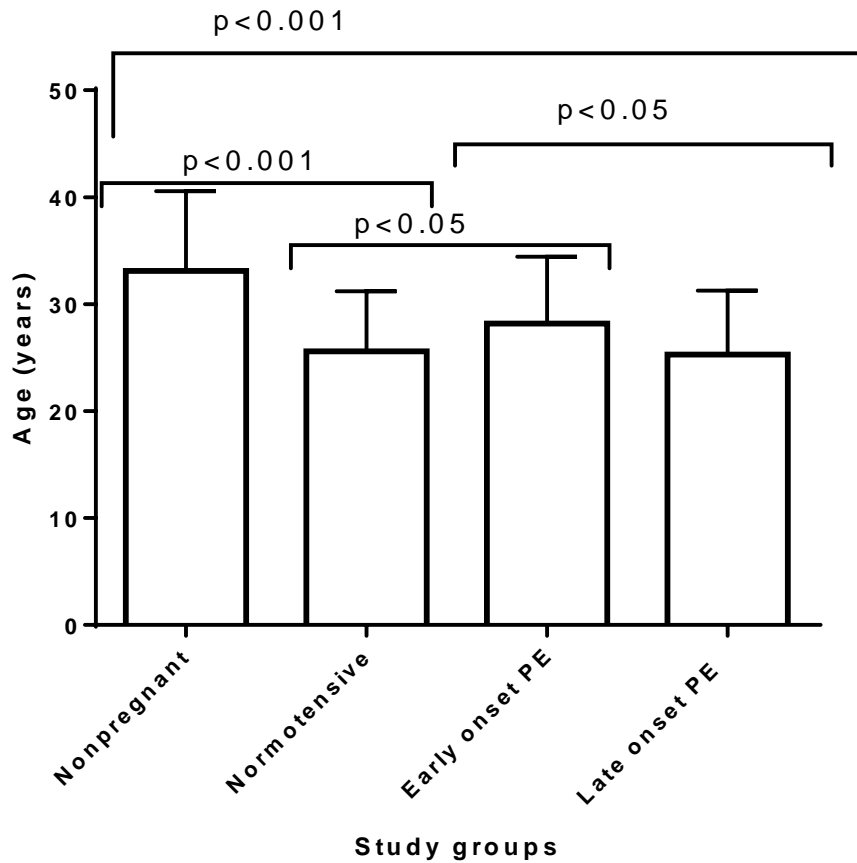


Figure 3.1: Age across study groups of pregnant and non-pregnant women.

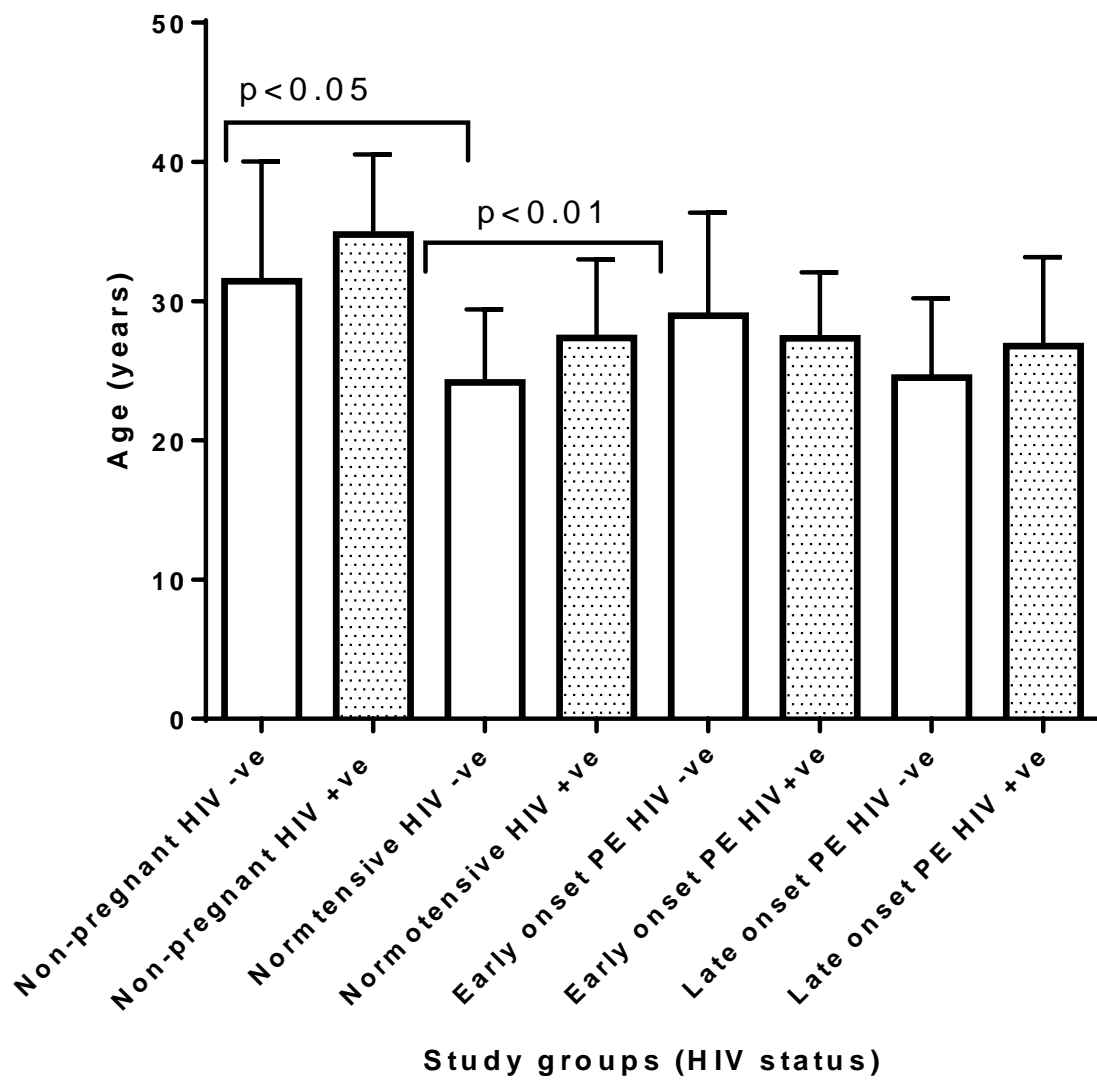


Figure 3.2: Age across study groups stratified by HIV status.

### ***3.1.2 Maternal weight***

The median maternal weight was 74.75kg (45-130kg). The Shapiro-Wilks test revealed that the distribution of maternal weight was not normally distributed hence non-parametric tests were used to assess the distribution of maternal weight across the study groups. A Kruskal-Wallis H test indicated no statistical significant in the distribution of maternal weight amongst the study groups ( $\chi^2(3)=5.245$ ;  $p>0.05$ ). However, a non-parametric Mann-Whitney U test found that there was a significance effect of HIV status on maternal weight across the study groups ( $U=1366.50$ ,  $p<0.01$ ). The test revealed that within the normotensive group, maternal weight was higher in the HIV negative group compared to HIV positive group.

### ***3.1.3 Gestational age (GA)***

Gestational age was not normally distributed amongst study groups. A Kruskal-Wallis H test revealed that there was a significant difference amongst the study groups ( $\chi^2(3)= 67.54$ ;  $p<0.001$ ; Figure 3.3).

The Mann-Whitney U test revealed no significant effect of HIV status on gestational age. Gestational age was higher in the normotensive compared to the non-pregnant group. Similarly gestational age was higher in the non-pregnant compared to the early-onset pre-eclamptic group. Additionally, GA was significantly higher in the normotensive compared to the early-onset ( $U=292.50$ ;  $p<0.001$ ) and late-onset pre-eclamptic group ( $U=2861.00$ ;  $p<0.001$ )

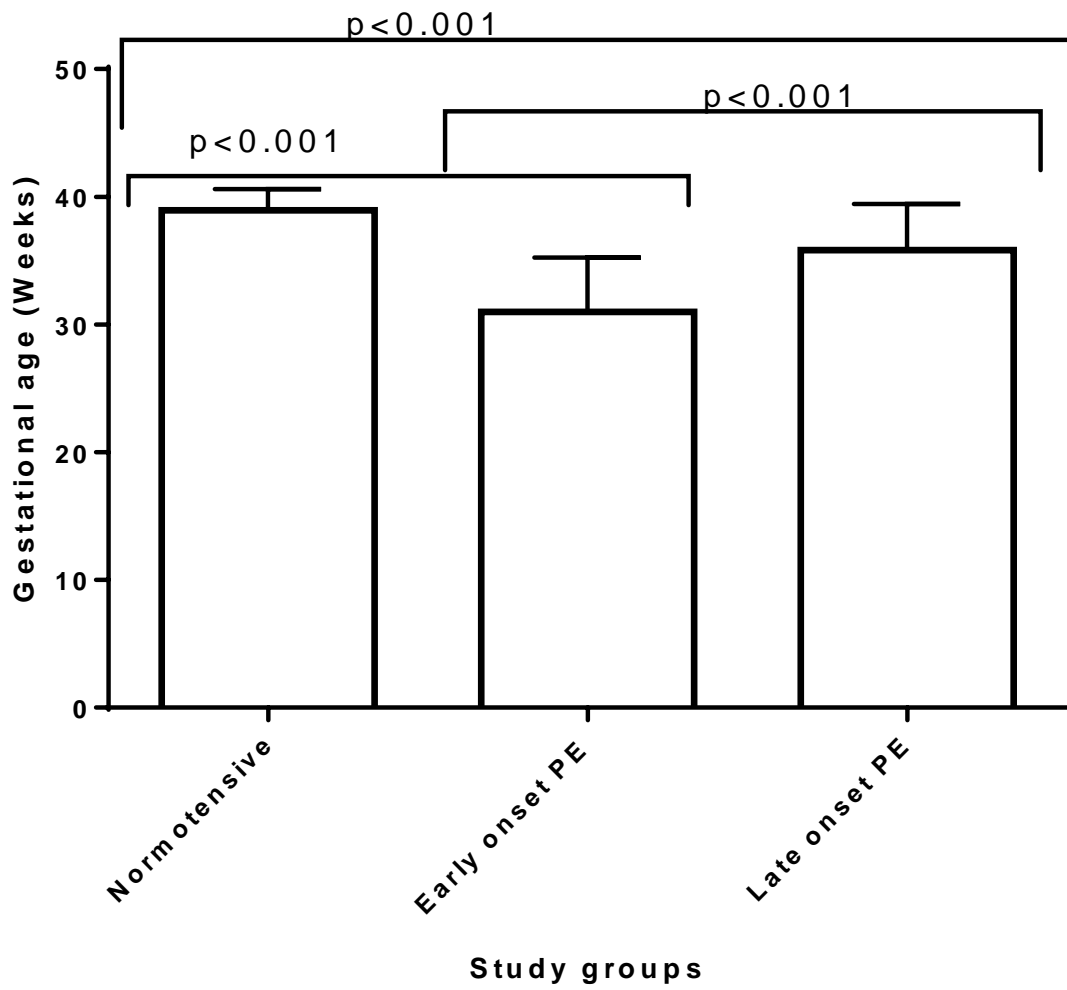


Figure 3.3: Gestational age in normotensive and pre-eclamptic pregnant groups.

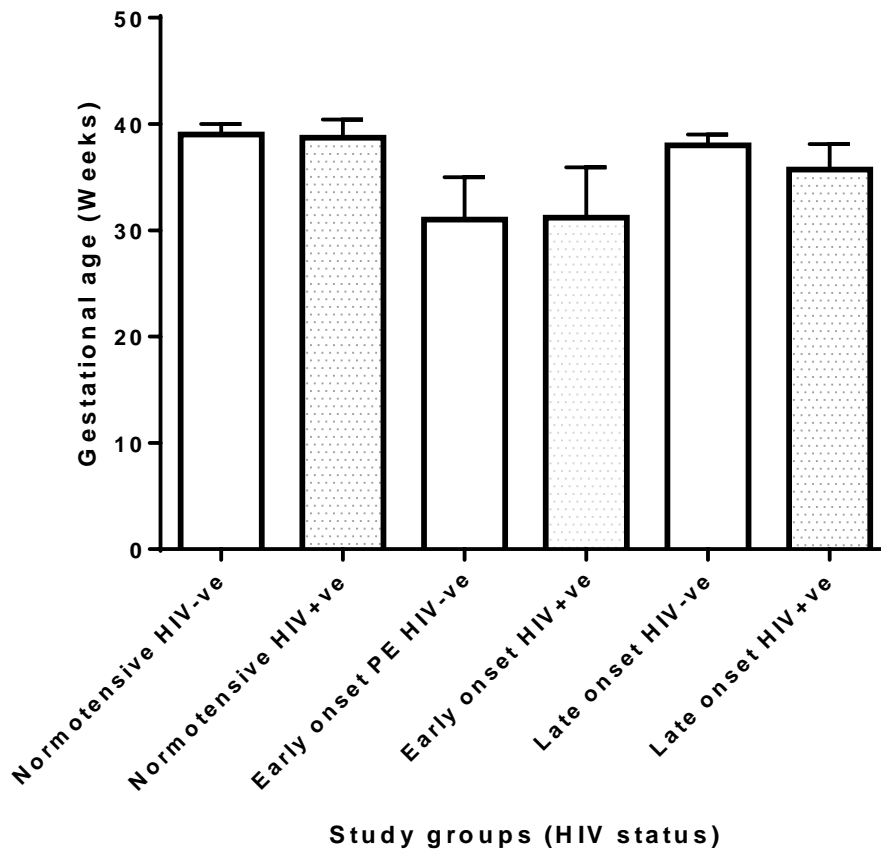


Figure 3.4: Gestational age in study groups subdivided by HIV status.

### 3.1.4 Maternal Blood Pressure

#### 3.1.4.1 Systolic pressure

The medians and IQR of systolic blood pressure is displayed on table 3.1 and 3.2. A Shapiro-Wilks test indicated that systolic blood pressure did not follow a normal distribution. Both log and square root transformation did not normalise the data hence non-parametric tests were used. A Kruskal-Wallis H test indicated that there was a significant difference in systolic blood pressure between the study groups ( $\chi^2(3) = 182.36; p < 0.001$ ). The Mann-Whitney U test showed a statistical significant higher systolic blood pressure ( $U = 9446; p < 0.05$ ) in the HIV negative compared to the HIV positive groups.

Systolic blood pressure was elevated in the early-onset ( $U=19.00$ ;  $p<0.001$ ) and late-onset ( $U=180.50$ ;  $p<0.001$ ) compared to the non-pregnant group. Furthermore, systolic blood pressure was significantly higher in the late-onset pre-eclamptic compared to the normotensive group which had a mean rank of 60.59; ( $U=70.50$ ;  $p<0.001$ ). Additionally, there was a significant difference in the early-onset pre-eclamptic versus normotensive groups ( $U=20.00$ ;  $p<0.001$ ).

#### *3.1.4.2 Diastolic pressure*

Medians and IQR of diastolic blood pressure are displayed on table 3.1 and 3.2. A Shapiro-Wilks test indicated that diastolic blood pressure did not follow a normal distribution; hence non-parametric tests were used for analysis. The Kruskal-Wallis H test indicated that there was a significance difference in diastolic blood pressure across all study groups ( $\chi^2(3)=183.36$ ;  $p<0.001$ ). The Mann-Whitney U test revealed no effect of HIV status on diastolic blood pressure. Of note, the test revealed that diastolic blood pressure was elevated in the early ( $U=130$ ;  $p<0.001$ ) and late-onset pre-eclamptic groups ( $U=271.00$ ;  $p<0.001$ ) compared to the non-pregnant group. Additionally, systolic blood pressure was significantly higher in the late-onset pre-eclamptic compared to the normotensive groups ( $U=70.50$ ;  $p<0.001$ ). Additionally, systolic blood pressure was elevated in the early-onset pre-eclamptic compared to the normotensive groups ( $U=20.00$ ;  $p<0.001$ ).

#### *3.1.5 Body mass index*

Body mass index (BMI) per group was not normally distributed. A Kruskal-Wallis test showed no significance difference in BMI amongst the groups ( $\chi^2(3)=2.89$ ;  $p>0.05$ ). A pair-wise comparison Mann-Whitney U test revealed a significantly higher BMI in the normotensive HIV negative

Compared to the normotensive HIV positive group ( $U=1379.00$ ;  $p<0.01$ ).

### ***3.1.6 Further analysis of clinical demographics***

#### ***3.1.6.1 Indication for delivery***

Within the pregnant groups (normotensive and pre-eclamptic), 65.9% of deliveries were performed in the interest of the baby compared to maternal indications. The Pearson Chi-square test showed a statistical significant difference between the normotensive and pre-eclamptic groups ( $p < 0.001$ ). Forty five percent pregnant women delivered by caesarean sections (C-section), whilst 27% required emergency C-section. Induction of labour was required in 4.6% of pregnant women whilst only 23% had a natural birth.

#### ***3.2.6.2 Clinical complications encountered***

Moreover, clinical complications encountered in our study included a case of abruption placentae, 9 cases of eclampsia, 5 cases of imminent eclampsia and 12 cases of severe pre-eclampsia. Additionally, there were 6 cases of still-births.

### ***3.1.7 Baby weight***

Baby weights were not normally distributed amongst the study groups. A Kruskal-Wallis H test indicated a significant difference in baby weight across study groups ( $\chi^2(2) = 15.801$ ;  $p < 0.001$ ; Figure 3.5). Additionally, the Mann-Whitney U test indicated no significant effect of HIV status on baby weight. However, the effect of pregnancy type (normotensive pregnant versus pre-eclamptic) on baby weight was statistical significant. Baby weight was lower in the pre-eclamptic group compared to the normotensive group. Additionally, within the pre-eclamptic group, babies of early onset women weighed more compared to babies of late onset pre-eclamptic women ( $U = 228$ ;  $p < 0.001$ ; Figure 3.6).



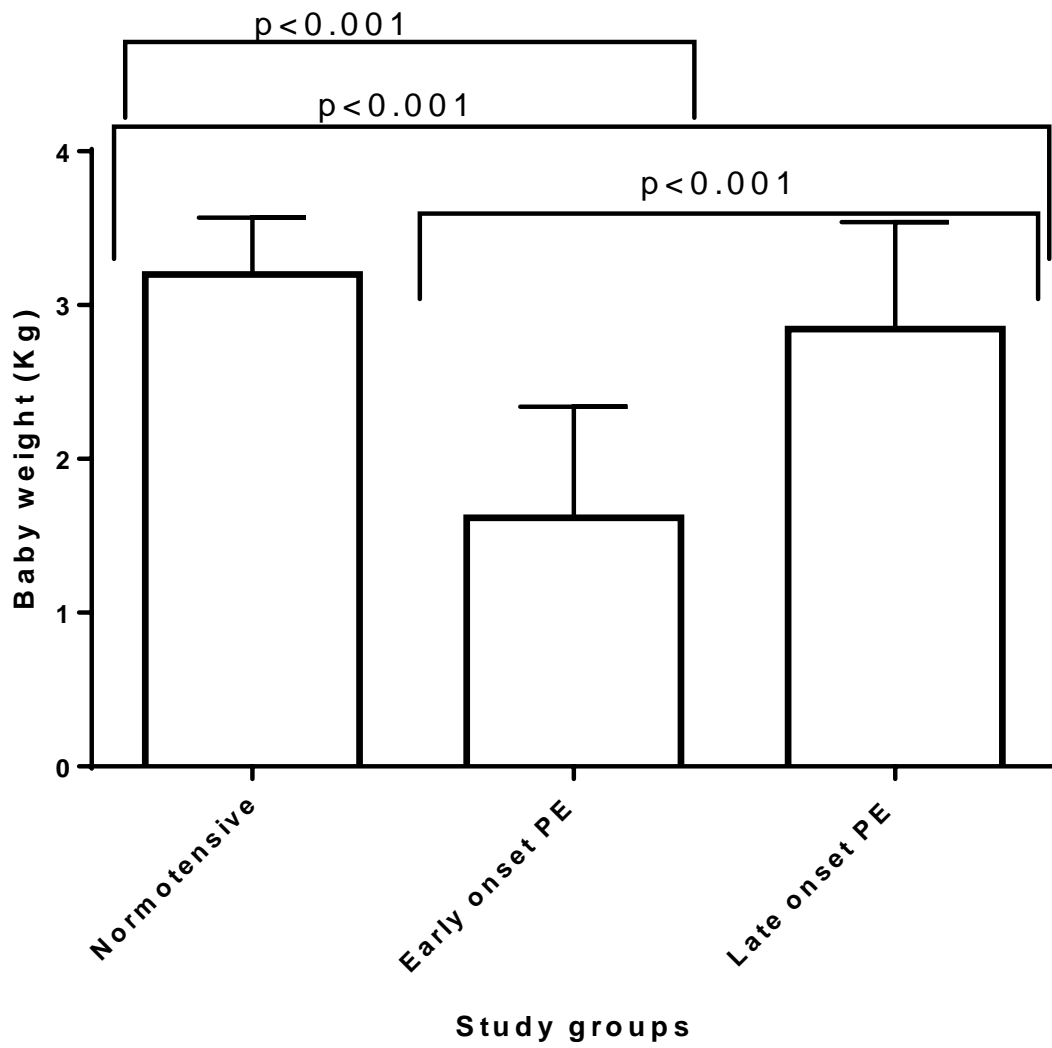


Figure 3.5: Baby weight (Kg) across the pregnant study groups

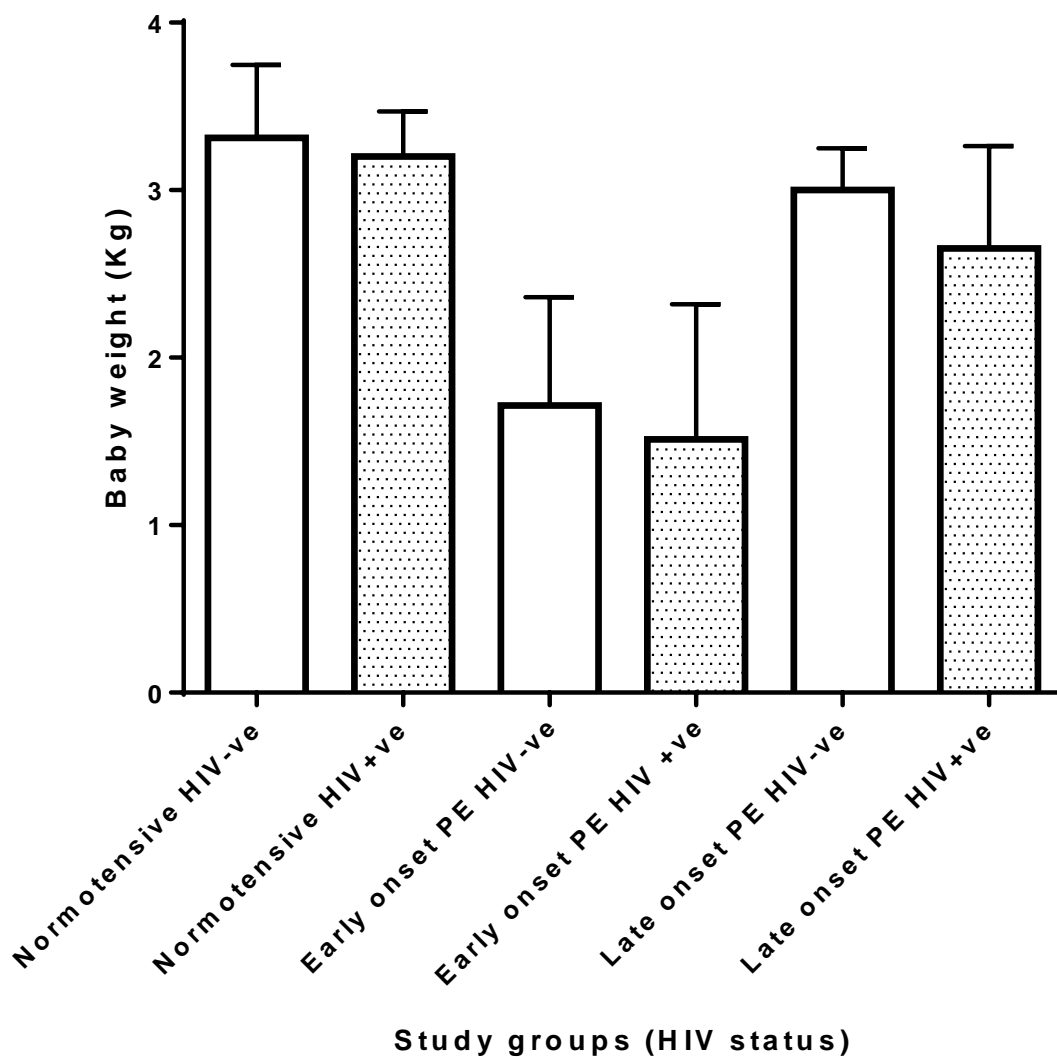


Figure 3.6: Baby weight (kg) across the pregnant study groups stratified by HIV status

### 3.1.8 Placental weight

One-way ANOVA showed a significance difference in placental weight across study groups ( $F(2.173) = 15.15; p < 0.001$ ). A multiple comparisons Tukey HSD test further revealed a significance difference in placental weight between normotensive HIV negative versus early onset HIV negative ( $p < 0.01$ ) and early onset HIV positive ( $p < 0.001$ ) groups. Additionally, there was a significant difference in placental weight between normotensive HIV positive versus early onset HIV negative

( $p < 0.05$ ) and early onset pre-eclamptic HIV positive ( $p < 0.001$ ) women. Also, there was a significant difference between the early-onset pre-eclamptic HIV negative versus late-onset pre-eclamptic HIV positive ( $p < 0.001$ ), early-onset pre-eclamptic HIV negative versus late onset pre-eclamptic HIV negative ( $p < 0.001$ ) and late-onset pre-eclamptic HIV positive versus late-onset pre-eclamptic HIV negative groups ( $p < 0.05$ ).

### **3.2 Analysis of adipokines**

Adipokine levels of C-peptide, ghrelin, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon, insulin, plasminogen activator inhibitor (PAI)-1 and visfatin are outlined in Tables 3.2 and 3.3 as medians and interquartile range (IQR).

Table 3.5: Adipokine levels across study groups, values are shown as medians and interquartile ranges (IQR). Kruskal Wallis H test was used, values highlighted in red indicate significant difference.

Adipokine	Non-pregnant n=90	Normotensive pregnant n=121	Early-onset pre-eclampsia n=32	Late-onset pre-eclampsia n=58	p- value
C-peptide	206 (32-363)	141.9 (94.7-79.5)	264.5 (104-379.1)	241.1 (116.520.2)	p< 0.01
Ghrelin	264.1 (102.9-428.3)	230.6 (98.70-375.2)	185.2 (74.31-366.3)	227 (86.42-358.2)	p>0.05
GIP	21.62 (10.84-53.31)	15.64 (5.19-37.47)	30.5 (12.32-66.3)	20.94 (15.26-44.78)	P<0.01
GLP-1	94 (75.31-116.4)	75.31 (21.01-115.3)	94 (75.31-116.4)	94 (21.01-115.3)	P<0.05
Glucagon	47.93 (41.94-55.83)	47.93 (9.92-49.39)	47.93 (41.94-55.83)	47.93 (9.92-49.39)	p>0.05
Insulin	141.9 (39.54-271.6)	74.26 (25.11-177.7)	103.7 (34.7-558.6)	74.5 (36.27-284.7)	p>0.05
PAI-1	5219 (311.6-15506)	6458 (1820-13695)	6661 (2077-15265)	6975 (573.6-148.95)	p>0.05
Visfatin	555.4 (153.7-5802)	744.4 (310.4-8233)	886.2 (262.1-6981)	2800 (487.5-10568)	P<0.05

Table 3.6: Adipokine levels across study groups based on HIV status (results are shown as median and interquartile range). Kruskal Wallis H test was used.

ADIPOKINES	NON-PREGNANT (n=90)		NORMOTENSIVE (n=121)		EARLY ONSET (n=32)		LATE ONSET (n=58)		P VALUE
	HIV <sup>+ve</sup> n=45	HIV <sup>-ve</sup> n=45	HIV <sup>+ve</sup> n=54	HIV <sup>-ve</sup> n=67	HIV <sup>+ve</sup> n=15	HIV <sup>-ve</sup> n=17	HIV <sup>+ve</sup> n=21	HIV <sup>-ve</sup> n=37	
C-peptide	200.1 (136.7-328.1)	211.7 (124-485.5)	131 (73.74-285)	153.1 (107.1-278.2)	284.3 (117.3-436.5)	235.4 (91.48-321.6)	254.3 (91.16-745.7)	231.1 (124.4-462.8)	p>0.05
Ghrelin	282.4 (126.1-491.2)	251.1 (102.9-388.4)	213.5 (101.0-384)	230.6 (98.43-377.8)	185.5 (130.4-459.3)	98.74 (48.75-297.5)	184.5 (90.54-381.9)	250 (92.43-342.9)	p>0.05
GIP	18.92 (15.64-65.22)	26.27 (8.51-48.11)	16 (5.04-39.26)	11.93 (5.62-19.97)	38.39 (17.19-69.51)	16 (3.11-52.95)	16.6 (9.06-39.65)	23.9 (15.54-61.5)	p<0.05
GLP-1	94 (94-116.4)	75.31 (75.31-115.3)	75.31 (21.01-115.3)	75.31 (21.01-116.4)	94 (75.31-116.4)	94 (75.31-116.4)	94 (75.31-115.3)	94 (21.01(115.3)	p<0.05
Glucagon	55.83 (47.93-55.83)	49.39 (47.93-55.83)	49.39 (44.94-55.83)	49.39 (47.93-163.9)	49.39 (47.93-55.83)	55.83 (47.93-55.83)	49.39 (47.93-55.83)	51 (44.94-55.83)	p>0.05
Insulin	147.8 (43.84-244.7)	136.3 (31.4-406.7)	43.28 (14.55-230.1)	82.83 (47.3-143.5)	105.1 (31.02-854.9)	102.3 (39.95-384.4)	68.9 (36.27-226.1)	82.95 (32.19-737.2)	p>0.05
PAI-1	4237 (11.36-10602)	6779 (742.8-19731)	6980 (1605-13291)	6367 (2377-15189)	7563 (11.36-16153)	6506 (3333-15040)	6056 (1168-17696)	7311 (464.8-14224)	p>0.05
Visfatin	373.3 (145.3-2052)	679.6 (225.2-8088)	714.4 (272.3-9701)	773.3 (389.9-7546)	1979 (359.7-17964)	555.4 (153.6-4577)	1523 (438-13187)	5050 (525.9-10222)	p<0.05

Table 3.7: Pairwise comparison/analysis of adipokines across stud groups. Statistical test used; Mann Whitney U test was used. Values in red=significant difference.

	Normotensive Vs Non-pregnant	Normotensive Vs Early onset PE	Normotensive Vs Late onset PE	Non-pregnant Vs Early onset PE	Non-pregnant Vs Late onset PE	Early onset Vs Late onset PE
C-peptide	4169 (p<0.01)	1597 (p>0.05)	2640 (p<0.01)	1353 (p>0.05)	2505 (p>0.05)	847 (p>0.05)
Ghrelin	5041 (p>0.05)	1727 (p>0.05)	3180 (p>0.05)	1197 (p>0.05)	2164 (p>0.05)	918.5 (p>0.05)
GIP	4151 (p<0.01)	1435 (p<0.05)	2614 (p<0.01)	1355 (p>0.05)	2573 (p>0.05)	892.5 (p>0.05)
GLP-1	4302 (p<0.01)	1539 (p>0.05)	3312 (p>0.05)	1424 (p>0.05)	2206 (p>0.05)	784.5 (p>0.05)
Glucagon	4415 (p<0.05)	1626 (p>0.05)	3394 (p>0.05)	1416 (p>0.05)	2173 (p>0.05)	795 (p>0.05)
Insulin	4328 (p<0.05)	1543 (p>0.05)	3145 (p>0.05)	1398 (p>0.05)	2413 (p>0.05)	842 (p>0.05)
PAI-1	5061 (p>0.05)	1921 (p>0.05)	3434 (p>0.05)	1334 (p>0.05)	2505 (p>0.05)	914.5 (p>0.05)
Visfatin	4532 (p<0.05)	1901 (p>0.05)	2964 (p<0.05)	1195 (p>0.05)	1806 (p<0.01)	785.5 (p>0.05)

Table 3.8: Analysis of adipokines in groups stratified by HIV status. Mann Whitney U test was used for pairwise comparison of the subgroups. Values highlighted in red indicate significant difference.

	Non-pregnant	Normotensive	Early onset PE	Late Onset PE
C-peptide	995 (p>0.05)	1556 (p>0.05)	101 (p>0.05)	376 (p>0.05)
Ghrelin	902 (p>0.05)	1797 (p>0.05)	94 (p>0.05)	360(p>0.05)
GIP	947.5 (p>0.05)	1591 (p>0.05)	80.50 (p>0.05)	328 (p>0.05)
GLP-1	688 (p<0.05)	1694 (p>0.05)	112 (p>0.05)	356.5 (p>0.05)
Glucagon	924 (p>0.05)	1679 (p>0.05)	95 (p>0.05)	357.5 (p>0.05)
Insulin	1001 (p>0.05)	1552 (p>0.05)	106 (p>0.05)	367.5 (p>0.05)
PAI-1	818 (p>0.05)	1713 (p>0.05)	122 (p>0.05)	368.5 (p>0.05)
Visfatin	766.5 (p<0.05)	1747 (p>0.05)	90.50 (p>0.05)	332 (p>0.05)



### 3.2.1 C-peptide

A Kruskal Wallis H test showed a difference in the level of c-peptide across all study groups ( $\chi^2(3)=11.71$ ;  $p<0.01$ ; Figure 3.7), additionally, the Dunn's multiple comparison test showed a difference between the non-pregnant versus normotensive and the normotensive versus late onset group. A Mann-Whitney U test found a significant difference between the non-pregnant and normotensive ( $p=0.01$ ), and between the normotensive and the late onset group ( $p=0.01$ ). There were no significant differences in the other groups. When considering HIV status, the Kruskal Wallis H test revealed that there was no significant differences in the levels of c-peptide amongst the groups ( $p>0.05$ ; Figure 3.8).

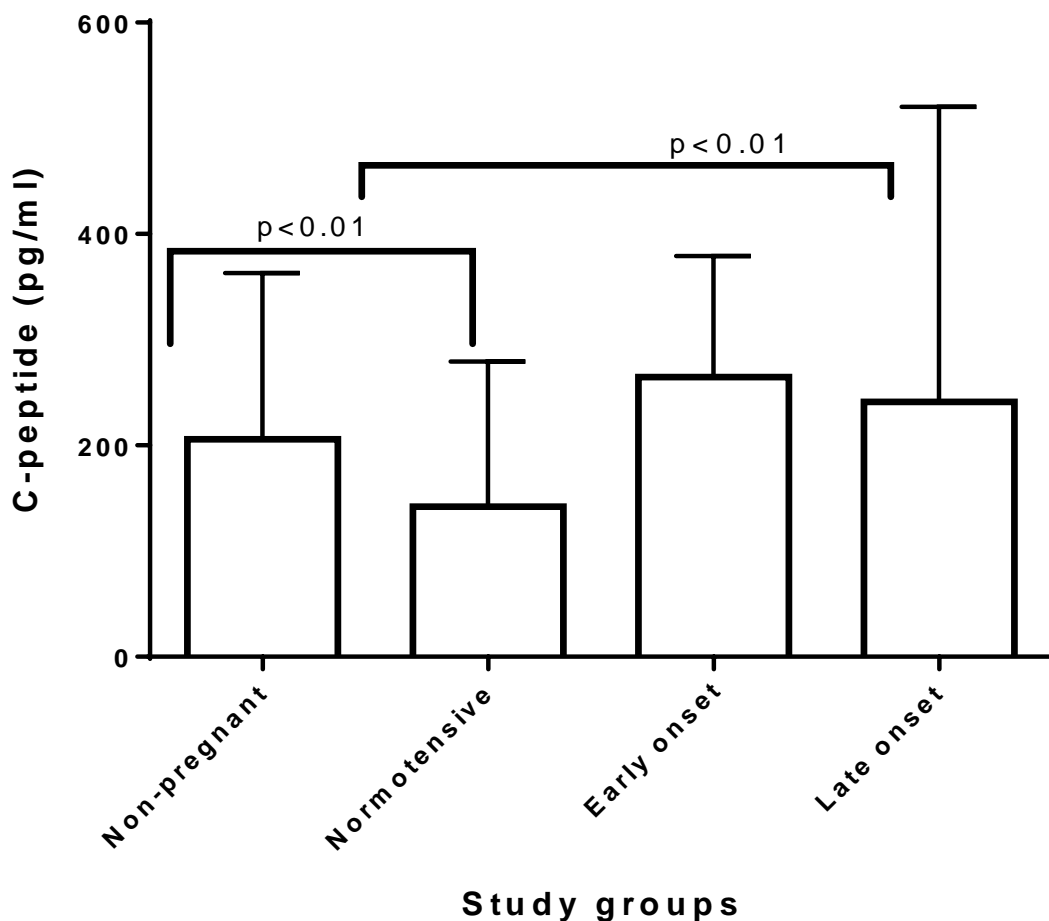


Figure 3.7: C-peptide across study groups. C-peptide is elevated in the early onset when comparing to the other groups, followed by late onset, non-pregnant and normotensive groups.

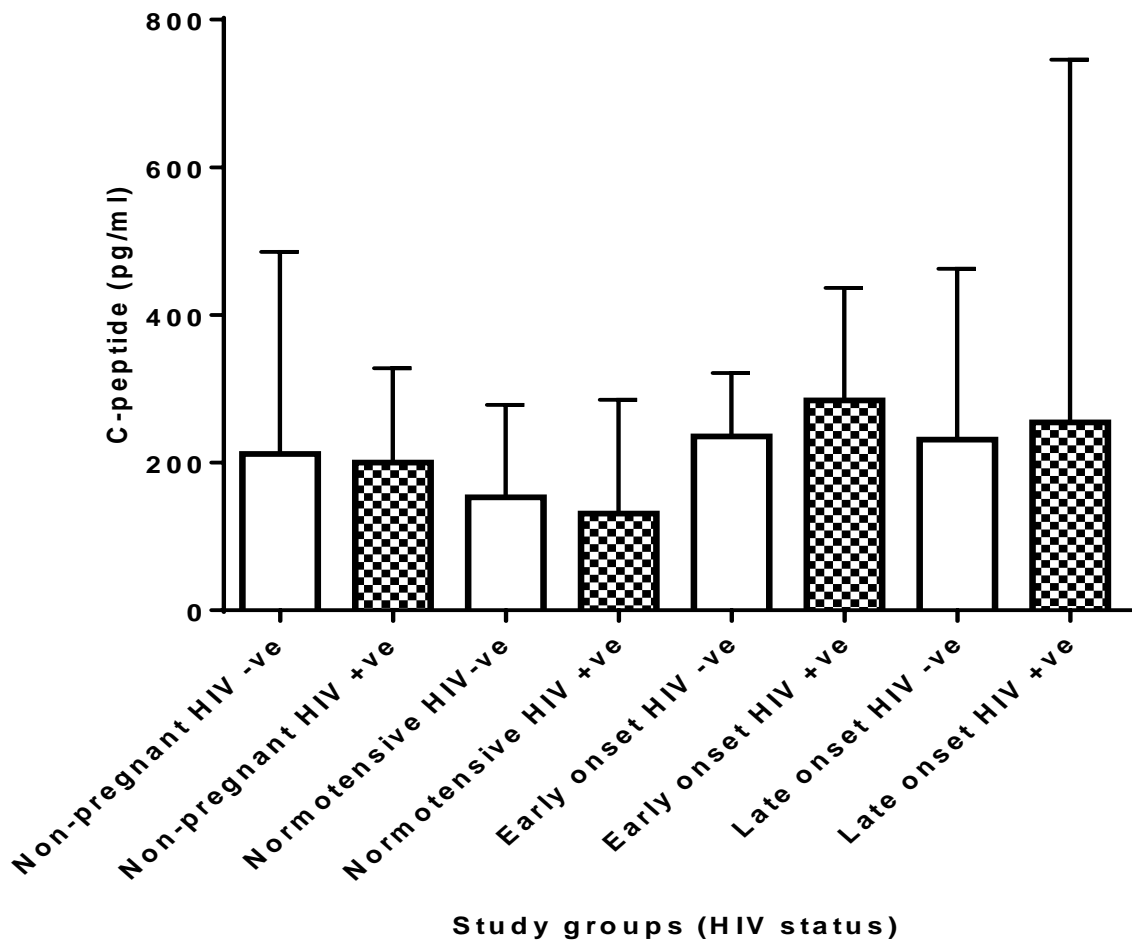


Figure 3.8: C-peptide across sub-stratified by HIV status. In the non-pregnant and normotensive, c-peptide is lower in the HIV negative groups compared to the HIV positive groups. Whilst, in the pre-eclamptic groups, it is higher in the HIV positive compared to the HIV negative subgroups.

### 3.2.2 Ghrelin

The medians and interquartile range of ghrelin are displayed on Table 3.5 and 3.6. The Kruskal Wallis H test showed no statistical differences in the levels of ghrelin across study groups ( $p>0.05$ ; Figure 3.9). Also, the Dunn's multiple comparison test indicated no statistical difference among the groups. Furthermore, HIV status had no effect on ghrelin levels across study groups ( $p>0.05$ ; Figure 3.10).

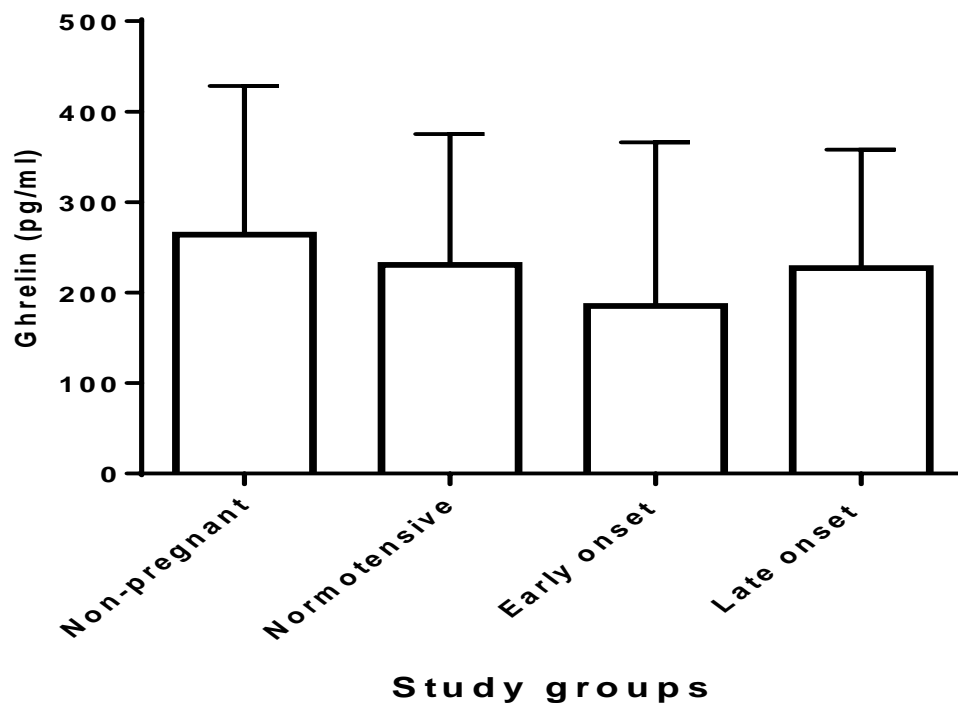


Figure 3.9: Levels of ghrelin across categories of study groups. The non-pregnant has the highest levels of ghrelin compared to the pregnant groups.

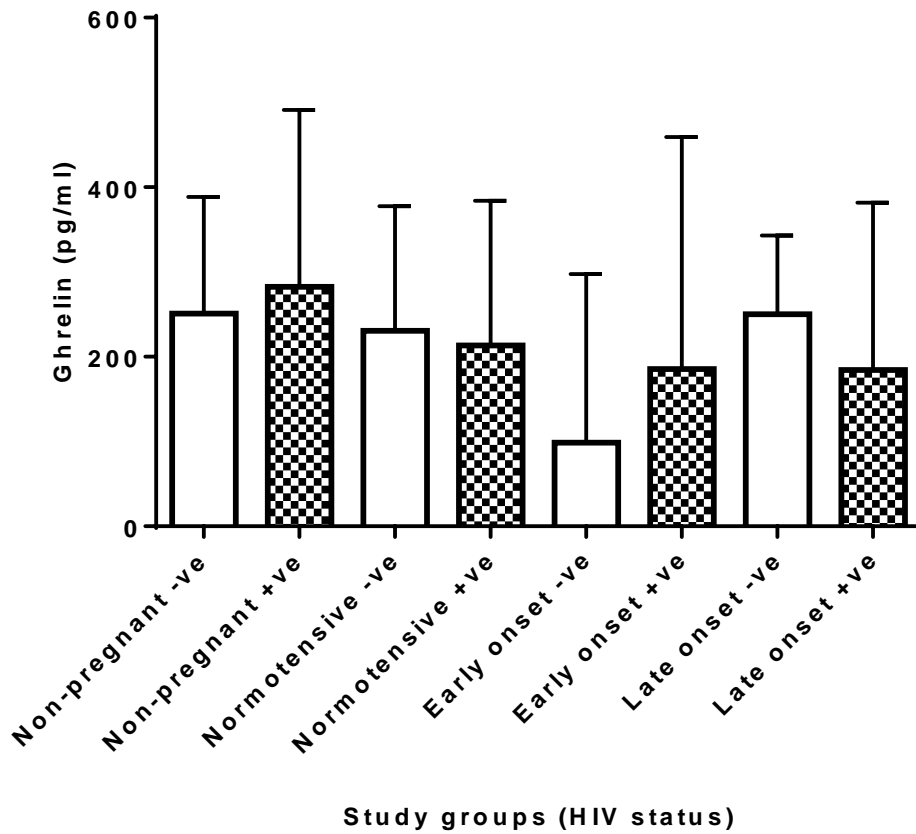


Figure 3.10: Levels of ghrelin in study groups sub-stratified by HIV status. Ghrelin was elevated in the HIV positive non-pregnant and early onset groups compared to the HIV negative groups, whilst it was higher in the HIV negative normotensive and late onset groups compared to the HIV positive groups.

### 3.2.3 Gastric inhibitory polypeptide

The median (IQR) for GIP are outlined on Tables 3.5 and 3.6. The Kruskal-Wallis H test and the Dunn's multiple comparison test found a statistical significant difference in GIP across the study groups ( $p < 0.01$ ; Figure 3.11). Furthermore, a pairwise comparison test found a significant difference between the non-pregnant versus normotensive ( $p < 0.01$ ); normotensive versus early onset ( $p < 0.05$ ; Figure 3.11) and normotensive versus the late onset group ( $p < 0.01$ ).

When considering HIV status, the Kruskal Wallis-H test revealed a statistical difference across the study groups ( $p < 0.001$ ; Figure 3.12).

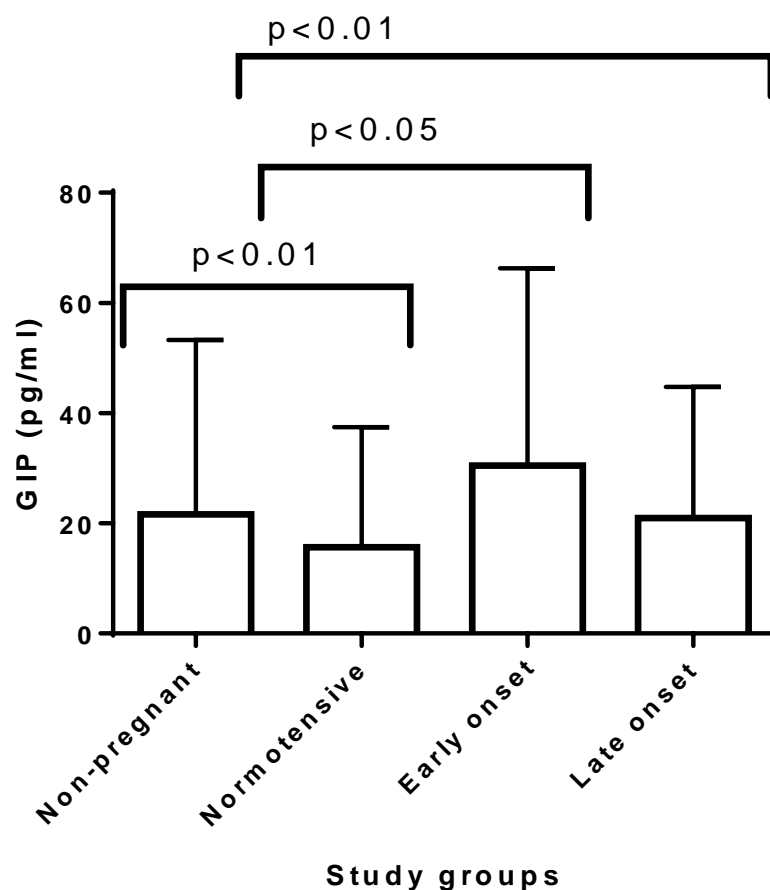


Figure 3.11: Levels of GIP across the categories of study groups.

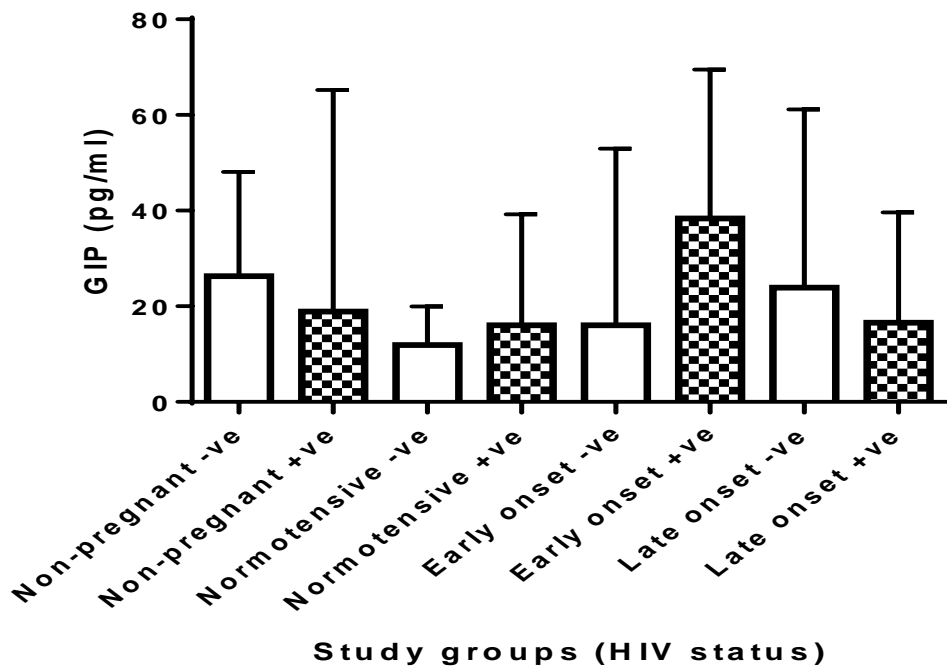


Figure 3.12: GIP levels in study groups stratified by HIV status.

### 3.2.4 Glucagon-like peptide 1

The median and IQR for GLP-1 are displayed on Tables 3.5 and 3.6. The data for GLP-1 was not normally distributed hence we performed a non-parametric test. The Kruskal-Wallis H test and the Dunn's multiple comparison test found a significant difference in GLP-1 across the study groups ( $p < 0.05$ ; Figure 3.13). Furthermore, a Mann Whitney U test found a significant difference between non-pregnant versus normotensive ( $p < 0.01$ ).

When considering HIV status, the Kruskal Wallis-H test revealed a statistical difference across the study groups ( $p < 0.05$ ; Figure 3.14). Additionally, the Mann Whitney U test showed a significant difference between the non-pregnant and the normotensive group ( $p < 0.01$ ).

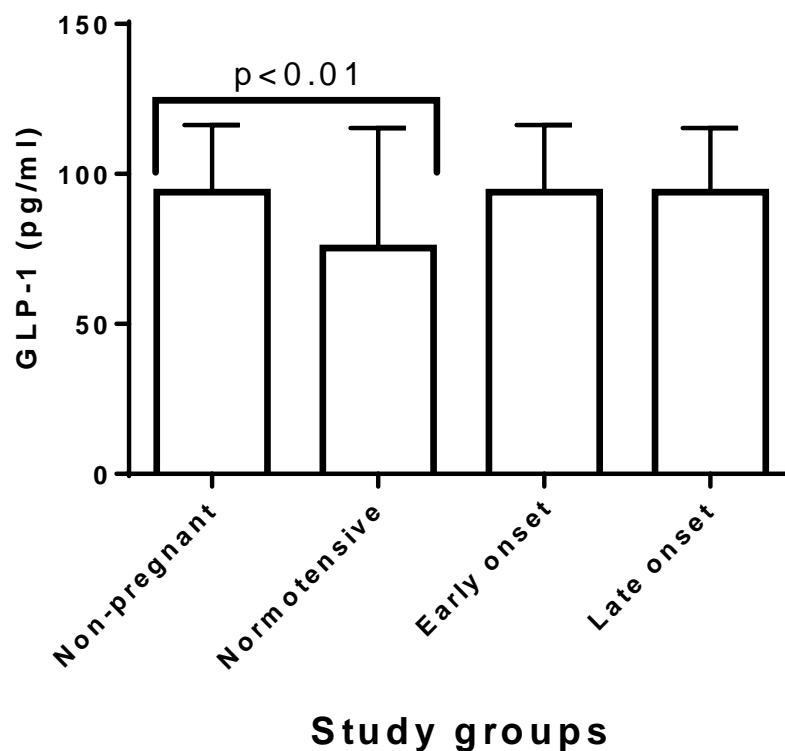


Figure 3.13: Levels of GLP-1 across categories of study groups. The levels of GLP-1 were lowest in the normotensive group.

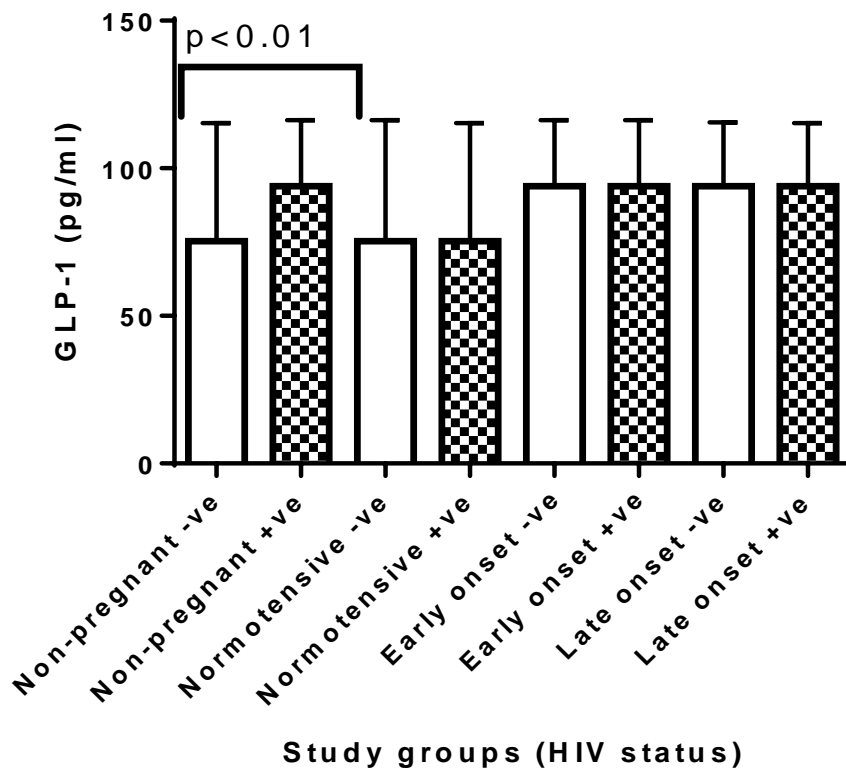


Figure 3.14: Levels of GLP-1 across study groups stratified by HIV status. There seem to be no difference between the HIV positive and negative in the pregnant (pre-eclamptic and normotensive) groups.



### 3.2.5 Glucagon

The median and IQR for glucagon are displayed on Tables 3.5 and 3.6. Glucagon was not normally distributed hence we performed a non-parametric test. The Kruskal-Wallis H test and the Dunn's multiple comparison test found no significant difference in the levels of glucagon across the study groups ( $p>0.05$ ; Figure 3.15). However, when performing a Mann Whitney U test, we found a significant difference between the non-pregnant and normotensive group ( $p<0.05$ ).

When considering HIV status, the Kruskal Wallis-H test indicated that HIV status does not affect glucagon levels ( $p>0.05$ ; Figure 3.16). However, a Mann-Whitney U test found a significant difference between HIV positive non-pregnant and HIV negative non-pregnant ( $p<0.05$ ).

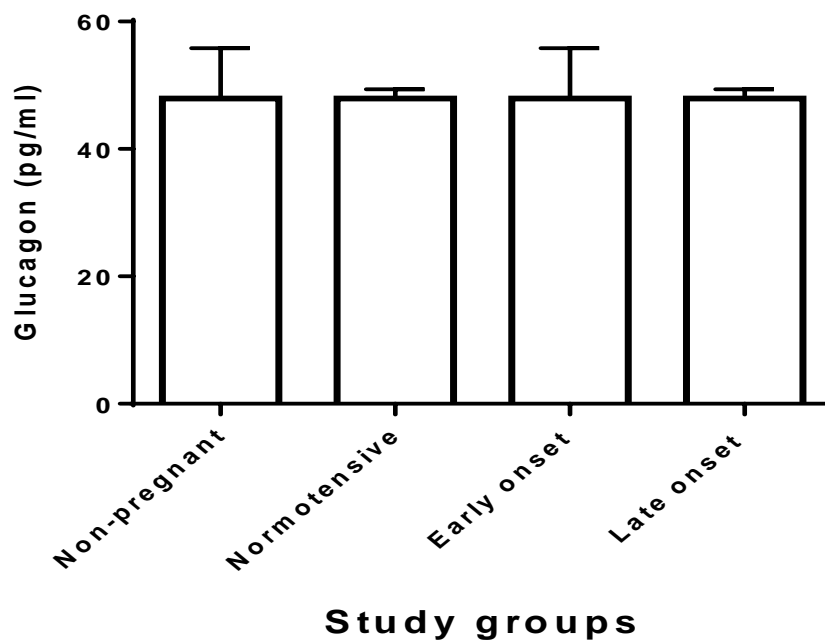


Figure 3.15: Glucagon across study groups. Glucagon did not differ between groups

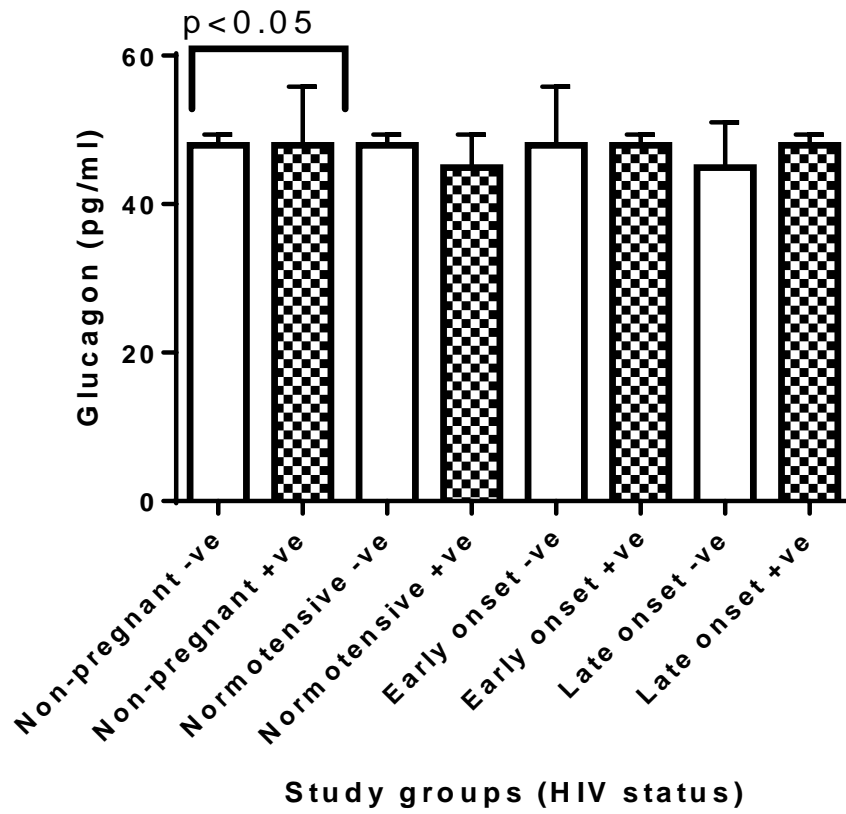


Figure 3.16: Glucagon levels in study groups based on HIV status. The non-pregnant and early onset groups have same levels of glucagon irrespective of HIV status.

### 3.2.6 Insulin

The median and IQR for insulin are displayed on Tables 3.5 and 3.6. The Kruskal-Wallis H test and the Dunn's multiple comparison test found no significant difference in the levels of insulin across the study groups ( $p>0.05$ ; Figure 3.17). However, a Mann Whitney U test, found a significant difference between the non-pregnant and normotensive group ( $p<0.05$ ).

When considering HIV status, the Kruskal Wallis-H test revealed a statistical difference across the study groups ( $p>0.05$ ; Figure 3.18).

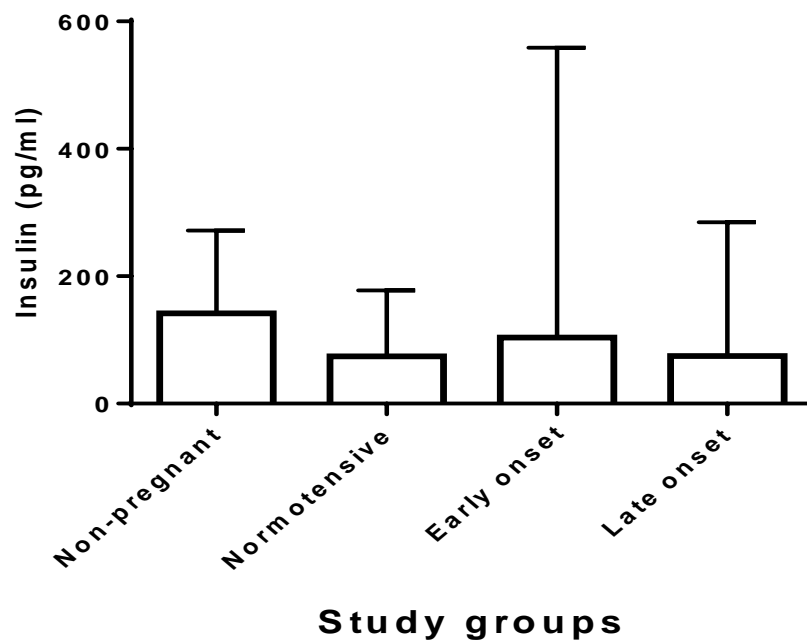


Figure 3.17: Insulin levels across study groups. The non-pregnant group have high levels of insulin compared to the pregnant groups, however, in the pregnant groups; the early onset has elevated levels of insulin compared to normotensive and late onset.

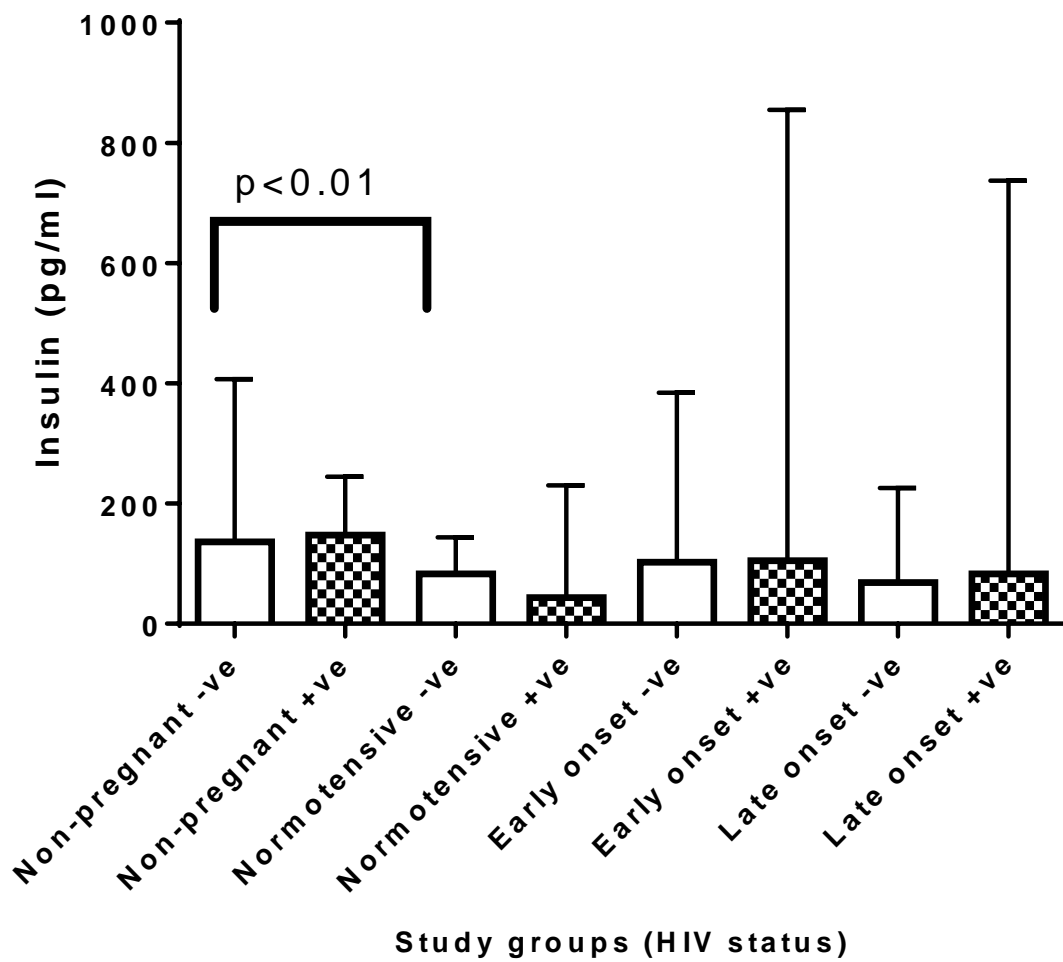


Figure 3.18: Insulin levels across categories of study groups stratified by HIV status. In the pre-eclamptic groups, insulin level is elevated in the HIV positive groups when comparing to the HIV negative.

### 3.2.7 Plasminogen activator inhibitor-1

The median and IQR for plasminogen activator inhibitor (PAI)-1 are displayed on Tables 3.5 and 3.6. The Kruskal-Wallis H test and the Dunn's multiple comparison test found no significant difference in the levels of PAI-1 across the study groups ( $p>0.05$ ; Figure 3.19). Furthermore, Mann Whitney U test found no significant difference between the groups ( $p>0.05$ ).

When considering HIV status, the Kruskal Wallis-H test revealed no significant difference across the study groups ( $p>0.05$ ; Figure 3.20).

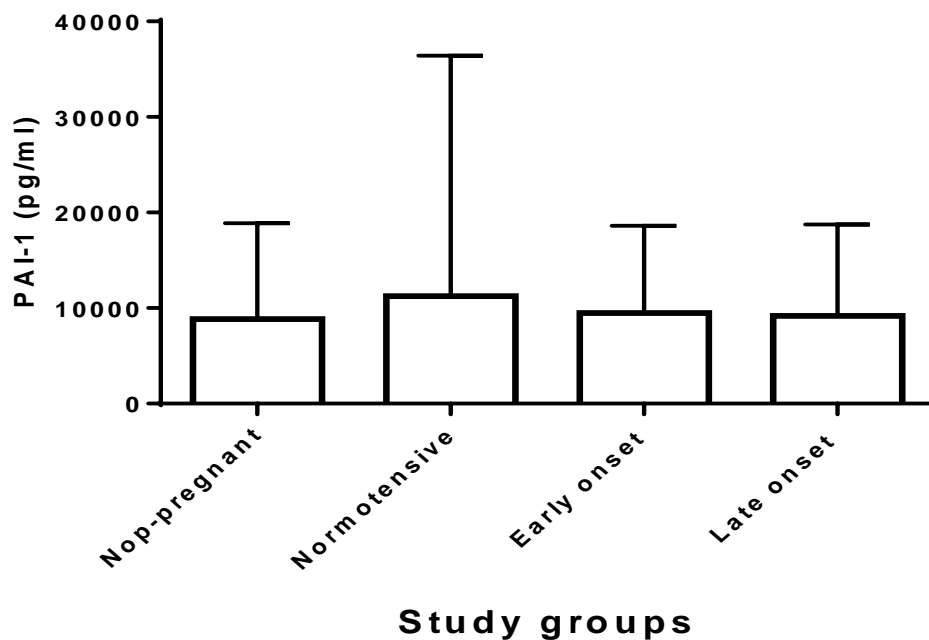


Figure 3.19: Levels of PAI-1 across study groups. The pregnant groups have the same levels of PAI-1, the non-pregnant group has elevated levels of PAI-1 compared to the pregnant groups.

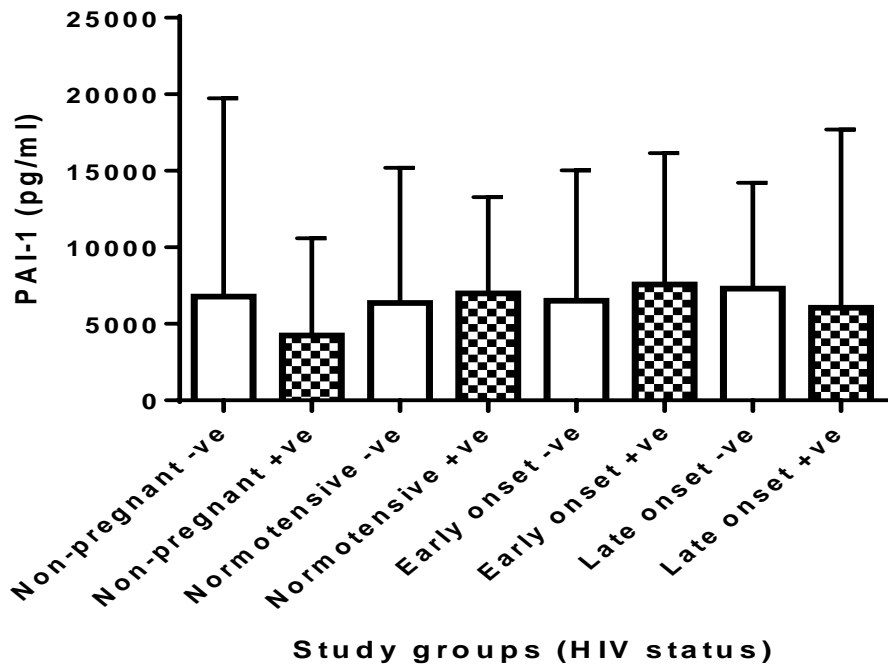


Figure 3.20: Levels of PAI-1 in study groups subdivided by HIV status.

### 3.2.8 Visfatin

The median and IQR of visfatin are displayed on Tables 3.5 and 3.6. The Kruskal-Wallis H test and the Dunn's multiple comparison test found a significant difference in the levels of visfatin across the study groups ( $p < 0.05$ ; Figure 3.21). Additionally, a Mann-Whitney U test found a significant difference between the non-pregnant versus normotensive group ( $p < 0.05$ ) and the late onset versus the non-pregnant group ( $p < 0.01$ ).

When considering HIV status, the Kruskal Wallis-H test revealed a statistical difference across the study groups ( $p < 0.05$ ; Figure 3.22). A Mann-Whitney U test revealed that there is a significant difference between the HIV positive versus negative non-pregnant groups ( $p < 0.05$ ).

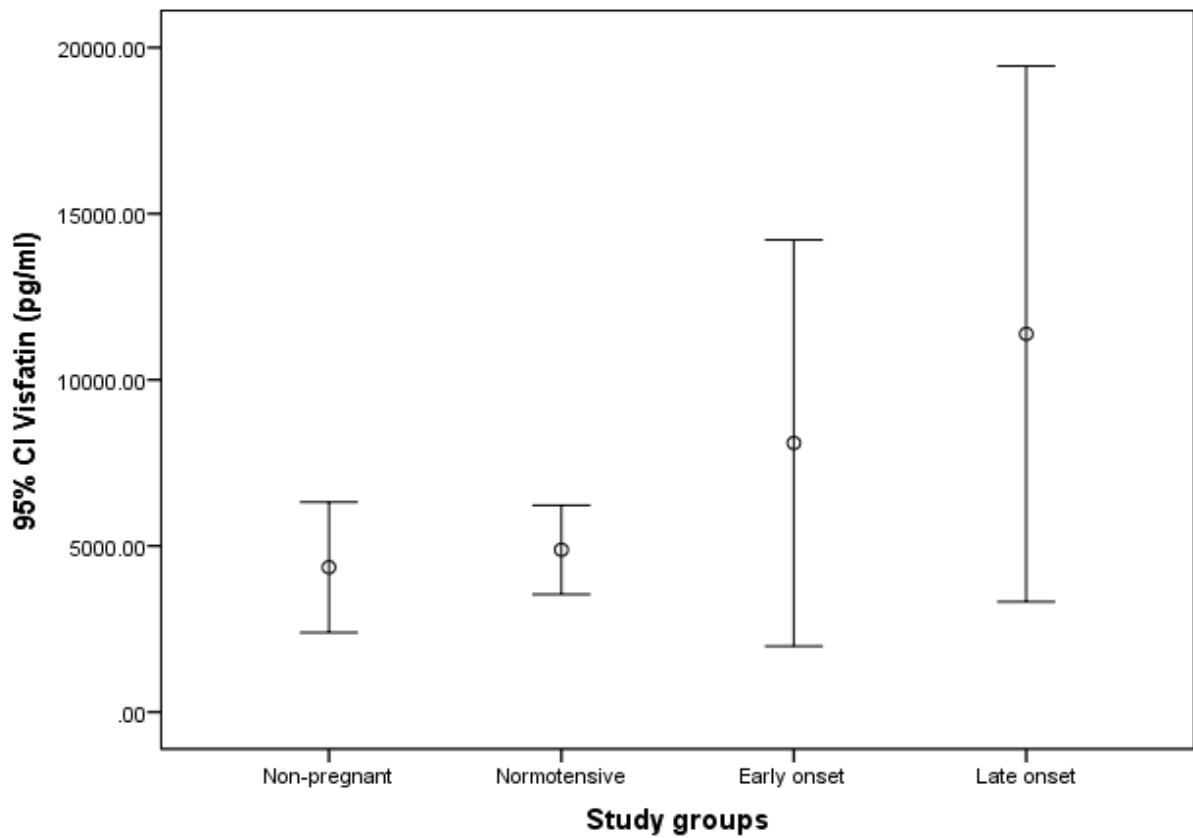


Figure 3.21: Levels of visfatin across study groups. Visfatin is elevated in the pre-eclamptic groups when comparing to the non-pregnant and the normotensive group.

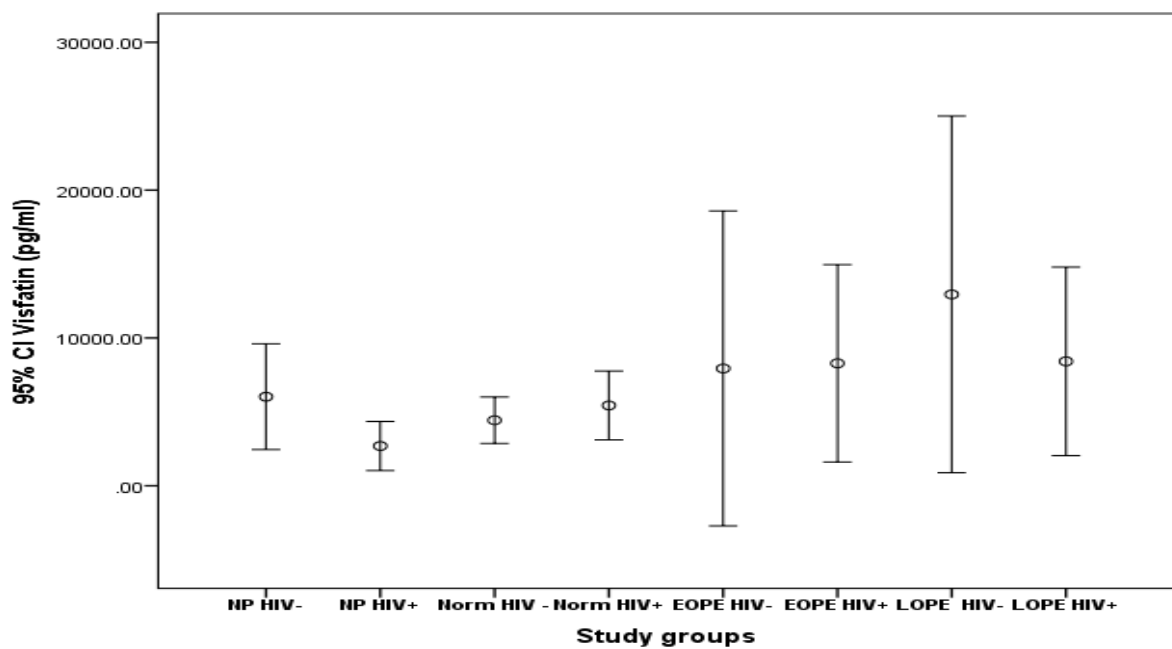


Figure 3.22: Visfatin across study groups stratified by HIV status. The pre-eclamptic and non-pregnant groups, the HIV positive groups have elevated levels of visfatin compared to the HIV negative.



## CHAPTER 4

## DISCUSSION

The millennium goal (1999) was developed to reduce maternal deaths by 75% by the year 2015. Since its establishment, maternal mortality has decreased by 50% (MDG Report, 2015). Sadly, a similar decline of maternal mortality cannot be reported for developing countries, especially the countries in the sub-Saharan region. In South Africa, despite a 50% decrease in maternal deaths for the period 2000-2015, maternal death is still a major health burden. The latest Saving Mothers report indicates that the top 3 causes of maternal death are non-pregnancy related infections (HIV, TB and pneumonia), 38% and obstetric haemorrhage (16%) and hypertension (14%) of all maternal deaths. Pre-eclampsia, accounts for 83% of deaths emanating from hypertension in pregnancy.

In South Africa, the national HIV prevalence amongst women has decreased from 30.2% to 29.1% (Udjo, 2006). Notably, the HIV epidemic has devastating consequences on women of reproductive age 15 - 24 years (Moodley and Moodley, 2005). Approximately 30% of South African parturients are co-infected with HIV (Kalumba *et al.*, 2013). The administration of ART to all HIV positive pregnant women with CD4 counts <400 has contributed to a decrease in neonatal and maternal deaths.

Globally, more than one billion people are overweight or obese (Obesity Task Force, 2005). South Africa is facing a new dilemma in that it is ranked as the third highest country in the world with obesity. In pregnancy, obese women have increased risk of suffering from complications due to caesarean section, sepsis, haemorrhage etc.

This study reports on adipokine dysregulation in the co-localisation of the three conditions (HIV, pre-eclampsia and obesity).

## 4.1. Maternal demographics

### 4.1.1 Maternal age

In this study the mean age $\pm$  standard deviation was  $33.10 \pm 7.47$  years,  $25.59 \pm 5.63$  years,  $28.19 \pm 6.30$  years and  $25.28 \pm 5.98$  years in the non-pregnant, normotensive pregnant, early-onset pre-eclamptic and the late-onset pre-eclamptic groups respectively. These maternal ages supports global trends of women delaying their first pregnancy (Benzies, 2008). Advanced maternal age has an increased risk of gestational diabetes (Jacobsson B, 2004) placenta previa (Jolly M *et al.*, 2000), preeclampsia (Ozalp *et al.*, 2003), miscarriage (Goldman *et al.*, 2005), pregnancy-induced hypertension and the need for caesarean deliveries (Lamminpää *et al.*, 2012).

We report that based on HIV status, there was a significant difference in maternal age across the study categories ( $p < 0.001$ ) and that the non-pregnant, normotensive and late-onset group were older in the HIV positive group compared to their corresponding HIV negative group. The distribution of HIV according to age demonstrates that the HIV positive participants tended to be older than the HIV negative participants. This attests to the fact that local incidence of the HIV disease seems to be stabilizing, as supported by local statistics (Lehola, 2014). In contrast, the early onset pre-eclamptic HIV positive group were younger than their corresponding HIV negative group. Maternal age appears to be independent obstetric risk factor for early onset of preeclampsia and impaired fetal growth (Lamminpää *et al.*, 2012). The young participants in our EOPE cohort is supported by classical risk factors such as nulliparity (Walsh, 2007; Luo *et al.*, 2007), Black ethnicity (ACOG Practice Guideline, 2002) and primipaternity (ACOG Practice Guideline, 2002; Zhang *et al.*, 1997), all of which are risk factors for pre-eclampsia development. The maternal age of the normotensive pregnant cohort in our study was similar to that of previous studies (Aksornphusitaphong & Phupong, 2013).

#### **4.1.2 Maternal weight**

The results of this study shows no statistical significant in the distribution of maternal weight amongst the study groups. Obesity is belied to increase the incidence of preeclampsia via an increased insulin resistance or inflammation (Walsh, 2007). Insulin resistance is associated with endothelial dysfunction and increased secretion of endothelin 1, a potent vasoconstrictor (Marasciulo *et al.*, 2006). We however, did not notice a correlation of increased maternal weight with pre-eclampsia development. A limitation of our study is that we do not have pre-pregnancy weight across all pregnant study groups.

We report a significance effect of HIV status on maternal weight across the study groups ( $p < 0.05$ ). Within the normotensive group, maternal weight was higher in the HIV negative group compared to HIV positive group. It is well documented that HIV infection results in wasting, generalised loss of fat-free and fat mass, augmenting morbidity and mortality (Macallan, 1999). An optimum maternal weight during pregnancy is important for optimal foetal development. An inadequate weight gain is associated with intrauterine growth retardation and perinatal mortality (World Health Organisation Report., 2005). Additionally, HIV-positive women in developing countries (like South Africa) are vulnerable to dietary deficiencies from insufficient dietary intake and HIV infection to meet the nutritional load of pregnancy (Brocklehurst P and French R, 1998; Scholl & Johnson, 2000).

#### **4.1.3 Body Mass Index**

In our study there was no significance difference in BMI amongst the study group. There was a significantly higher BMI in the normotensive HIV negative compared to the normotensive HIV positive group ( $p < 0.01$ ). In this study, BMI was calculated in the third trimester of pregnancy. BMI is based on population and ethnicity specific charts. A baseline pregnancy BMI has only recently been outlined for our Black ethnic group within KwaZulu-Natal, South Africa and are congruent with this study due to use of same patient intake (Govender, 2014).

The relationship between BMI and hypertension in sub-populations of Ethiopian, Vietnamese and Indonesian population has been investigated (Tesfaye *et al.*, 2006). In our study, the comparison between the pregnant cohorts allowed for a more uniform comparison, however the majority of the participants fell into the overweight, pre-obese and grade 1 obesity category. It is also plausible to link the finding of similar BMI amongst the normotensive and pre-eclamptic cohorts in this study to a high pre-pregnancy weight. Pre-pregnancy weight was not available for the participants. A consideration of high pre-pregnancy weight is conceivable since 70 % of South African women are considered overweight. Also a strong association between pre-eclampsia and high BMI has been reported (Duckitt, K & Harrington, 2005; Machado Elizabeth Stankiewicz *et al.*, 2014).

#### **4.1.4 Blood pressure**

In our study, there was a higher blood pressure in the pre-eclamptic groups compared to the non-pregnant and normotensive groups. Currently both the International Society for the Study of Hypertension in Pregnancy and the Working Group of the National High Blood Pressure Education Program in the United States both define pre-eclampsia as either systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mm Hg, with associated proteinuria after 20 weeks' gestation (Brown *et al.*, 2001, 2000).

We also report a significantly higher systolic and diastolic blood pressure in our pre-eclamptic cohort compared to the normotensive non-pregnant and normotensive pregnant population. Furthermore, we found that a significant difference with respect to HIV status and blood pressure across the non-pregnant, pre-eclamptic and normotensive cohorts ( $p < 0.001$ ).

A posturing inspection of BP in this study revealed a trend towards a lower systolic and diastolic blood pressure in HIV naive individuals. This trend is in keeping with the development of insulin resistance, obesity and hypertension in HIV infection (Gazzaruso *et al.*, 2003). Notably metabolic

syndromes such as obesity, impaired glucose tolerance, diabetes, hypertriglyceridemia, and reduced levels of high-density lipoprotein are risk factors for cardiovascular disease development, including blood pressure elevation (Hadigan *et al.*, 2001; Samaras *et al.*, 2007).

#### **4.1.5 Gestational Age**

In our study, gestational age was significantly different across the study groups ( $p < 0.001$ ). Gestational age was higher in the normotensive compared to the early-onset ( $p < 0.001$ ) and late-onset pre-eclamptic group ( $p < 0.001$ ). Our results are congruent with the classical theory in that pre-eclampsia is associated with lower gestational age. However, the converse has been demonstrated in that babies born to mothers with pre-eclampsia at term have fetal growth similar to that of babies born to normotensive mothers (Xiong *et al.*, 1999). Also early-onset pre-eclampsia is associated with adverse perinatal outcomes, such as small for-gestational-age infants, than late onset pre-eclampsia due to inadequate placental blood flow (Van Rijn *et al.*, 2006). Moreover, placental lesions are higher the earlier the gestational age at the time of delivery, compared with normotensive control subjects (Moldenhauer *et al.*, 2003).

Additionally, we report no significant effect of HIV status on gestational age in our study. In 2010, a study by Boyajian, reported to have found preterm birth did not differ between HIV positive women when compared to the negative women, however it found lower birth weight among babies born by HIV positive mothers.

#### **4.1.6 Birthweight**

In our study, baby weight was significant different across study groups ( $p < 0.001$ ). Baby weight was lower in the pre-eclamptic group compared to the normotensive group. Our results are supported by previous studies in that the mean birth weight of newborns and mean placental weight are significantly lower in pre-eclampsia than normotensive pregnancies ( $p < 0.001$ ) suggesting that

impaired placental function in preeclampsia is directly related to low placental and birth weights and poor fetal outcome (Kaur *et al.*, 2013). However, in contrast to these findings, the birth weight of pre-eclamptic women at term were found to be similar to that of babies born to normotensive mothers (Xiong *et al.*, 2001), advocating against the reduced uteroplacental perfusion being the unique pathophysiologic process in pre-eclampsia. Our finding of low birth weight is in keeping with other studies, where babies from the EOPE cohort fell into the very low birth weight category (Kucukgoz Gulec *et al.*, 2013, Lisonkova & Joseph, 2013).

Our study also reports no significant effect of HIV status on baby weight across the study groups. In contrast to our study, a large scale Tanzanian study (2001) found that HIV positivity was not a risk factor for lower birth weight. Although earlier studies by Minkoff *et al.*, (1990) found no association between HIV infection and risk for low birth weight, these were small studies. Notably they did not consider the stage of HIV disease (Minkoff *et al.*, 1990). Likewise, later studies also did not show a correlation (Castetbon *et al.*, 1999). In contrast, studies by Stratton *et al.*, (1999) support the correlation of low birth weight with HIV infection (Stratton *et al.*, 1999).

Infant birth weight does not differ among women in the earlier stages of HIV disease, but has been shown to decrease significantly in stage III than in stage I infection (Dreyfuss *et al.*, 2001). This may be attributed to the fact that with increased HIV infection, the immune system is further, down regulated eventuating in a deterioration of nutritional reserves.

#### **4.1.7 Placental weight**

In this study we observed a significant difference in placental weight across study groups ( $p < 0.001$ ). Supporting evidence is that the placental weight is has been previously reported to be significantly less in pre-eclampsia than normotensive pregnancies (Mallik *et al.*, 1979; Udainia & Jain, 2001; Majumdar *et al.*, 2005). Additionally, early onset pre-eclampsia is characterized by abnormal

placentation and resultant hypoxia. Poor fetal/neonatal outcomes particularly, lower birth weight and lower birthweight has been previously reported in the EOPE group (Lisonkova *et al.*, 2013). The shallow placentation with resultant decreased blood flow and ensuing lower placental weight in the early onset pre-eclampsia group expounds placental inefficiency (Naicker *et al.*, 2003).

#### **4.1.8 Mode of delivery**

Timing of delivery depends on several factors, including gestational age, fetal lung maturity, and most importantly, disease severity. In the pregnant groups (normotensive and pre-eclamptic) of our study, 65.9% of deliveries were performed in the interest of the baby compared to maternal indications. In our study there was a significant difference between the normotensive and pre-eclamptic groups ( $p < 0.001$ ). Off note, pre-eclampsia may lead to significant fetal morbidity and mortality, including an increased incidence of placental abruption, fetal growth restriction, and preterm delivery (Turner, 2010). In our study, forty five percent pregnant women delivered by caesarean sections (C-section), whilst 27% required emergency C-section. Induction of labour was required in 4.6% of pregnant women whilst only 23% had a natural birth. Moreover, clinical complications encountered in our study included a case of abruption placentae, 9 cases of eclampsia, 5 cases of imminent eclampsia and 12 cases of severe pre-eclampsia. Additionally, there were 6 cases of still-births. In the early-onset pre-eclamptic group, there were 5 women that developed eclampsia and one case of abruption placentae.

#### **4.1.9 HELLP**

In a study by Minire *et al.*, (2013), maternal complications in pre-eclampsia were examined precisely and showed that liver damage occurred in 4.9% and 12.3% had renal impairment. Detachment of the placenta (abruption) was encountered in 7% of cases and 0.7% of patients had an epilepsy. HELLP syndrome was present only in 4.2%, pulmonary edema occurred in 5.6% of subjects, DIC was found in 2.8% of cases and only one patient (0.2%) was registered with encephalopathy (Minire *et al.*,



2013). A limitation of this study is that the data on liver and renal function tests were not available for all participants hence it was not possible to speculate on the true incidence of liver and renal dysfunction in the local setting. This would have been an interesting area of future investigation, as there may be contributory disease to these organs with use of various antiretroviral drugs.

#### **4.1.10 HIV infection**

As per study design and inclusion criteria, forty five percent (45.1%) of our overall study population was HIV infected. It must be noted that the study was conducted at a large referral hospital, there is a higher number of HIV positive individuals in such a setting. Furthermore, in the Obstetric ward, a younger population tends to be the norm and this is a high risk group for the HIV infection. This study specifically included participants in the 18-45 years age group, an age group that carries the highest incidence of HIV nationwide (Lehola, 2014). As participants were of a similar age throughout the study groups their time of exposure to HIV infection may be similar.

As the national government of South Africa had instituted policy at the time of initiation of this study, all pregnant women with a CD4 count less than 350 cells/mm<sup>3</sup> commenced an ARV triple regimen therapy and those with a CD4 >350 received PMTC this may explain the significant difference in ARV usage between the non-pregnant and pregnant populations.

## **4.2 Adipokines**

### **4.2.1 C-peptide**

We report a significant difference in c-peptide levels across study groups ( $p < 0.01$ ). We also demonstrated a significant difference in c-peptide between the non-pregnant versus normotensive ( $p < 0.01$ ) and the normotensive versus late onset pre-eclamptic group ( $p < 0.01$ ). In addition to that, we have found that the levels of c-peptide are elevated in the pre-eclamptic groups when compared to normotensive pregnant and non-pregnant groups. According to (Schipper *et al.*, 2010), adipokines

play a huge role in the pathophysiology of obesity, which in turn plays a crucial role in pre-eclampsia development. Moreover, several studies have reported to have found a strong association between adipokines and pre-eclampsia development. This correlates with other studies that have reported that c-peptide plays a crucial role in glucose homeostasis since it is responsible for cleaving the A and B chain of pro-insulin. Additionally, insulin has been reported to be one of the most important adipokines that plays a role in the pathophysiology of pre-eclampsia by causing insulin resistance (Shangguan *et al.*, 2009). Furthermore, pregnancy itself is a state of physiological insulin resistance, in pre-eclampsia, the levels of insulin are exaggerated. In contrast to our findings, serum C-peptide has been shown to be significantly lower in women with severe compared to mild pre-eclampsia and normotensive pregnant women (Sayed *et al.*, 2015).

When considering HIV status, there was no significant differences in the levels of c-peptide amongst the groups ( $p>0.05$ ). Additionally, we found high levels of c-peptide in the HIV positive pre-eclamptic groups. Our findings correspond with other studies that have reported high levels of adipokines in pregnant HIV positive women, owing to the fact that HIV does cause dysregulation of adipokines. Our results corresponds with the literature that has reported that HIV is characterized by changes in fat distribution and insulin resistance (Koutkia *et al.*, 2003). Both HIV infection and its management therapies are known to contribute to metabolic and morphologic alterations that may increase risk dyslipidemia, altered glucose metabolism, central fat accumulation, and inflammation (Grunfeld *et al.*, 2008). All three major classes of antiretroviral therapy (ART), including protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), and non-nucleoside reverse transcriptase inhibitors (NNRTI), have been implicated in these complications, with the nature and magnitude of effects differing among drugs in a given class (Mulligan *et al.*, 2010).

### **4.2.2 Ghrelin**

In our study we with have found low levels of ghrelin in the pregnant groups, compared to the non-pregnant. Ghrelin is a hormone that makes you want to eat, it is an antagonist of leptin. Both hormones play a role in energy homeostasis. In mid-pregnancy, ghrelin levels increase, but a later gestation, it is said that the levels of ghrelin decrease and then returns to normal after delivery (Fuglsang, 2008).

Additionally, we found that HIV status does not impact levels of ghrelin. However the early onset pre-eclampsia has shown higher levels of ghrelin in the HIV positive group when comparing to the HIV negative group. According to Falasca and associates, HIV-related metabolic abnormalities include hypertriglyceridemia, hypercholesterolemia, insulin resistance, and diabetes mellitus (Falasca *et al.*, 2006). Recent studies have reported that ghrelin plays a role in elevating hypertriglyceridemia promoting the deposition of triglycerides (TG) in the liver among HIV positive people (Falasca *et al.*, 2006).

### **4.2.3 Gastric inhibitory polypeptide (GIP)**

We demonstrate a significant difference in GIP across the study groups ( $p < 0.01$ ). Furthermore, we also show a significant difference between the non-pregnant versus normotensive pregnant ( $p < 0.01$ ); normotensive pregnant versus early onset ( $p < 0.05$ ) and the normotensive pregnant versus the late onset pre-eclamptic group ( $p < 0.01$ ). Additionally, our study have found higher levels of GIP in pre-eclamptic groups compared to normotensive pregnant groups. Studies done by Chen and associates have reported elevated levels of GIP in pregnancy when comparing to non-pregnant (Chen *et al.*, 1995). Furthermore, Studies have reported that adipokines are elevated in pregnancy and even more so in pre-eclampsia.

GIP was statistically different based on HIV status ( $p < 0.001$ ). Interestingly in fasting or 2-h glucose, insulin, pro-insulin and C-peptide concentration did not differ amongst antiretroviral naïve, on an ART regimen that contained an NNRTI but no protease inhibitors (PI), on an ART regimen that included a PI but no NNRTI, on a non-PI/non-NNRTI containing regimen and a HIV seronegative control groups (Grunfeld *et al.*, 2008). All three major classes of antiretroviral therapy (ART), including protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), and non-nucleoside reverse transcriptase inhibitors (NNRTI), have been implicated in dyslipidemia, with the nature and magnitude of effects differing among drugs in a given class (Grunfeld *et al.*, 2008).

#### **4.2.4 Glucagon-like peptide 1 (GLP-1)**

GLP-1 is a hormone that regulates glucose levels in the body by stimulating the secretion of insulin, and GLP-1 also inhibits appetite. In our study, we noted a significant difference in GLP-1 across the study groups ( $p < 0.05$ ) and between non-pregnant versus normotensive pregnant groups ( $p < 0.01$ ). GLP-1 is reduced by 25% among people with pre-diabetes and up to 20% among obese people compared to normal weight people. This specifies that the decrease in GLP-1 is not a result of type 2 diabetes, but appears much earlier in the disease development and may dispose people to type 2 diabetes development (Science Daily, 2015). Notably, the combined use of hormones glucagon and glucagon-like peptide 1 (GLP-1) may form the basis for a new treatment for obesity and diabetes in the future (Science Daily, 2013). GLP-1 promotes weight loss by central suppression of feeding and via delayed gastric emptying promoting early satiety (Näslund *et al.*, 1999). A study by Bende and associates (2006) has reported that pregnancy does not alter secretion of incretin hormones.

GLP-1 was statistically different based on HIV status ( $p < 0.05$ ) specifically, between the non-pregnant versus the normotensive groups ( $p < 0.01$ ). According to our knowledge, there is no literature reporting the association of GLP-1 and HIV. However, GLP-1 is involved in the secretion of insulin, and there have been several studies that have reported that HIV is associated with dysregulation of adipokines,

which can alter glucose homeostasis. It is therefore plausible to suggest that HIV does affect GLP-1 levels. Additionally, HIV has been reported to be involved a number of chronic metabolic disorders of multifactoria which include increased prevalence of impaired glucose tolerance, diabetes mellitus (Gutierrez & Balasubramanyam, 2012).

#### **4.2.5 Glucagon**

We report no significant difference in glucagon across the study groups ( $p>0.05$ ). Glucagon controls food intake and satiety, through signaling the body to decrease levels of other appetite hormones like ghrelin (Science Daily, 2013). Glucagon induces the liver's production of glucose into the bloodstream and maintains the fuel supply for the brain. In this study, we found a significant difference between the non-pregnant and normotensive group ( $p<0.05$ ).

Also we show that HIV status does not affect glucagon levels ( $p>0.05$ ). However, glucagon differed significantly between HIV positive non-pregnant versus HIV negative non-pregnant ( $p<0.05$ ). Metabolic abnormalities (hyperlipidaemia and insulin resistance) and body fat redistribution (central adiposity and peripheral fat wasting) has been reported with increasing incidence in HIV-1 infected patients, receiving highly active antiretroviral therapies including HIV-1 protease inhibitors (Esteban & Jose, 1999). There haven't been studies that report the impact of HIV on glucagon secretion.

#### **4.2.6 Insulin**

Insulin blocks the secretion of glucagon, opposes glucagon action on the liver, and instructs the body to take up glucose from the blood (Science Daily, 2014). Glucagon and insulin normally counteract each other to stabilize blood-sugar levels. Glucagon is released by the pancreas in response to low concentrations of insulin and, conversely, glucagon release is suppressed by high levels of insulin in the bloodstream. In our study, we report no significant difference in insulin across the study groups

( $p>0.05$ ). However, we found a significant difference between the non-pregnant versus normotensive group ( $p<0.05$ ).

We have also found that HIV status did not affect insulin levels ( $p>0.05$ ). Insulin plays a crucial role in glucose homeostasis by Insulin regulates how the body uses and stores glucose and fat. Many of the body's cells rely on insulin to take glucose from the blood for energy. In HIV positive individuals, Insulin ability to maintain glucose homeostasis is altered. Rondanelli and associates have reported that recently reported  $\beta$  cell dysfunction in HIV-infected individuals especially for individuals on anti-retroviral treatment (Rondanelli *et al.*, 2004).

#### **4.2.7 Plasminogen activator inhibitor (PAI)-1**

In our study, PAI-1 did not differ across the study groups ( $p>0.05$ ) and between the groups ( $p>0.05$ ). Significantly elevated levels of PAI-1 and fibronectin occurring early in pregnancies that subsequently develop pre-eclampsia suggest that these variables may have predictive values (Halligan *et al.*, 1994). During pregnancy, profound changes take place in the hemostatic system, the levels of PAI-1 increases (Kruithof *et al.*, 1987). The fibrinolytic activity during pregnancy decreases, due to that fact that plasma levels of fibrinogen factor VII- VIII and von Willebrand factor increases. It is said that the decrease levels of fibrinolytic activity observed during pregnancy is due to increase levels of PAI-1 derived from the placenta (Kruithof *et al.*, 1987). High levels of PAI-1 have been observed in pregnant women compared to non-pregnant women. A common deletion polymorphism that results in a sequence of 4G instead of 5G in the promoter region of the gene associated with small increase in the risk of venous thromboembolism (Said *et al.*, 2012). Some studies have reported that this polymorphism is associated with adverse pregnancy outcome, however, the topic remains controversial. Moreover, a study by Linjen *et al.*, (2005) reports that PAI-1 levels correlate with insulin resistance, can be used to predict the development of type 2 diabetes independently of other known risk factors (Linjen *et al.*, 20015; Festa *et al.*, 2002).

In our study, when considering HIV status, there was no effect on PAI-1 ( $p > 0.05$ ). In HIV-associated lipodystrophy PAI-1 is augmented via dysregulation of the TNF-system (high TNF alpha and high sTNFR1) may play a role in up-regulating PAI-1 (He *et al.*, 2005). In contrast, no association between PAI-1 levels with HIV or HCV (Keifer *et al.*, 2013), whilst an association between worsening HIV disease and higher PAI-1 levels has been demonstrated (Schved *et al.*, 1992).

#### **4.2.8 Visfatin**

We observed a significant difference in the levels of visfatin across the study groups ( $p < 0.05$ ) and between the non-pregnant versus normotensive pregnant group ( $p < 0.05$ ) and the late onset pre-eclamptic versus the non-pregnant group ( $p < 0.01$ ). Maternal plasma visfatin peaks between 19–26 gestational weeks in normal weight pregnant women (Mazaki-Tovi *et al.*, 2009). There are only a few reports regarding circulating visfatin concentrations in pregnant women (Chan *et al.*, 2006; Fasshauer *et al.*, 2008). Indeed, only a single study reported the results of comparison of maternal visfatin concentrations between the three trimesters of pregnancy (Mastorakos *et al.*, 2007) and none included a comparison of circulating maternal visfatin concentrations between normal and overweight pregnant women. Given the diabetogenic effect of visfatin, it is tempting to suggest that the median visfatin concentration increase during pregnancy in association with insulin resistance and the increase in maternal weight.

In our study there was no effect of HIV status on the level of visfatin across the study groups ( $p < 0.05$ ). There was a significant difference between the HIV positive versus negative non-pregnant groups ( $p < 0.05$ ). Visfatin has the tendency to mimic insulin and activate insulin receptors

### **4.3 Obesity and pre-eclampsia**

Several studies have reported a strong association between insulin resistance and elevation of adipokines during pregnancy. A number of studies have analysed the association of obesity and the development of diabetes type 2. Women who develop gestational diabetes are at high risk of developing pre-eclampsia and also diabetes type 2 after delivery. Women who are obese and diabetic are associated with an increase in circulation adipokines (Saucedo *et al.*, 2011). Insulin resistance is one of the major metabolic disorders that is related to gestational diabetes.

In obesity, there are high level of circulating cytokines which can lead into inflammation, metabolic syndrome. Recently, studies have shown that the elevation of c-reactive protein and ADMA are a major factor contributing to this metabolic dysfunction in obesity. Additionally, another main factor is the infiltration of the endothelium by neutrophils which cause inflammation and hence increase high risk of pre-eclampsia development. This correlates with studies that have reported that pre-eclampsia is associated with high markers of endothelial inflammation (Innes & Wimsatt, 1999; Lorentzen *et al.*, 1998). It is therefore not surprising that individuals with higher BMI and are overweight have high levels of adipokines such as C-peptide and visfatin and therefore likely candidates for developing pre-eclampsia. It has been reported in several studies that among the many features of pre-eclampsia, hyperinsulinemia is common (Martinez-Abundis, 1996).

### **4.4 HIV and obesity**

Obese or overweight individuals have been reported to have high levels of adipokines like insulin, c-peptide, GIP and GLP-1 which suggest that HIV positive individuals have high incidence of insulin resistance, hence higher risk of developing hypertension. Additionally, pregnancy on its own is associated with some level of insulin resistance leading to diabetes mellitus, this suggest that HIV positive pregnant individuals have even higher risk of developing insulin resistance and if they are



obese/overweight, the risk even higher (Kaaja *et al.*, 1999; Innes *et al.*, 1999; Lorentzen *et al.*, 1999). Additionally, high levels of adhesion markers have been reported in HIV positive cohorts, this means that inflammation is also high.

#### **4.5 HIV and pre-eclampsia**

In our study, we report no significant differences in the levels of c-peptide ( $p>0.05$ ); ghrelin ( $p>0.05$ ); glucagon ( $p>0.05$ ) and PAI-1 groups ( $p>0.05$ ) between HIV negative and positive groups, irrespective of non-pregnancy or type of pregnancy (pre-eclamptic or normotensive). On the other hand we show significant differences between HIV positive and negative groups for, GIP ( $p<0.001$ ); GLP-1 ( $p<0.05$ ); insulin ( $p<0.05$ ) and Visfatin ( $p<0.05$ ) irrespective of non-pregnancy and type of pregnancy.

A study done by Suy and associates found that women who are HIV positive and on anti-HIV treatment have more incidence of pre-eclampsia than women who are HIV negative. Moreover, the introduction of ARV treatment to be administered to all pregnant HIV positive women have led to a decrease in mortality and morbidity among HIV infected individuals (Suy *et al.*, 2006). Additionally, it has also led to lower incidences of mother-to-child HIV transmission. Moreover, this has been a major achievement in the department of obstetrics and gynaecology because not only did it led to decrease in maternal mortality and mother-to-child HIV transmission, it also allowed women of fertile age, who are HIV positive with the chance to have babies who can be born without the virus.

Sadly though, despite the positive effect of ARV's in management of HIV infection, it has been reported that long term exposure to the anti-HIV treatment is reportedly associated with long term complications such as lipodystrophy and cardiovascular diseases (Martinez *et al.*, 1999). Obstetric complications associated with fetal death are and not limited to preterm birth, low birthweight, intrauterine, growth retardation and may lead to fetal death if they are severe. Interestingly, Stratton and associates (1999), have reported that adverse pregnancy outcomes due to pre-eclampsia among

HIV positive individuals was a common problem before it was compulsory for all HIV positive women to take HAART but now it is not. Additionally, Wimalasundera *et al.*, (2002), have found high incidence of pre-eclampsia among HIV positive women, suggesting that HIV does not give immunity against pre-eclampsia. A short survey done by the European collaborative study in 2003, have further reported that they identify pre-eclampsia as one of the most common condition among HIV positive women but only those who are on the HAART treatment. This was further supported by the study done by Suy and associates in 2003 who reported to have found high incidence in the number of cases of pre-eclampsia in HIV positive individuals on HAART. Additionally, they have also found incidence of fetal deaths to be high in individuals with pre-eclampsia and HIV positive. Pre-eclampsia and cases of fetal deaths was found to coexist more frequently among HIV positive individuals.

In 2001, Martinez study have found that not only does HIV individuals on HAART have high risk of developing pre-eclampsia, they are also at risk of developing lipodystrophy and myocardial infection. Moreover, a systematic review on maternal infection and pre-eclampsia found no association between pre-eclampsia and HIV (Conde-Agudelo *et al.*, 2008). Furthermore, another more recent study argue that women have high risk of developing pre-eclampsia only if they have been on HAART for a long time before pre-gnancy, additionally, have shown a strong association between pre-eclampsia and high BMI (Machado *et al.*, 2014). Additionally, Suy and associates in 2006, reported that HAART increase risk of pre-eclampsia. However, a study done by Frank and associates to determine whether HIV positive women have lower rates of pre-eclampsia than HIV negative have found no reduction (Frank *et al.*, 2004). Additionally, the same results were found by Boer and associates (Boer *et al.*, 2007).

## 4.6 Conclusion

This study reports no significant difference in the levels of c-peptide ( $p>0.05$ ); ghrelin ( $p>0.05$ ); glucagon ( $p>0.05$ ) and PAI-1 groups ( $p>0.05$ ) between HIV negative and positive groups, irrespective of non-pregnant or type of pregnancy (pre-eclamptic or normotensive). On the other hand we show significant differences between HIV positive and negative groups for, GIP ( $p=0.00065$ ); GLP-1 ( $p=0.03$ ); insulin ( $p>0.05$ ) and Visfatin ( $p=0.0108$ ) irrespective of non-pregnant and type of pregnancy. In terms of HIV status, we have observed that many of the adipokines were elevated in the HIV positive compared to the HIV negative group. These novel results implicate a role of HIV infection in the dysregulation of adipokines.

We report that c-peptide, GIP, GLP-1 and glucagon differed between the non-pregnant and normotensive groups ( $p<0.05$ ). There was no dysregulation of ghrelin and PAI-1 amongst non-pregnant, normotensive pregnant and pre-eclamptic pregnancies ( $p>0.05$ ). Additionally, c-peptide, GIP and GLP-1 differed between the normotensive and the late onset group respectively ( $p<0.05$ ). Visfatin varied between the late onset versus the non-pregnant group and GIP between the normotensive versus early onset pre-eclamptic groups ( $p<0.05$ ).

These adipokines play a role in glucose homeostasis contribute to the development of insulin resistance, a high risk factor pre-eclampsia development. Additionally, elevated adipokines in individuals with high BMI (obese and overweight) predisposes one to pre-eclampsia development.

In conclusion, our study is the first to examine adipokine dysregulation in the triad of HIV infection, pre-eclampsia and obesity. Furthermore, we have established that adipokines: C-peptide, GIP, GLP-1, PAI-1 and visfatin were significantly dysregulated hence they may have predictor test value in

diagnosing pre-eclampsia development. Based on the results we conclude that c-peptide, visfatin and gastric inhibitory polypeptide may be used as biomarkers to predict pre-development.

#### **4.7 Limitations of the study**

These include:

- Pre-pregnancy BMI- this was not available for all study participants.
- Duration of anti-HIV therapy- were not available all HIV positive participants.
- Family history with hypertension- most women were not able to contribute to this question.
- Levels of adipokines at each trimester- these results would strengthen our findings
- Adipokine levels post-partum adipokine levels for at least a short period postpartum would be of immense value.
- HAART pregnancy correlate adipokine levels with HIV management therapy
- Viral load-in each trimester is required as pregnancy itself, may accelerates HIV replication and progression
- Neonatal care- adherence to treatment is not known.

#### **4.8 Further direction**

We envisage further studies to compare levels of adipokines at each trimester as well as in HIV positive women stratified with regards to CD4 T cell count as well as duration of ARV treatment.

## **CHAPTER 5**

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## CHAPTER 6

## APPENDIX