MATERNAL SERUM LEVELS OF ADIPONECTIN AND LEPTIN IN NON-PREGNANT, NORMAL PREGNANT AND PREECLAMPTIC WOMEN

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BY

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Molecular Medicine, KNUST. This thesis has not been submitted for any other degree in this Institution or elsewhere.

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ABSTRACT

Adiponectin and leptin, bioactive substances produced and secreted by the adipose tissue and the placenta, are involved in the regulation of a variety of endocrine processes in the body. Limited and contradictory data exist, regarding the roles of adiponectin and leptin in normal pregnancy and preeclampsia. This study, therefore, sought to investigate the maternal serum levels of adiponectin and leptin during normal pregnancy and preeclampsia. A non-randomized case-control study was conducted among pregnant women who sought antenatal care at the Manhyia Government Hospital between January 2014 and March 2014. Forty non-pregnant women, sixty normal first trimester pregnant women, sixty normal second trimester pregnant women, sixty normal third trimester pregnant women and sixty preeclamptic third trimester women were enrolled in the study. The medical history, Body Mass Index and Blood Pressure of each participant were measured; and their blood and urine samples were collected for biochemical analysis. Among the non-pregnant women, adiponectin correlated significantly but inversely with leptin ($r=-0.402$, $p<0.05$) and BMI ($r=-0.684$, $p<0.05$), and leptin correlated significantly and directly with BMI ($r=0.571$, $p<0.05$). Among the pregnant women, adiponectin correlated significantly and inversely with leptin ($r=-0.669$, $p<0.05$), BMI ($r=-0.620$, $p<0.05$), and leptin correlated significantly and directly with BMI ($r=0.710$, $p<0.05$). Among the preeclamptic women, adiponectin correlated insignificantly with leptin ($r=-0.290$, $p>0.05$) but significantly and inversely with BMI ($r=-0.521$, $p<0.05$), and leptin correlated significantly and directly with BMI ($r=0.700$, $p<0.05$). While adiponectin levels ($p<0.05$) and the adiponectin-leptin ratio ($p<0.05$) were higher in the normal-weight women than in their overweight counterparts, the exact opposite held true for leptin levels ($p<0.05$). With regards to the levels of the adipokines in the participants, adiponectin levels were highest in the first trimester pregnant women, higher in the non-pregnant women, high in the second trimester pregnant women and low in the third trimester pregnant women ($p<0.05$).Leptin levels, on the other hand, remained comparable between the non-pregnant and the first trimester pregnant women ($p>0.05$), high in the second trimester pregnant women and highest in the third trimester pregnant women ($p<0.05$).The adiponectin-leptin ratio was comparable between the non-pregnant women and the first trimester pregnant women ($p>0.05$), low in the second trimester pregnant women and lowest in the third trimester pregnant women ($p<0.05$). The levels of adiponectin

($p<0.05$), leptin ($p<0.05$) and the adiponectin-leptin ratio ($p<0.05$) were higher in the preeclamptic women than in the normal pregnant women. In assessing overweight, gravidity and parity as possible determinants of preeclampsia, it was observed that overweight women are at a higher risk to developing preeclampsia than their normal-weight counterparts [OR=2.14 (0.93 – 9.84), $p<0.05$)], primigravida pregnant women are at a higher risk of developing preeclampsia than multigravida pregnant women [OR =3.57(1.524-8.37), $p<0.05$]. It was also observed that nulliparous pregnant women are at a higher risk of developing preeclampsia than multiparous pregnant women [OR =3.88 (1.30-11.62), $p<0.05$], but at comparable risk with primiparous pregnant women at developing preeclampsia [OR=1.9(0.87-4.18), $p>0.05$]. However, primigravidity and nulliparity, as risk factors to developing preeclampsia, were observed to be independent on the pre-pregnancy levels of adiponectin and leptin, due to the comparable levels of each adipokine among the pregnant women on the bases of gravidity and parity ($p>0.05$). Among non-pregnant and normal pregnant women, adiponectin and leptin exhibit an inverse relationship, and while adiponectin varies inversely with BMI and gestational age, leptin varies directly with BMI and gestational age. However, on the emergence of preeclampsia, a condition whose occurrence is possibly dependent on the gravidity and parity status of a woman, and alterations in the adiponectin-leptin ratio which is partly influenced by body weight, some of these relationships become blunted, as the levels of adiponectin and leptin rise significantly. The fluctuations in the levels of adiponectin and leptin during normal pregnancy indicate that adiponectin and leptin may play vital roles during pregnancy; and the observed elevated levels of adiponectin, leptin and the adiponectin-leptin ratio during preeclampsia, indicates that knowledge of the levels and ratio of the two adipokines during pregnancy, could assist in the diagnosis and treatment of preeclampsia.

DEDICATION

This thesis is dedicated to the Members of Staff

Department of Molecular Medicine

School of Medical Sciences

College of Health Sciences

KNUST

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ABBREVIATIONS

- AdipoR1 Adiponectin Receptor 1
- AdipoR2 Adiponectin Receptor 2
- Ad^{-1-} Adiponectin deficient
- AICAR 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide
- AMP Adenosine Monophosphate
- AMPK Adenosine Monophosphate Kinase
- AP-1 Activation Protein 1
- APPL1 Adaptor protein, Phosphotyrosine interaction, PH domain and Leucine zipper containing 1
- BBB Blood Brain Barrier
- BMI Body Mass Index
- CNS Central Nervous System
- eNOS Nitric Oxide Synthase
- ERK Extracellular Signal-regulated Kinases
- gp 130 Glycoprotein 130
- hCG Human Chorionic Gonadotropin
- HMW-Ad High Molecular Weight Adiponectin
- HPA Hypothalamic-Pituitary-Adrenal
- HRP Horseradish Peroxidase
- ICAM Intercellular Adhesion Molecule
- IL-1 Interleuken-1
- IL-6 Interleuken-2
- IRS-1 Insulin Receptor Substrate-1
- IRS-2 Insulin Receptor Substrate-2
- JAK Janus Kinase
- JNK Jun NH_2 terminal Kinase
- LMW-Ad Low Molecular Weight Adiponectin
- LPS Lipopolysaccharidase

- mRNA messenger Ribonucleic acid
- MAP Mitogen-activated protein
- MMW-Ad Medium Molecular Weight Adiponectin
- MnSOD Manganese Superoxide Dismutase
- MAPK Mitogen Activated Protein Kinase
- NF-kB Nuclear factor kappa-light-chain-enhancer of activated B cells
- NK Natural Killer
- NO Nitric Oxide
- PAI-1 Plasminogen Activator Inhibitor
- PAP1 Poly(A) polymerase
- PI-3 Phosphoinositide-3
- PPAR Peroxisome Proliferator-activated Receptors
- RAS Renin-Angiotensin System
- SAPK Stress activated protein kinase
- STAT Signal transducer and activator transcription factor
- TMB 3,3,5,5 -Tetramethylbenzidine
- TNF-α Tumour Necrosis Factor alpha
- tPA Tissue Plasminogen Activator
- VCAM Vascular Cell Adhesion Molecule
- VLCD Very Low Calory Diet
- ZO-1 Zonula Occludens -1

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Advances in the field of biomedical science are regularly altering and adding to existing knowledge on the roles of different tissues and organs in the human body; and the adipose tissue represents one of the most typical examples of the tissues that have received that attention. The human adipose tissue had, previously, been noted as a reservoir of fat for energy storage alone (Havel, 2002). However, recent investigations of the biology of the adipose tissue have revealed that it is not only an energy storage tissue, but also a secretory tissue that synthesizes and secretes multiple cytokine-proteins which modulate various biological functions (Jürimäe et al., 2005; Lecke et al., 2011). The number and range of identified adipocytokines – also called adipokines – is expanding rapidly (Scherer et al., 1995).

Two of these adipokines are adiponectin and leptin. They are plasma proteins that have, of late, drawn substantial attention in the research of metabolic syndrome, which is a combination of the existence of conditions like insulin resistance, dyslipidaemia, and hypertension (Berg et al., 2002; Havel, 2002; Reaven, 2005). These peptides are released by the adipose tissue in response to specific extra-cellular stimuli or changes in metabolic status (Zhang et al., 1994; Maeda et al., 1996), and it is because they seem to share some structural properties of cytokines that they are called adipocytokines (Matsuzawa et al.,

1999). Since their actions are similar (Nedvidkova et al., 2005), adiponectin and leptin may have synergistic effects.

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Adiponectin and leptin are involved in the endocrine processes of regulating glucose and fat metabolism, energy expenditure, inflammatory response, immunity, cardiovascular function and reproduction. They have been implicated in both the physiological adaptation to normal pregnancy and obstetric complications. During normal pregnancy, they seem to act in an autocrine/paracrine fashion in the placenta and adipose tissue to, possibly, play roles in the maternal-foetal interface, and contribute to glucose metabolism and foetal development (Lecke et al., 2011).Their levels are dysregulated in pathological conditions such as preeclampsia (an unpredictable suddenly-occuring and life-claiming pregnancy complication of placental origin but with seemingly elusive pathogenesis, and characterized by hypertension, proteinuria, inflammation, endothelial dysfunction and, sometimes, fluid retention), gestational diabetes and intrauterine growth restriction, representing an effect or a cause of disturbances in the feto/placento/maternal unit (Lea et al., 2000; Laivuori et al., 2006; Khosrowbeygi et al., 2009; Abd-Alaleem et al., 2011; Miehle et al., 2012).

1.2 PROBLEM STATEMENT

Pregnancy is characterized by profound local and systemic physiologies which are initiated after conception, and which continue throughout gestation. During pregnancy, there are series of metabolic changes that enhance adipose tissue accretion in early gestation, followed by insulin resistance, dyslipidaemia and facilitated lipolysis in late pregnancy (Buchanan et al., 1990; Catalano et al., 1991; Catalano et al., 1999). In normal pregnancy,

there is a physiological 50% decrease in insulin-mediated glucose disposal and a 200–250% increase in insulin secretion to maintain euglycaemia in the mother (Kühl, 1998; Catalano et al., 1999).

Compelling evidence suggests that some of these changes don't get corrected physiologically and so exacerbate into metabolic syndrome and other pregnancy complications, which affect the mother and the foetus; and the pregnant women who mostly suffer this effect are the overweight and first time pregnant women (Reaven, 2007; Owiredu et al., 2012 Puhkala et al., 2013). The mechanisms involved in the pathophysiological progression of some of these metabolic changes, and why the overweight and first time pregnant women are at increased risk of this occurrence than their normal-weight counterparts, have not been fully clarified. If the actual biomolecules that are associated with the metabolic alterations are known, then monitoring of their levels could be integrated into the routine obstetric investigations that form the basis of antenatal care and, hence, used as biomarkers for predicting pregnancy complications. Nevertheless, the biomolecules, as well as the mechanisms involved in that occurrence, have not been fully identified and expounded. Due to this, predicting pregnancy complications and, hence, delivering proactive medical care is difficult (Steegers et al., 2010; Al-Jameil et al., 2014). But since we live in the age of preventive medicine, several attempts to solve this problem have been underway. Researchers have hypothesized that variations in the concentrations of various hormones produced by the placenta may be responsible for the pregnancy-induced changes (Ong et al., 2000; Girling, 2004; Spencer et al., 2005); and following the discovery that adiponectin and leptin are not only produced by the adipose tissue but also by the placenta (Fantuzzi, 2005; Chen et al.,

2006), it has become imperative to investigate the interplay between the two adipokines

and pregnancy (both normal pregnancy and preeclampsia) since that would help comprehend the roles of adiponectin and leptin in normal pregnancy, unravel the mystery behind the actual pathogenesis and pathophysiology of preeclampsia, and then suggest whether or not adiponectin, leptin and the adiponectin-leptin ratio could be used as biomarkers for predicting and diagnosing preeclampsia. The dearth and paucity of information in this area, as well as the contradictory nature of the little available information, necessitates this investigation. For instance, while Nien et al., (2007) observed that the levels of adiponectin decrease proportionally with gestational age, Mazaki-Tovi et al., (2007) observed otherwise, and while Schubring et al., (1999) reported that leptin levels rise proportionally with gestational age, Herse et al., (2009) reported that leptin levels rather fall proportionally with gestational age. And concerning the levels of the adipokines in preeclampsia, whereas some investigators have reported significantly higher levels of the two adipokines in preeclampsia than in normal pregnancy (McCarthy et al., 1999; Khosrowbeygi et al., 2009; Abd-Alaleem et al., 2011), and others have reported comparable levels of the two adipokines between preeclampsia and normal pregnancy (Martinez-Abundis et al., 2000; Hendler et al., 2005), the rest have reported lower levels of the two adipokines in preeclampsia than in normal pregnancy (Mazaki-Tovi et al., 2009; Vaisbuch et al., 2009; Dalamaga et al., 2011).

It is very obvious from the available information that even the little attention, which has been given to the interplay between the two adipokines and pregnancy, is biased against Africans, since none of the studies reviewed was carried out in the African setting. Even if similar studies have been carried out in Africa, the findings might have not been published and, hence, not available. Thus, regarding the physiological profiles and roles of adiponectin and leptin in pregnancy, and the possibility of these adipokines being used

as biomarkers for pregnancy complications like preeclampsia, wide gaps are yet to be bridged.

1.3 HYPOTHESIS

Maternal serum levels of adiponectin and leptin are influenced by body weight, normal pregnancy and preeclampsia.

1.4 GENERAL OBJECTIVE

The aim of this study is to investigate the maternal serum levels of adiponectin and leptin in normal-weight and overweight non-pregnant, normal pregnant and preeclamptic women.

1.5 SPECIFIC OBJECTIVES

1. To characterize the relationship among adiponectin, leptin, body mass index and blood pressure in non-pregnant, normal pregnant and preeclamptic women
2. To describe the physiological profiles of adiponectin, leptin and the adiponectin- leptin ratio from pre-pregnancy state to the third trimester pregnancy state
3. To compare the levels of adiponectin, leptin and the adiponectin-leptin ratio between normal pregnant women and preeclamptic women
4. To determine whether or not maternal weight gain, gravidity and parity are possible risk factors of preeclampsia
5. To determine the effects of weight gain, gravidity and parity on the serum levels of adiponectin, leptin and the adiponectin-leptin ratio among non-pregnant, normal pregnant and preeclamptic women

CHAPTER TWO

REVIEWED LITERATURE

2.1 PREGNANCY

Pregnancy, a common name for gestation in humans, is the development of one or more embryos or foetuses in the womb of a female, following sexual intercourse or assisted reproductive technology. The pregnant woman undergoes several physiological and homeostatic changes. These include cardiovascular, haematologic, metabolic, renal and respiratory changes that ensure that the foetus is provided for. There is also cessation of menstrual flow during pregnancy; and this is attributable to the continual rise of oestrogen and progesterone which suppresses the concerned hypothalamic axis (Hytten et al., 1964; Enkin et al., 2000; Girling, 2004).

Pregnancy is detected by using various pregnancy tests. These tests detect hormones that are produced by the placenta. Examples of these hormones are human chorionic gonadotropin and progesterone. These hormones serve as biomarkers for pregnancy. Pregnancy is not detected immediately after fertilization, but few days (about 12 days) after implantation (Qasim et al., 1996; Poikkeus et al., 2002). In terms of sensitivity, blood pregnancy tests are highly recommendable. A quantitative blood test can even be used to determine the date on which the embryo was conceived. Urine tests are less sensitive, and are mostly used to detect pregnancies at home. A single test of progesterone levels also helps to determine the chances of survival of the foetus in pregnancies that are threatened by miscarriages (Verhaegen et al., 2012).

2.1.1 THE TRIMESTERS

Pregnancy is typically divided into three periods, called trimesters, each of which spans within three months; and obstetricians define each trimester as lasting for 14 weeks (Gerard, 1993; Girling, 2004; Cox et al., 2005).

2.1.1.1 First trimester

This ranges from the first week of conception to about the fourteenth week. At this stage, there is 40% increase in minute ventilation (Gerard, 1993). By week eight, the uterus attains a size that is comparable to the size of a lemon. It is at this stage that morning sickness and many of the symptoms and discomforts which signal pregnancy, appear. The symptoms comprise; nausea and vomiting, excessive tiredness and fatigue, frequent urination (particularly during the night), and cravings for certain foods that are not normally sought out (Campbell et al., 2001; Girling, 2004).

Some medical signs are also observed at this period. Some of these signs are; cessation in menstrual flow, the presence of human chorionic gonadotropin (hCG) in the urine and blood, rise in basal body temperature after ovulation (this lasts for over two weeks), implantation bleeding (which occurs within the third or fourth week after the last menstrual period), Goodell's sign (softening of the cervix), Chadwick's sign (darkening of the vulva, vagina and cervix), and Hegar's sign (softening of the isthmus of the uterus) (Hytten et al., 1980). At this trimester, breast tenderness also occurs; and is predominant in women who are pregnant at young ages. Due to a temporary increase in hormones shortly after conception, the nipples and areolas begin to darken. This process continues throughout pregnancy (Hytten et al., 1980; Girling, 2004).

2.1.1.2 Second trimester

Week thirteen to week twenty eight of pregnancy constitutes the second trimester. As the signs and symptoms of morning sickness begin to fade, most women start to put on extra weight. The uterus undergoes hypertrophy, and reaches about twenty times its original size. The foetus then begins to move by a process called "quickening". This mostly happens in the 4th month, more especially, in the 20th to 21st week, or by the 19th week, if the woman is not nulligravida. By the way, some women do not feel the movement of the foetus at this stage (Gerard, 1993; Girling, 2004).

2.1.1.3 Third trimester

This is the period within which the pregnant woman gains the final weight. The woman's belly changes in shape as the belly drops, due to the foetus turning in a downward position in readiness for birth. During the second trimester, the woman's belly would have been very upright, whereas in the third trimester, it would drop down quite low, and the woman would be able to lift her belly up and down (Gerard, 1993; King, 2000). The foetus usually moves, and this movement is felt by the woman. These foetal movements can be very intense, and hence very disturbing to the pregnant woman. Due to the gradual increase in size of the abdomen, the woman's navel sometimes becomes convex. “Head engagement”, a process where the foetal head descends into cephalic presentation, decreases pressure on the upper abdomen with enhanced ease in breathing. However, it exerts pressure on the pelvic floor and rectum, and severely decreases the capacity of the bladder (Girling, 2004). Moreover, it is at this period that unchecked sleep positions and maternal activity may affect the development of the foetus. For example, the enlarged uterus may restrict the flow of blood by compressing the lower pressured vena cava (King, 2000; Girling, 2004).

2.1.2 WEIGHT GAIN DURING PREGNANCY

Normal weight gain occurs during pregnancy, and this is partly determined by the weight of the foetus, the placenta and accumulated fluid. During pregnancy, maternal weight loss or excessive weight gain can affect the health of the mother and the foetus. Excessive weight gain during pregnancy can predispose the mother to pregnancy complications like preeclampsia, gestational diabetes, gestational hypertension, macrosomia and shoulder dystocia. It can also make postpartum weight loss become difficult (Oken et al., 2009; Siega-Riz et al., 2009).

2.1.2.1 BMI AS A MARKER OF WEIGHT GAIN

The BMI, also called, Quetelet index was invented between 1830 and 1850 by the Belgian polymath, Adolphe Quetelet, during the course of developing "social physics"(Eknoyan, 2008). Body mass index is defined as an individual's body weight divided by the square of his or her height. The formula has a unit of kg/m ^{2}. BMI can also be determined by using a BMI chart. This chart displays BMI as a function of weight (horizontal axis) and height (vertical axis), using contour lines for different values of BMI or colours for different BMI categories (Pietrobelli et al., 1998; Eknoyan, 2008).

Body mass index (BMI) has been widely accepted and used in most epidemiological studies as a surrogate measure of body weight; and is frequently used to estimate the prevalence of obesity within a population (Deurenberg et al., 1998; Pietrobelli et al., 1998). The criteria for classifying underweight, normal-weight, overweight and obesity among humans by using BMI is; underweight (<18.5kg/m ^{2}) , normal-weight (18.5-24.9kg/m^2), overweight (25.0-29.9kg/m ^{2}) and obese (≥30.0kg/m ^{2}) . BMI of 30–35kg/m ^{2} reduces life expectancy by two to four years, while severe obesity (BMI > 40)

reduces life expectancy by 10 years (Weisell, 2002). BMI has been found to be consistently associated with an increased risk of cardiovascular diseases (CVD) and type 2 diabetes (Savva et al., 2000; Baker et al., 2007), yet this measurement fails to differentiate fat weight from lean weight, and so does not take into consideration, the distribution of body fat which is believed to have significant health implications (Akpinar et al., 2007). BMI, therefore, does not account for variation in body fat distribution and abdominal fat mass, which can differ greatly across populations and can vary substantially within a narrow BMI range. Due to this, individuals with a similar BMI may show variations in their abdominal-fat mass, with premenopausal women typically having half the abdominal-fat mass of men (Daniels et al., 1997; Akpinar et al., 2007).

The World Health organization (2000) recommends an overall pregnancy weight gain for those of normal-weight (Body mass index of 18.5kg/m ^{2}– 24.9kg/m ^{2}) , of 11.3kg–15.9 kg (25–35 pounds) having a singleton pregnancy; women who are underweight (BMI of less than 18.5 kg/m ^{2}) should gain between 12.7kg–18 kg (28–40 lbs), while those who are overweight (BMI of 25 kg/m –^{2} 29.9 kg/m)^2 are advised to gain between 6.8kg –11.3 kg (15–25 lbs) and those who are obese (BMI > 30 kg/m ^{2}) should gain between 5kg–9kg (11–20 lbs) (Weisell, 2002).

2.1.3 PREECLAMPSIA

Preeclampsia is a pregnancy-specific disorder which is characterized by high blood pressure and significant proteinuria. It is associated with alterations in maternal physiological characteristics and metabolism; and is a major cause of maternal mortality and mobidity. Preeclampsia was formerly referred to as toxaemia of pregnancy, since it was erroneously believed to be caused by toxins released into the maternal circulation. It

affects between 2-8% of pregnancies worldwide, and it is associated with multiple maternal and foetal adverse effects (Eiland et al., 2012). Commonly, preeclampsia occurs in first pregnancies than in subsequent pregnancies with the mother's blood pressure returning to normal after delivery (Sibai et al., 2005). It may develop after 20 weeks of gestation, though most commonly after 32 weeks. When it occurs before 32 weeks of gestation, it is considered as early-onset, and that is associated with increased morbidity (Lisonkova et al., 2013).

Central to the effects of preeclampsia are the resulting presence of uteroplacental hypoxia (inadequate oxygen supply), an imbalance in angiogenic and anti-angiogenic proteins, oxidative stress, maternal endothelial (lining of blood vessels) dysfunction, and elevated systemic inflammation (Steegers et al., 2010; Al-Jameil et al., 2014).

Blood pressure equal to or higher than 140/90 mmHg and proteinuria higher than 100 mg/dl by urinalysis, in conjunction with the presence of a low blood platelet count (thrombocytopenia), impaired liver function, the development of new kidney dysfunction, fluid accumulation in the lungs (pulmonary oedema), and/or new-onset brain or visual disturbances are used to diagnose this condition (Steegers et al., 2010). If left untreated, preeclampsia can develop into eclampsia, the life-threatening occurrence of seizures during pregnancy. Removal of the foetus and placenta is the only known treatment for preeclampsia. Rarely, preeclampsia may also occur in the postpartum period (Arulkumaran et al., 2013).

The pathogenesis of preeclampsia is not known. Nevertheless, it has been hypothesized that abnormal placentation (development and arrangement of the placenta) and placental function are strong predisposing factors to developing preeclampsia. A number of factors

are responsible for the mystery behind the pathogenesis of preeclampsia. These include immunologic, heamatologic, genetic, and environmental factors. A major consequence of all these factors is generalized endothelial dysfunction, which results in hypertension and the other symptoms and complications associated with preeclampsia (Eiland et al., 2012; Al-Jameil et al., 2014). Given the syndromic and mulitfactorial nature of the disease, it is not yet possible to routinely predict preeclampsia (Steegers et al., 2010; Al-Jameil et al., 2014), however, a number of risk factors associated with preeclampsia have been put forward (Conde_Agudelo et al., 2000). These risk factors comprise maternal, paternal, genetic, environmental and/or obstetric factors. Preeclampsia is more prevalent in primiparous women than in multiparous women; and in nulliparous women than in multiparous women, complicating 25-30% of nulliparous pregnancies. Due to this, it is said that the first pregnancy of every woman is considered to be a risk factor for preeclampsia (Trupin et al., 1996; Skjærven et al., 2002; Duckitt et al., 2005).

It has been hypothesized that susceptibility to preeclampsia is the maternal-foetal conflict between the mother and the foetus. After the first trimester, trophoblasts enter the spiral arteries of the mother to alter the spiral arteries and, thereby, gain more access to maternal nutrients. However, occasionally, there is impaired trophoblast invasion that results in inadequate alterations to the uterine spiral arteries. Thus, the developing embryo releases biochemical signals that result in the woman developing hypertension and preeclampsia so that the foetus can benefit from a greater amount of maternal circulation of nutrients, due to increased blood flow to the impaired placenta (Lie et al., 1998; Sargent et al., 2006; Rusterholz et al., 2007).

It has been proposed that the presence of paternal genes in the foetus and the placenta poses a challenge to the immune system of the pregnant woman (Lie et al., 1998). As a buttressing

fact to this theory, some investigators have observed that continual exposure to a partner's semen several years before conception (so as to establish immunological tolerance) reduces the risk of developing preeclampsia, but exposure to a partner's semen few months before conception, increases the risk of developing preeclampsia. These investigators then concluded that long periods of sexual cohabitation with the same partner fathering a woman's child, is protective against preeclampsia, and this protective effect is broken when there is a change of partner. They, therefore, suggested that condom use is not a good habit as far as avoiding preeclampsia is concerned (Trupin et al., 1996; Hernandez-Valencia et al., 2000; Einarsson et al., 2003). The occurrence of pair-bonding between the mother and the father and paternal investment in the foetus has, thus, been suggested to account for the vulnerability to preeclampsia. The occurrence of preeclampsia is, therefore, an adaptation for the mother to terminate investment in a foetus that might have an unavailable paternal donor, as determined by repeated semen exposure of the paternal donor to the mother (Davis et al., 2006).

Following the relevance of a woman's immunological tolerance to her baby's paternal genes, and the fact that the human immune system tolerates things better when they enter the body through the mouth (Challacombe, 1987), some investigators have conducted a series of studies that have, surprisingly, found a strong correlation between diminished incidence of preeclampsia, and the swallowing of a partner's semen, through oral sex. Those researchers concluded that while any exposure to a partner's semen during sexual activity appears to decrease a woman's chances for the various immunological disorders that can occur during pregnancy, immunological tolerance could be most quickly established through oral introduction and gastrointestinal absorption of semen (Koelman et al., 2000; Bonney, 2007).

2.2 ADIPOSE TISSUE

Since the adipose tissue has the capacity to secrete hormones and cytokines, it is currently recognized as a dynamic organ. There exist the white adipose tissue and the brown adipose tissue. The white adipose tissue is the predominant form found in adults while the brown adipose tissue is the predominant form found in neonates (Cannon et al., 2008). The white adipose tissue consists of preadipocytes and adipocytes, macrophages, endothelial cells, fibroblasts, and leukocytes, and secretes a variety of adipocytokines, namely; adiponectin, leptin, tumour necrosis factor–alpha (TNF-α), interleukin-6 (IL-6), resistin, plasminogen-activating inhibitor–I (PAI-1), and angiotensinogen (Galic et al., 2010).

These low and medium-weight molecular proteins play an important role in the physiology of the adipose tissue. They are involved in a range of endocrine processes that regulate glucose and fat metabolism, energy expenditure, inflammatory response, immunity, cardiovascular function, and reproduction, among other functions; and are believed to be a link between obesity, insulin resistance and endothelial dysfunction. The uncontrolled expansion of adipose tissue is the key feature of obesity (Cannon et al., 2008; Galic et al., 2010).

2.2.1 ADIPONECTIN

This adipokine is also referred to as GBP-28, apM1, AdipoQ or Acrp30 (Maeda et al., 1996; Ukkola et al., 2002).

2.2.1.1 Discovery

In 1995, Scherer et al., reported the cDNA that encodes the Acrp30 protein in mice (adipocyte complement–related protein) (Scherer et al., 1995). In 1996, the same protein

was characterized in mice as the mRNA transcript most highly expressed in adipocytes, and was called apM1 (Adipose Most Abundant gene transcript) (Maeda et al., 1996). In the same year, another group identified the protein in mice and termed it adipoQ (Hu et al., 1996). Finally, Nakano and his group isolated the human homologue of adiponectin from plasma (Nakano et al., 1996). In 2007, adiponectin was identified as a transcript highly expressed in preadipocytes (Lara-Castro et al., 2007), differentiating into adipocytes (Ukkola et al., 2002; Matsuzawa et al., 2004).

2.2.1.2 Structure and Functions

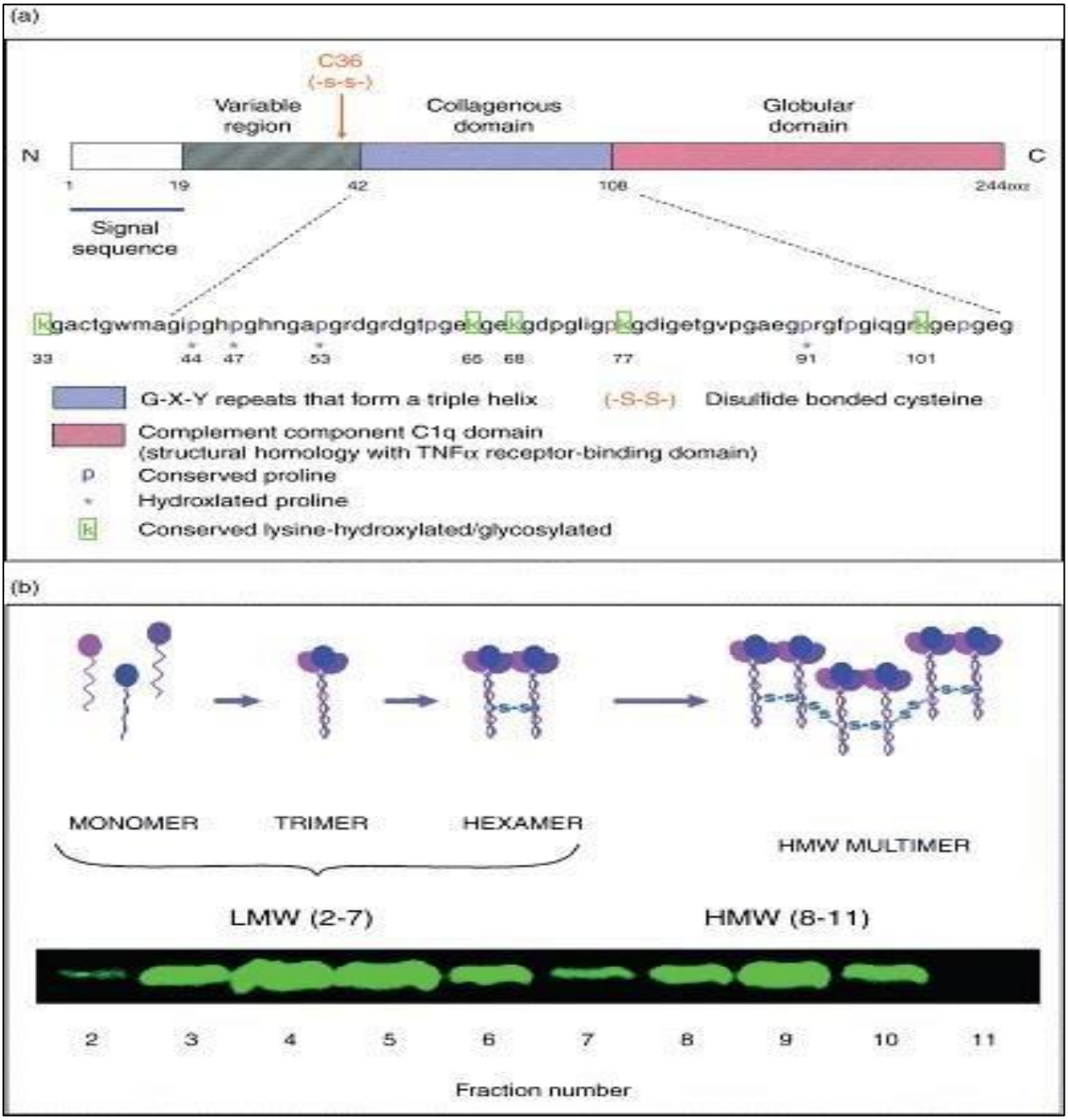

Figure 2.1 The structure and multimerization of adiponectin (a) The domain structure of human adiponectin. **(b)** Adiponectin multimerization and analysis by velocity sedimentation. Separation of the low-molecular weight (LMW) and high-molecular weight (HMW) multimers was performed by centrifugation on a 5 to 20% sucrose density gradient. Fractions 2 to 7 of the gradient contain the LMW multimers, while the HMW multimers are found in fractions 8 to11.
Reproduced from Whitehead JP and his colleagues (Whitehed et al., 2006).

Adiponectin, a protein of 244 amino acids, is one of the most abundant adipose tissuespecific proteins. It has four distinct regions. The first region is a short signal sequence (20-residue signal sequence) that targets the hormone for secretion outside the cell, the second is a short region (an N-terminal region without homology to any known protein) which varies between species, the third is a 65-amino acid region with similarity to collagenous proteins, and the last is a C-terminal globular domain. The three dimensional structure of its C-terminal globular domain is similar to that of tumour necrosis factor– alpha , even though there is no sequence homology at the primary structure level (Shapiro et al., 1998).

Adiponectin is encoded by the ADIPOQ gene (Maeda et al., 1996). Of the number of receptors that adiponectin binds to, only three of them have been identified. They are: adiponectin receptor 1 – ADIPOR1, adiponectin receptor 2 – ADIPOR2 and T-cadherin – CDH13. The first two bear homology to G protein-coupled receptors and the third one is similar to the cadherin family (Yamauchi et al., 2003b; Hug et al., 2004)

These have distinct tissue specificities within the body and have different affinities to the various forms of adiponectin. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. They serve as receptors for globular and full-length adiponectin, and mediate increased AMP kinase, PPAR ligand activity, fatty acid oxidation, and glucose uptake by adiponectin (Yamauchi et al., 2003b). Expression of the receptors is correlated with insulin levels, and reduced in mouse models of diabetes, particularly in the skeletal muscle and the adipose tissue (Fang et al., 2006; Bonnard et al., 2008).

After post-translational modifications, adiponectin is secreted from the adipose tissue and the placenta of pregnant women into the bloodstream in three different multimeric forms: a trimeric or low molecular weight form (LMW-Ad), a hexameric or medium molecular weight form (MMW-Ad), and a high molecular weight form (HMW-Ad); and is very abundant in plasma relative to many hormones. Adiponectin automatically self-associates into these larger structures. Initially, three adiponectin molecules bind to form a homotrimer. The trimers continue to self-associate and form hexamers or dodecamers. The amount and distribution of these molecular forms can determine the activity of adiponectin in different tissues (Chen et al., 2006; Neumeier et al., 2006; Kovacova et al., 2009).

Studies have shown that the high-molecular weight form may be the most biologically active form regarding glucose homeostasis (Tabák et al., 2009); and that it associates with a lower risk of diabetes, with similar magnitude of association as total adiponectin (Zhu et al., 2010). However, coronary artery disease has been found to be positively associated with the high molecular weight adiponectin, but not with the low molecular weight adiponectin (Rizza et al., 2010).

In the blood stream, adiponectin accounts for approximately 0.01% of the total plasma proteins (Nedvidkova et al., 2005). It exists in circulating plasma at concentrations ranging from 4 to 30µg/mL, which is higher than the concentrations of the other hormones and cytokines. The relative levels of the higher-order structures are sexually dimorphic, where females have increased proportions of the high-molecular weight forms

(Coppola et al., 2009).

Adiponectin has a sticky nature and so binds to collagen I, III, and V, which are present in vascular intima. It exhibits various anti-atherogenic effects on vascular cells by suppressing the expression of adhesion molecules in vascular endothelial cells, proliferation of smooth muscle cells, and accumulation of cholesteryl-esters in macrophages; however, its plasma

levels are low in subjects with excess intra-abdominal fat (Ouchi et al., 2001; Arita et al., 2002; Yamauchi et al., 2003a; Shimomura et al., 2006).

Results from animal studies suggest that adiponectin is likely to play an important role in regulating insulin action (Hotta et al., 2001; Maeda et al., 2002). It was reported by Hotta and his research team that a decline in adiponectin concentration coincides with the development of hyperinsulinaemia and insulin resistance in rhesus monkeys (Hotta et al., 2001). Adiponectin exhibits anti-diabetic properties, and plays a role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues. Thus, hypoadiponectinaemia is an independent risk factor for developing metabolic syndrome (Nedvidkova et al., 2005; Lara-Castro et al., 2007).

2.2.1.3 Maternal serum levels of Adiponectin during Normal Pregnancy

Normal pregnancy is associated with alterations in maternal circulating adiponectin, and with changes in the relative distribution of its isoforms (Mazaki-Tovi et al., 2008). Maternal levels of adiponectin correlate inversely with gestational age, such that as pregnancy advances, adiponectin levels fall (Nien et al., 2007b). This has been contradicted by the findings of Mazaki-tovi et al., (2007) which indicate that, despite increased insulin resistance during pregnancy, no significant alteration in adiponectin occurs as pregnancy advances; and this may imply that the regulation of adiponectin during gestation is altered. These investigators then added that slightly elevated gestational adiponectin levels are consistent with increased 'adiponectin resistance' during pregnancy (Mazaki-Tovi et al., 2007).

2.2.1.4 Adiponectin and Body Weight

Plasma levels of adiponectin are inversely correlated with body fat percentage in adults while the association in infants and young children is less clear (Ukkola et al., 2002). The levels are lower in obese than normal-weight people, even though obese people tend to have more adipose tissue (Hotta et al., 2001; Ukkola et al., 2002; Nedvidkova et al., 2005). Weight reduction significantly increases circulating levels of adiponectin (Coppola et al., 2009). Unlike other adipocytokines, such as leptin, plasma adiponectin is inversely correlated with body mass index (BMI), intraabdominal fat, and indices of insulin resistance (Cnop et al., 2003).

It has been reported that weight loss results in increased adiponectin levels in men exposed to acute exercise (Saunders et al., 2012). In a study by Yang and his research team to determine the relationship between weight loss and adiponectin levels, it was observed that a 21% reduction in mean body mass index was accompanied by a 46% increase in mean plasma adiponectin level. The change in plasma adiponectin levels was significantly correlated with the changes in the various anthropometric markers of obesity. It was also evident in that study that reduction of body weight after gastric bypass surgery was associated with a similar increase in plasma adiponectin levels (Yang et al., 2001).

Additionally, among normal pregnant women, overweight pregnant women have a lower adiponectin concentration than normal-weight pregnant women (Hendler et al., 2005; Nien et al., 2007b). By investigating the influence of BMI on the levels of adiponectin in preeclamptic women, Hendler et al., (2005) observed a decreased level of adiponectin in overweight women with severe preeclampsia compared to their normal-weight counterparts; but no difference in the levels of adiponectin in overweight women with mild

preeclampsia compared to their normal-weight counterparts. This study was partially confirmed by Khosrowbeygi et al., (2009) who reported a lower level of adiponectin in overweight preeclamptic women than the normal-weight preeclamptic women.

2.2.1.5 Adiponectin and Blood Pressure

Adiponectin correlates inversely with systolic blood pressure and diastolic blood pressure (Kazumi et al., 2002; Adamczak et al., 2003). Adamczak et al., (2003) showed for the first time that plasma adiponectin levels are significantly lower in patients with essential hypertension compared with those in body mass index-matched normotensive subjects. And following this, a number of clinical studies have also demonstrated that an inverse relationship exisits between plasma adiponectin concentration and hypertension (Iwashima et al., 2004; Chow et al., 2007).

By prospective analysis of some Chinese subjects for 5 years, Chow et al., (2007) demonstrated an inverse relationship between plasma adiponectin concentration and the future development of hypertension. In that study, 70 normotensive, non-diabetic subjects, who developed hypertension by the end point, were compared to 140 age and sex-matched subjects who were normotensive during the observed periods. They concluded that hypoadiponectinaemia at baseline is a strong predictor of future hypertension even after adjusting the confounding factors such as mean blood pressure, C-reactive protein, body mass index, and waist circumference; and that subjects with hypoadiponectinaemia show three times higher morbidity of future hypertension than those with normal range of adiponectin levels.

A***diponectin and the renin-angiotensin system***

The Renin Angiotensin System (RAS) is an important regulatory system for blood pressure and extracellular volume control, through angiotensin II signaling (Stroth et al., 1999). However, during pathophysiological conditions, such as obesity-related metabolic disorders, the excessive production of angiotensin II plays an important role in the development and progression of comorbidities like hypertension and insulin resistance. Evidence from clinical investigations explains that inhibition of RAS with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers can decrease blood pressure in hypertensive people. This is consistent with the important role of RAS on blood pressure control (Kintscher et al., 2005).

Growing evidence from other clinical studies shows that exposure to angiotensin II receptor blockers increases circulating levels of adiponectin. The angiotensin II receptor blocker–mediated increase of adiponectin may contribute to the additional beneficial effects that these drugs exhibit in hypertensive people (Furuhashi et al., 2003). In a study to compare the impact of five antihypertensive drugs on adiponectin regulation, angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker administration showed increased adiponectin levels, compared with other common antihypertensive drugs (Yilmaz et al., 2007). Angiotensin II receptor blockers may, collectively, perform multiple functions, including antihypertensive effects by inhibiting the angiotensin II receptor subtype 1 and angiotensin II signaling and antidiabetic effects by activating PPAR nuclear receptor and concomitant adiponectin production. Also, adiponectin induction may facilitate the antihypertensive effects of these agents by ameliorating endothelial dysfunction. Thus, hyperadiponectinaemia may be protective against hypertension (Scherer & Zhao, 2008).

2.2.1.6 Adiponectin and Inflammation

Adiponectin exerts anti-inflammatory effects through activation of all of its three receptors. Several mechanisms have been suggested; including direct actions on inflammatory cells, actions on NF-κB, and interaction with TNF-α (Robinson et al., 2011).

2.2.1.6.1 Direct actions on inflammatory cells

Peripheral blood mononuclear cells (monocytes, natural killer cells and T and B lymphocytes) express adiponectin receptors 1 and 2 (Pang et al., 2008). Adiponectin inhibits the growth of myelomonocytic progenitors and the functioning of mature macrophages (Yokota et al., 2000), suppresses macrophage-to-foam cell transformation (Ouchi et al., 2001), stimulates macrophage production of the anti-inflammatory cytokine, IL-10 (Kumada et al., 2004), inhibits both TNF-α-induced monocyte adhesion and adhesion molecule {vascular cell adhesion molecule (VCAM)-1, E-selectin and intercellular adhesion molecule (ICAM)-1} surface expression on endothelial cells (Ouchi et al., 1999) and inhibits Toll-like receptor mediated NF-κB activation in macrophages (Yamaguchi et al., 2005). Adiponectin also inhibits the production of reactive oxygen species in human neutrophils (Magalang et al., 2006).

2.2.1.6.2 Actions on NF-κB

NF-κB, a key player in the inflammatory response, is involved in the regulation of cellular functions, including the stress response. The action of adiponectin on NF-κB pathways is complex, being both stimulatory and inhibitory. Initially, higher order multimers of adiponectin were reported to activate NF-κB pathways in myocytes (Tsao et al., 2002). Activation of NF-κB pathways by adiponectin has also been observed in

vascular endothelial cells (Tomizawa et al., 2009) and fibroblasts (Tang et al., 2007), as well as hepatocytes where NF-κB activation induces secretion of a protective chemokine (Wanninger et al., 2009). Counteracting the above evidence is the demonstration of an inhibitory effect of adiponectin on the NF-κB pathways. Adiponectin suppresses lipopolysaccharidase (LPS)-induced NF-κB activation in adipocytes (Ajuwon et al., 2005), suppresses TNF-α-induced NF-κB pathways in endothelial cells (Ouchi et al., 2000) and inhibits NF-κB pathways in macrophages (Wulster-Radcliffe et al., 2004). Adiponectin is also able to regulate the cytotoxicity of NK cells. It activates AMP-activated kinase pathways in NK cells, which, in turn, inhibit IL-2-induced NF-κB activation. This results in the suppression of the IL-2-enhanced cytotoxic activity of NK cells without affecting basal NK cell cytotoxicity (Kim et al., 2006).

2.2.1.6.3 Interaction with TNF-α

Adiponectin may play a role in the down-regulation of inflammatory responses involving TNF-α. An inverse relationship has been shown to exist between TNF-α and adiponectin (Bruun et al., 2003; Kern et al., 2003). This effect is bi-directional; that is, primary changes in TNF-α can influence adiponectin concentrations and vice versa. TNF-α suppresses the expression and secretion of adiponectin from murine and human adipocytes in cell cultures (Maeda et al., 2001). Conversely, primary alterations in adiponectin result in inverse correlation with TNF-α. Adiponectin KO mice show high levels of TNF-α mRNA in adipose tissue and high plasma TNF-α concentration, with reversal of these changes seen, following viral-mediated adiponectin expression in these mice. Additionally, adiponectin strongly inhibits LPS-induced TNF-α gene expression in macrophages (Yokota et al., 2000).

The inverse correlation demonstrated between adiponectin and TNF-α in cell cultures has not been consistently reproduced in human subjects. Keller and his colleagues (Keller et al., 2003) induced endotoxaemia in 23 normal humans. Following endotoxin injection, TNF-α and IL-6 levels increased; however, there was no significant change in the level of adiponectin. Anderson and his research team explored the relationship between acute inflammation and adipokines within a cohort of 20 normal humans. Endotoxaemia was associated with increases in whole blood leukocyte, monocyte and adipose tissue TNF-α and IL-6 but with no significant changes in total plasma adiponectin or complex distribution. AdipoR1 and AdiporR2 receptor down-regulation, however, was evident. Although adiponectin levels did not change, the down-regulation of adiponectin receptors following endotoxaemia suggested attenuation of adiponectin's insulin sensitizing and inflammatory signaling functions (Anderson et al., 2007). In animal models of sepsis, adiponectin deficiency is associated with a marked increase in inflammatory mediator status and exacerbation of hepatic injury (Uji et al., 2010). This consistent finding raises the possibility that modulation of adiponectin in sepsis may be a potential therapeutic target to attenuate the inflammatory response (Robinson et al., 2011).

2.2.1.7 Adiponectin and Endothelial Dysfunction

The endothelium is not only the inert interface between circulating blood and the vessel wall, but is also a major paracrine organ that plays critical roles in controlling vascular tone, inflammation, and smooth muscle cell proliferation. Under physiological conditions, nitric oxide (NO) functions as a mediator for vasoconstriction and vasodilation, adhesion molecule expression and leukocyte transmigration, and smooth muscle cell growth control. The endothelial NO synthase (eNOS) activity and NO production can be controlled by the

availability of substrates and cofactors, transcription of eNOS, mRNA stability of eNOS, subcellular localization of eNOS protein, enzymatic uncoupling, and posttranslational modifications (Dudzinski et al., 2006; Searles, 2006). Recently, a growing body of evidence shows that hypoadiponectinaemia is associated with endothelial dysfunction. By measuring forearm blood flow in response to reactive hyperemia, Ouchi et al., (2003) found that plasma adiponectin levels are correlated with an endothelial vasodilation response. These results were confirmed in animal studies by showing that adiponectin-deficient mice display impaired endothelium-dependent vasodilation and NO production (Ouedraogo et al., 2007). Similarly, in 2004, Tan and his colleagues showed that hypoadiponectinaemia is associated with a lower vasodilation response in diabetic patients. The same group found that adiponectin administration increases NO production in human aortic endothelial cells (Tan et al., 2004). By analyzing nitrate/nitrite as NO metabolites, Ohashi and his research team showed that adiponectin-knockout mice had decreased nitrite and nitrate levels compared to wild-type littermates after high-salt feeding (Ohashi et al., 2006). The obesity-associated metabolic syndrome causes a decrease in the expression and secretion of adiponectin. The dysregulated production of adiponectin may be one of the critical factors mediating obesity-associated NO decrease, endothelial dysfunction, and cardiovascular disease. Adiponectin regulates eNOS enzymatic activity and NO production by several mechanisms. Globular adiponectin has been found to stimulate NO production in bovine and human aortic endothelial cells. It has also been shown that adiponectin stimulates eNOS phosphorylation at Ser1179, which is a modification linked to the increase of eNOS enzymatic activity (Chen et al., 2003; Dudzinski et al., 2006).

Interestingly, the phosphorylation stimulated by adiponectin is also phosphatidylinositol 3-kinase dependent, further underlining the parallel mechanism to insulin signal transduction.

However, in contrast to insulin signaling, Akt is not involved in this process. Instead, AMPK may mediate the phosphorylation downstream of adiponectin signaling. Both adiponectin receptors are expressed in human endothelial cells (Tan et al., 2004). Knockdown of either receptor decreases the production of NO and phosphorylation of eNOS after adiponectin treatment (full-length and globular) in human umbilical vein endothelial cells (Cheng et al., 2007). The stimulatory effect of adiponectin on eNOS is also dependent on the adaptor molecule, APPL1. Knockdown of APPL1 significantly decreases the production of NO. In addition, globular adiponectin treatment of human umbilical vein endothelial cells also results in a doubling of eNOS mRNA primarily because of increased mRNA stability, since actinomycin D

coincubation did not affect the half-life (Hattori et al., 2003). Thus, as to whether or not full-length adiponectin stimulates the transcription of eNOS mRNA remains to be determined. Moreover, adiponectin treatment can regulate the subcellular localization and enzymatic activity of eNOS. In the resting state, eNOS is associated with caveolin-1 in membrane rafts structures. Agonist stimulation increases calcium release and the dissociation of eNOS. Subsequently, eNOS is phosphorylated by kinases in a heat-shock protein (Hsp) 90–mediated process. In this manner, Hsp90 plays a scaffolding role in the continuous activation of eNOS after calcium release. Either globular or full-length adiponectin induces the association between Hsp90 and eNOS, which may facilitate the recruitment of kinases to phosphorylate eNOS (Xi et al., 2005). Knockdown of APPL1, the adaptor molecule downstream of AdipoR1, results in a decreased association of Hsp90 with eNOS and reduced NO production. All these results suggest that adiponectin regulates eNOS enzymatic activity by various mechanisms, including an increase in the mRNA stability, Ser1179 phosphorylation, and an association with the scaffolding

molecule Hsp90. As a consequence, elevated plasma adiponectin levels increase NO production and vasodilation to prevent the occurrence of endothelial dysfunction and subsequent cardiovascular disease (Cheng et al., 2007).

2.2.1.8 Adiponectin and Proteinuria

According to Sharma et al., (2008), plasma adiponectin is inversely related to urinary albumin secretion. To establish a role of adiponectin in the pathogenesis of albuminuria, Sharma et al., (2008) compared wild-type mice with adiponectin-deficient mice. They found that blood pressure, glucose levels, and lipid levels were not affected in Ad $^{-/-}$ mice fed with a regular rodent diet; however, albuminuria was significantly higher and was exacerbated by diabetes. Hydrogen peroxide levels increased in the urine of Ad $^{-/-}$ mice, consistent with oxidative stress. Electron microscopic examination revealed segmental fusion of the feet processes of podocytes (interdigitated cells that closely invest the glomerular capillary network in the kidney, and act in part as a filter for large macromolecules) in Ad$^{-/-}$ mice, although the thickness of the glomerular basement membrane and the structures of endothelial and mesangial cells were not altered by adiponectin deficiency. Albumin permeability was increased in a monolayer culture of podocytes from Ad$^{-/-}$ mice, consistent with albuminuria in this model. They showed that AdipoR1 was highly expressed in podocytes. AMPK phosphorylation was increased in podocytes by adiponectin or the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-d-ribonucleoside. The distribution of the tight junction protein zonula occludens–1 (ZO-1) was disrupted in podocytes from Ad$^{-/-}$ mice and restored by adiponectin or AICAR treatment. These results demonstrate major effects of adiponectin on the kidney. To determine whether adiponectin is causally related to kidney disease, the authors assessed

the effects of adiponectin treatment on urine albumin and hydrogen peroxide excretion. These parameters were related to podocyte structure, activity of AMPK, and expression of oxidative enzymes. Ad $^{-/-}$ mice were treated with the full-length or globular forms of adiponectin. Restoration of plasma adiponectin to levels measured in wild-type mice blunted albuminuria in nondiabetic as well as diabetic Ad$^{-/-}$ mice. Remarkably, adiponectin reversed the fusion of podocyte foot processes in Ad $^{-/-}$ mice. AMPK activity was attenuated in podocytes and glomeruli, and the expression of the NADPH oxidase Nox4, but not Nox1 or Nox2, was enhanced in the absence of adiponectin in Ad$^{-/-}$ mice. As predicted, adiponectin treatment restored AMPK activity and decreased Nox4 expression in parallel with improvements in urinary albuminuria and hydrogen peroxide levels. This, therefore, indicates that a fall in adiponectin levels may predispose to proteinuria and a rise in adiponectin levels may be protective against proteinuria (Sharma et al., 2008).

2.2.1.9 Maternal serum levels of Adiponectin in Preeclampsia

Experimental and clinical studies have indicated that plasma levels of adiponectin are associated with obesity-related metabolic and vascular diseases (Ouchi et al., 2003), both of which are risk factors for preeclampsia. These evidences suggest that adiponectin may play a role in the syndrome. However, reports about the levels of adiponectin in preeclampsia are conflicting.

Ramsay et al., (2003) were the first to study the levels of adiponectin in preeclampsia. They reported that adiponectin levels are significantly elevated in preeclamptic patients compared to normal pregnant women. These investigators proposed that exaggerated release of adiponectin by adipocytes is one possible mechanism for the elevation. In 2005, Naruse et al., also reported that adiponectin levels are significantly increased in women

with preeclampsia than normal pregnant women, even after correcting for haematocrit. These investigators then suggested that adiponectin may act as an inhibitor of inflammatory cytokine production in preeclamptic patients (Naruse et al., 2005). However, in 2005, investigators headed by Hendler found that there is no difference in the concentrations of adiponectin between the women with preeclampsia and the normal pregnant women (Hendler et al., 2005). But after this, the findings of the studies conducted by Lu et al., (2006), Takemura et al., (2007) and Nien et al., (2007) consolidated the earlier observation that adiponectin levels are significantly higher in preeclamptic women than in normal pregnant women. Later, Ouyang et al., (2007), Ouyang et al., (2009), Mazaki-Tovi et al.,(2009), and Herse et al., (2009) reported a contradictory finding when they observed that preeclamptic women rather have significantly lower circulating concentrations of adiponectin than normal pregnant women. These obvious contradictory reports about the comparative levels of adiponectin in normal pregnant women and preeclamptic women make it difficult to determine whether adiponectin acts to correct or exacerbate the pathophysiology of preeclampsia.

2.2.2 LEPTIN

2.2.2.1 Discovery

It was in 1950 that a study conducted in the Jackson laboratory on mutant obese mice that the effects of leptin were observed (Ingalls et al., 1950). A subsequent study by Douglas Coleman in the same Laboratory indicated that the ob gene encoded a hormone regulating body weight and that a second mutation, also causing obesity, the db/db gene, encoded the receptor for this hormone. Leptin was finally co-discovered in 1994 by Jeffrey M. Friedman, Rudolph Leibel, Douglas Coleman and their research teams at the

Rockefeller University, through the study of mice (Zhang et al., 1994; Zhang et al., 1997; Shell, 2003).

2.2.2.2 Structure and Functions

Leptin, a 16-kDa adipocytokine, is a 167-amino-acid-long polypeptide. Analysis of this ob gene product reveals a high degree of homology among species, showing that human leptin is 84% identical to mouse leptin and 83% identical to rat leptin (Williams et al., 2009). However, there are clear differences in the relative levels of placental leptin expression between species (Hoggard et al., 1997; Masuzaki et al., 1997).

Human Leptin has structural homology to tumour necrosis factor alpha (TNFα), interleukin 6 (IL-6), leukaemia inhibiting factor, granulocyte-colony stimulating factor, glycoprotein 130 (gp130) and other cytokine family proteins, and is therefore considered a cytokine-like substance. Nuclear magnetic resonance and crystal structure analysis revealed a four-helix bundled structure for leptin with four antiparallel helices linked with two long crossover arms and one short loop (Ahima et al., 2000).

Although leptin is manufactured primarily in the adipocytes of white adipose tissue, it is also produced by the brown adipose tissue, the placenta (syncytiotrophoblasts), the chorionic villi, the chorion and amnion, the ovaries, the skeletal muscle, the stomach (the lower part of the fundic glands), the mammary epithelial cells, the bone marrow, the pituitary gland, and the liver (Margetic et al., 2002). Human leptin mRNA and protein are also localized to the villous vascular endothelial cells, which are in direct contact with the foetal blood. In normal individuals, baseline leptin levels are between 1 and 5 ng/dl in men and 7 and 13 ng/dl in women. The human leptin gene has a placental-specific

upstream enhancer, suggesting that placental leptin is differentially regulated from leptin of adipose origin (Lea et al., 2000).

After production, leptin is secreted into both the maternal and foetal circulation (Masuzaki et al., 1997). It circulates in plasma in both free and bound forms. Binding of leptin to its membrane receptor results in homodimerization. So far, six isoforms of leptin receptors have been identified, and they are, Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re and Ob-Rf. The Ob-Rb is a long isoform (125 kDa), the Ob-Ra, Ob-Rc, Ob-Rd and Ob-Rf are short isoforms (100 kDa), and the Ob-Re is a soluble isoform. All the receptor isoforms share the same extracellular hormone-binding domain, but differ in the structure and signaling potential of their intracellular domains. The leptin receptor is a trans-membrane protein with structural homology to that of the gp130 receptor family. The extracellular domain of the leptin receptor contains two cytokine binding regions with one region being the specific leptin-binding site. The trans-membrane domain consists of 23 amino acids and the intracellular domain is variable in length. The intracellular domain is usually associated with one or two box motifs, whereas most gp130 receptors have three box motifs. The cytoplasmic motifs allow leptin receptors to interact with intracellular messengers such as mitogen-activated protein kinases (MAPK), adenosine monophosphate (AMP)-activated protein kinase (AMPK), insulin receptor substrate (IRS-1 and IRS-2), nitric oxide (NO), Janus kinase (JAK) and signal transducer and activator transcription factor (STAT). While the short isoforms trigger the above signaling mechanisms, the soluble isoforms seem to play important roles in transporting leptin across the blood–brain barrier (BBB) (Ahima et al., 2000; Hileman et al., 2000).

Also, while the box 1 motif is necessary for the short form of leptin receptor (Ob-Rs) activation of JAK2, the box 2 motif is required for full transduction of the JAK-STAT signal. Both the long and the short leptin receptor (Ob-R) isoforms are present in the placenta, and are co-localized with leptin to the syncytiotrophoblast at the maternal interface (Bodner et al., 1999; Lea et al., 2000; Bjorbaek et al., 2004), implicating a potential autocrine or paracrine effect of leptin on placental function. In the brain, leptin receptors are expressed not only in the hypothalamus but also in other brain regions, particularly in the hippocampus. Because the blood–brain barrier is attenuated in the area of the median eminence, close to where the neuropeptide Y (NPY) neurons of the arcuate nucleus are located, it is not known whether leptin can cross the blood–brain barrier to access receptor neurons. Leptin is generally thought to enter the brain at the choroid plexus, where the intense expression of a form of leptin receptor molecule could act as a transport mechanism. The hypothalamic receptors for leptin are expected to assist the transfer of the hormone across the blood–brain barrier. The transfer is believed to occur at a slow rate. Therefore, the levels of leptin in the cerebrospinal fluid are lower than those in the plasma. The central leptin action is usually associated with elevated cerebrospinal leptin and an increased JAK/STAT3 signaling, leading to increased sympathetic tone (Wang et al., 1996; Lieb et al., 2009). Ob-Rb is the only receptor isoform that can signal, intracellularly, via the Jak-Stat and MAPK signal transduction pathways and is present in hypothalamic nuclei (Bjorbaek et al., 2004; Malendowicz et al., 2006).

Upon binding of leptin, the Ob-Rb receptor has been demonstrated to activate STAT1, STAT3, STAT5, and STAT6 in vitro but only STAT3 in vivo. The downstream signaling of STAT is not completely understood, but has been somewhat revealed in the heart, brain and other cell lines. Earlier studies identified the MAP kinase pathway as a downstream

signaling mechanism of Ob-Rs. In addition, the extracellular signal regulated kinase (ERK) was activated with leptin stimulation. While this is enlightening for Ob-Rs, the long form Ob-Rb receptor has also been demonstrated to stimulate ERK activity, although at a higher potency. Binding of leptin to its receptors directly enhances the activity of IRS-1 and IRS-2 (Bjorbaek et al., 2004). Phosphorylation of IRS on the serine/threonine residue may lead to decreased phosphatidylinositol-3 (PI-3) kinase and subsequently deficient PI-3 kinase activation. Therefore, hyperleptinaemia may be associated with suppressed PI-3 kinase activity and reduced cardiovascular inotropicity since PI-3 kinase is a known positive inotropic mediator for the heart and vasculature (Zhao et al., 2004).

After in vivo activation of STAT3 by leptin, STAT3 is phosphorylated and travels to the nucleus to presumably effect changes in gene expression. One of the main effects on gene expression is the down-regulation of the expression of endocannabinoids, responsible for increasing appetite. Circulating leptin levels give the brain input regarding energy storage, so that it can regulate appetite and metabolism. Leptin produces a feeling of satiety by signaling to the brain that the body has had enough food. This fullness hormone may make it easier for people to resist the temptation of foods high in calories (Baicy et al., 2007). The absence of leptin (or its receptor) results in unchecked food intake. This can then lead to obesity. Several studies have shown that fasting or following a very-low-calorie diet (VLCD) lowers leptin levels (Dubuc et al., 1998). Leptin is, therefore, considered as an indicator of energy balance. Interestingly, it is more sensitive to starvation than to overfeeding. Its levels change more when food intake decreases than when it increases (Chin-Chance et al., 2000). It has been hypothesized that the dynamics of leptin due to an acute change in energy balance may be related to appetite and eventually to food intake (Mars et al., 2005). Leptin and insulin are the only hormones known to act as adiposity

signals. In animal models, leptin deficiency (ob/obmice) and leptin resistance (db/dbmice having a defective leptin receptor) lead to hyperphagia and decreased energy expenditure. Consequently, affected animals become obese and develop insulin resistance (Williams et al., 2009).

Other intracellular pathways are activated by leptin, but less is known about how they function in this system. In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them. The action of leptin is recognized to be more decentralized than previously assumed. In addition to its endocrine action at a distance (from adipose tissue to brain), leptin also acts as a paracrine mediator (Margetic et al., 2002).

Leptin, once released from fat tissue, acts through the sympathetic nervous system to regulate bone metabolism (Takeda et al., 2002). Leptin can affect bone metabolism via direct signalling from the brain; and although leptin acts to reduce cancellous bone, it conversely increases cortical bone. A number of theories have been put forward concerning the cortical-cancellous dichotomy, including a theory suggesting that increased leptin during obesity may represent a mechanism for enlarging bone size and, thus, bone resistance to cope with increased body weight (Hamrick et al., 2008).

Factors that acutely affect leptin levels are also factors that influence other markers of inflammation, e.g., testosterone, sleep, emotional stress, caloric restriction, and body fat levels. While it is well established that leptin is involved in the regulation of the inflammatory response, it has been further theorized that leptin's role as an inflammatory marker is to respond specifically to adipose-derived inflammatory cytokines (Fantuzzi et al., 2000).

Circulating leptin seems to affect the HPA axis, suggesting a role for leptin in stress response (Heiman et al., 1997). In vascular endothelial cells, leptin can activate the stress activated protein kinase (SAPK) and Jun NH_2 - terminal kinase (JNK) transduction pathways of the MAP kinase family. Leptin may also up-regulate the transcription factor activation protein 1 (AP-1). While the SAPK/JNK pathway appears to be associated with leptin signaling, it may be directly activated by reactive oxygen species (ROS), thus making sustained hyperleptinaemia potentially hazardous. Activation of SAPK/JNK may contribute to the pro-oxidant effects of leptin, especially at chronic high circulating levels (Bouloumie et al., 1999).

Accumulating evidence have suggested a close interaction between leptin signaling and insulin signaling, or between hyperleptinaemia and hyperinsulinaemia (Bjorbaek et al., 2004). However, investigations of whether leptin is a diabetogenic or an anti- diabetogenic hormone have produced conflicting results (Ceddia et al., 2002). Obese individuals may often display both hyperleptinaemia and hyperinsulinaemia concurrently, although neither is likely to be permissive to the onset of the other comorbidity (Hintz et al., 2005)

Elevated leptin concentrations are associated with elevated white blood cell counts in both men and women (Mabuchi et al., 2005). Leptin modulates the immune response to atherosclerosis, of which obesity is a predisposing factor. Exogenous leptin can promote angiogenesis by increasing vascular endothelial growth factor levels (Taleb et al., 2007).

In some epidemiological studies, hyperleptinaemia is considered as a risk factor.

However, a few animal experiments demonstrated that systemic hyperleptinaemia produced by infusion or adenoviral gene transfer decreases blood pressure in rats (Zhang et al., 2010).

Leptin plays a pivotal role in a wide variety of organ systems, including the reproductive, renal and cardiovascular systems (Ahima et al., 2000). In mice, leptin is required for male and female fertility, but in humans, it has a lesser effect. In humans, ovulatory cycles in females are linked to energy balance (positive or negative, depending on whether a female is losing or gaining weight) and energy flux (how much energy is consumed and expended) much more than energy status (fat levels). When energy balance is highly negative (meaning the woman is starving) or energy flux is very high (meaning the woman is exercising at extreme levels, but still consuming enough calories), the ovarian cycle stops and females stop menstruating. Only if a female has an extremely low body fat percentage does energy status affect menstruation. Some studies have indicated that leptin levels outside an ideal range can have a negative effect on egg quality and outcome during in vitro fertilization (Anifandis et al., 2005).

2.2.2.3 Maternal serum levels of Leptin during Normal Pregnancy

Serum leptin concentration rise slightly during pregnancy. This rise in serum leptin concentration is about 2 to 3 fold above non-pregnancy concentrations, with the peak occurring around 28 weeks of gestation (Schubring et al., 1999). The level then falls to below pre-pregnancy levels at around birth and after parturition (Lage et al., 1999) .These variations are unrelated to changes in body composition, and may be responsible for the postpartum weight gain observed in some women (Moynihan et al., 2006). In mice, serum leptin has been reported to increase 25-fold in the maternal circulation and this compares with a threefold reported rise in humans (Hardie et al., 1997; Tomimatsu et al., 1997). However, the large increase in bound circulating leptin observed during late pregnancy in mice does not occur in humans or rats (Gavrilova et al., 1997). This may be due to the

fact that, in humans, the levels of soluble leptin receptor fall between weeks 20 and 30 of gestation (Lewandowski et al., 1999).

There are a number of explanations for the increase in leptin towards the end of pregnancy. One of them is that during pregnancy, there is increased secretion of leptin by specific adipose tissue depots (Tomimatsu et al., 1997) Also, there is increased synthesis of leptin by the placenta or the release of the soluble leptin receptor (Ob-Re) by the placenta (Gavrilova et al., 1997). The placenta has been shown to express both the leptin and leptin receptor genes, though the relative contribution of adipose and placental- derived leptin to circulating levels is uncertain. Also, it is not only the adipose tissue and placenta that produces leptin, but also the foetus and the amniotic fluid (Hoggard et al., 1997; Masuzaki et al., 1997; Hoggard et al., 1998).

Changes in circulating leptin concentrations in pregnant women are generally consistent with changes in maternal fat stores and glucose metabolism. And in spite of the fact that maternal fat deposition increases during pregnancy, the gestational increase in circulating leptin does not appear to be correlated with changes in maternal Body mass index (Lage et al., 1999; Ahima et al., 2000).

2.2.2.4 Leptin and Body Weight

A very small group of humans possesses homozygous mutations of the leptin gene, leading to a constant desire for food and resulting in severe obesity (Farooqi et al., 2007).The level of circulating leptin is proportional to the total amount of fat in the body, since leptin levels

increase exponentially with increased fat mass (Margetic et al., 2002). The body's fat cells, under normal conditions, are responsible for the constant production and release of leptin (Zhao et al., 2004). Leptin mRNA is expressed predominantly by subcutaneous rather than visceral fat cells. This suggests a role for leptin in modulating adipose tissue mass and distribution (Prins et al., 1997).

Blache et al., (2000) found a strong correlation between plasma leptin and back fat thickness, explaining 30% of the variation in leptin concentrations. Similarly, in non-pregnant women, Delavaud et al., (2000) observed a strong positive relationship between plasma leptin and body weight, body condition score and body fatness. Results from numerous metabolic studies document a positive association between direct and indirect measures of adiposity with plasma leptin concentrations. Considine et al., (1996) found that the mean serum leptin concentrations were 7.5 ng per millilitre in normal-weight subjects and 31.3 ng per millilitre in obese subjects. The obmRNA content of adipocytes was twice as high in the obese subjects as in the normal-weight subjects. This observation suggests that obese persons are insensitive to endogenous leptin production, indicating the possible existence of a leptin resistant state. After making adjustment for BMI, women seem to have higher leptin levels than men (Saad et al., 1997). This could be either related to the increased percentage of peripheral body fat in women, or a result of stimulation of leptin production by oestrogen/progesterone and/or by androgens (Shimizu et al., 1997).

Chronically elevated leptin levels are associated with over-eating and obesity (Hamilton et al., 1995). Although leptin reduces appetite, obese individuals, generally, exhibit an, unusually, high circulating concentration of leptin (Considine et al., 1996). These people are said to be resistant to the effects of leptin, in much the same way that people with type

2 diabetes are resistant to the effects of insulin. The persistent high levels of leptin from the enlarged adipose stores imply leptin desensitization. The pathway of leptin control in obese people might be flawed at some point, so the body does not adequately receive the satiety feeling subsequent to eating.

"A signal-to-noise ratio" theory has been proposed to explain the phenomenon of leptin resistance. Although leptin resistance is sometimes described as a metabolic disorder that contributes to obesity, similar to the way insulin resistance is sometimes described as a metabolic disorder that has the potential to progress into type 2 diabetes, it is not certain in most cases. The mere fact that leptin resistance is extremely common in obese individuals suggests that it may simply be an adaptation to excess body weight. Leptin does not function as a "satiety signal" to prevent obesity in times of energy excess, but as a "starvation signal" to maintain adequate fat stores for survival during times of energy deficit. Due to this, leptin resistance in overweight individuals is considered as a standard feature of mammalian physiology, which possibly confers a survival advantage (Banks et al., 2006; Myers et al., 2008).

Weight loss, physical exercise, fasting, and starvation are known to induce reductions in leptin concentrations, whereas leptin concentrations are increased with weight gain and hyperinsulinaemia. Weight loss is directly proportional to a drop in levels of circulating leptin. This drop causes reversible decreases in thyroid activity, sympathetic tone, and energy expenditure in skeletal muscle, and increases in muscle efficiency and parasympathetic tone. The result is that a person who has lost weight attains a lower basal metabolic rate than an individual with the same weight that has never lost weight (Havel et al., 1996). These changes are leptin-mediated homeostatic responses meant to reduce

energy expenditure and promote weight regain. Many of these changes are reversed by peripheral administration of recombinant human leptin to restore pre-diet levels (Ahima, 2008).

Since leptin can affect bone metabolism via direct signalling from the brain to increase cortical bone, it has been suggested that a rise in leptin levels during weight gain may represent a mechanism for enlarging bone size and, thus, bone resistance to cope with increased body weight (Hamrick et al., 2008)

2.2.2.5 Leptin and Blood Pressure

Leptin has been shown to associate positively with blood pressure in adult females. This association between leptin and blood pressure (BP) seems to be independent on some potentially important variables such as BMI (Patel et al., 2008). In 1997, Kennedy et al., demonstrated the relationship between elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP) and plasma leptin levels in hypertensive men, whereas in the subsequent year, Suter et al., (1998) reported a significant relationship between SBP and plasma leptin levels in hypertensive women. And since leptin has been found to stimulate the renin–angiotensin system, the sympathetic system, and natriuresis, it is possible that a blunted effect of leptin may predispose to hypertension in humans (Stenvinkel, 2000). Based on the significant correlation observed between blood pressure and leptin levels in hypertensive subjects, but not in normotensive subjects, it is suggested that a rise in leptin levels may be a regulator of blood pressure in hypertensive subjects only (Patel et al., 2008).

2.2.2.6 Leptin and Inflammation

2.2.2.6.1 Leptin and acute inflammation

Leptin regulates the production of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin 1 (IL-1), and interleukin (IL-6). These cytokines also regulate the expression of leptin, which sustains a chronic pro-inflammatory state. Many genes related to inflammation, including genes encoding acute phase response proteins such as tPA (tissue plasminogen activator), fibrinogen-β, lipocalin-2, PAP1, preprotachykinin, and MnSOD (manganese superoxide dismutase) are induced by leptin (Hekerman et al., 2007). Leptin levels rapidly increase in acute inflammatory conditions, such as cholecystectomy, acute infection, and sepsis, particularly favoured by cytokines, such as TNF-α, IL-6, and IL-1 β (Fernández-Riejos et al., 2010). Parenteral administration of LPS, commonly used to experimentally induce systemic inflammation, leads to a rise in plasma leptin. A protective role of leptin in the clearance of pathogens is observed in leptin-deficient ob/ob mice, which develop severe disease and die of infection with Klebsiella more rapidly than WT mice. The ob/ob mice are also highly susceptible to LPS-induced lethality, which can be reversed by the administration of leptin. The protective effects of leptin in these cases seem to occur by means of a modulation of TNF-α and IL-6 responses after endotoxin priming (Bernotiene et al., 2006). Elevated levels of leptin are found in sepsis and may be predictive of the severity of sepsis and increased survival (Bornstein et al., 1998). Leptin is a critical factor in host resistance and in its absence, sepsis-induced organ damage is increased, whereas neutrophil function is diminished. Furthermore, there is an important role of leptin in the central nervous system (CNS) in regulating survival and systemic immune response in sepsis. Selective leptin administration into the CNS controls systemic immune response in a functionally relevant manner with

significant protection from sepsis. A leptin- dependent neurocircuit in the CNS is required for efficient coordination of the immune response in sepsis to limit organ damage and prevent mortality (Tschöp et al., 2010). On the other hand, some studies have not found increased leptin levels in specific inflammatory conditions, including acute experimental endotoxemia in humans, HIV infection, and newborn sepsis (Iikuni et al., 2008).

2.2.2.6.2 Leptin and chronic inflammation

Together with the established function of leptin as a pro-inflammatory cytokine that helps the host fight infection, there is increasing evidence of an association between leptin and increased risk of chronic inflammatory disease. Serum leptin levels are elevated in many chronic inflammatory conditions (Gilbert et al., 2013).

2.2.2.6.3 Leptin as an anti-inflammatory cytokine

According to Konturek et al., (2002), exogenous leptin may exert an anti-inflammatory role in the exocrine pancreas, protecting rats against acute pancreatitis mediated by the nitric oxide pathway. Leptin also played an anti-inflammatory effect in an experimental model of acute colitis via a neutrophil-dependent mechanism that depends on the HPA (hypothalamic-pituitary-adrenal) axis (Çakır et al., 2004). Another study showed that endogenous leptin may locally protect the joints from the most severe form of erosive rheumatoid arthritis in humans (Bokarewa et al., 2003).

2.2.2.7 Leptin and Endothelial Dysfunction

Human leptin mRNA and protein are also localized in the villous vascular endothelial cells, which are in direct contact with the foetal blood (Lea et al., 2000). Leptin may alter vascular tone and vasoreactivity. These changes together with altered fibrinolysis are considered main features of endothelial dysfunction. Various mechanisms have been

proposed to underlie this association, including SNC activation (Hall et al., 2010), altered vascular tone and vasopressor effects (Singhal et al., 2008), enhancement of platelet aggregation and arterial thrombosis (Konstantinides et al., 2001), impairment of fibrinolysis (Eriksson et al., 2008), pro-angiogenic actions and systemic inflammation (Shamsuzzaman et al., 2004), or promoting oxidative stress (Bouloumie et al., 1999).

2.2.2.8 Leptin and Proteinuria

A rise in leptin levels causes renal dysfunction Carlyle et al., (2002). Additionally, leptin has been found to enhance natriuresis, increase sympathetic nervous activity, and stimulate reactive oxygen species. These findings collectively suggest that the kidney is not only a site of leptin metabolism, but also a target organ for leptin action in pathophysiological conditions (Wolf et al., 2002).

2.2.2.9 Maternal serum levels of Leptin in Preeclampsia

In 1998, Sattar et al., reported a significantly higher level of leptin in preeclamptic women than normal pregnant women. And in 2004, a longitudinal study conducted by Kolyingit et al., confirmed this finding. These investigators then concluded that increased plasma leptin level with severity of preeclampsia could be taken as a marker of placental hypoxia in severe preeclampsia. However, the findings of the study conducted by Martinez-Abundis et al., (2000), Lalm et al., (2001) and Masuyama et al., (2010) contradict the findings of Sattar et al., (1998) and Kolyingit et al., (2004). Martinez- Abundis et al., (2000) found that the levels of leptin are not different among the different grades of preeclampsia and normotensive pregnant women; and Lalm et al., (2001) and Masuyama et al., (2010) reported decreased levels of leptin in preeclampsia as compared to normal pregnancy.

Incidentally, the finding that leptin stimulates the rennin-angiotensin system (Kim et al., 2002), and that higher leptin levels can cause hypertension (Stenvinkel et al., 2000), proteinuria (Wolf et al., 2002), and endothelial dysfunction (Hall et al., 2010), together with the finding that leptin plays a vital role during inflammation (Fernández-Riejos et al., 2010), may justify the higher levels of leptin seen in the preeclamptic women.

2.3 GRAVIDITY

This refers to the number of times a woman has been pregnant, regardless of whether the pregnancy was interrupted or resulted in live birth. The following are the terms used to classify women as far as gravidity is concerned;

- Nulligravida refers to a woman who has never conceived.
- Primigravida refers to a woman who has conceived for the first time, or has been pregnant one time.
- Mulitgravida (secundogravida) refers to a woman who has had several conceptions (Borton & Chloe, 2009).

2.4 PARITY

This refers to the number of pregnancies a woman has carried to viable gestational ages. The following are descriptive terms of women in relation to their parity statuses.

- A woman who has never been pregnant beyond 20 weeks is said to be nulliparous, and is referred to as a nullipara or para 0.
- Also, a woman who has given birth once is said to be primiparous, and is referred to as a primipara or primip.
- Moreover, a woman who has given births two or more times is multiparous and is called a multip.
- Finally, grand multipara is the condition of having given birth five or more times (Cox et al., 2005).

CHAPTER THREE

METHODOLOGY

3.1 STUDY SITE

The study was conducted at the antenatal clinic of the Manhyia Government Hospital. This hospital is located in the Manhyia sub Metropolis of the Kumasi Metropolitan Assembly. Majority of the people who seek medical care at this hospital reside in the suburbs of the Manhyia sub metropolis. These suburbs include Asawase, Aboabo, Ashanti New Town, Dichemso and New Tafo. People from various ethnic backgrounds reside in this metropolis, with majority of them being Akans.

3.2 STUDY DESIGN

A non-randomized case-control study design was used. Pregnant women attending the Polyclinic were examined by an Obstetrician/Gynaecologist and Obstetric nurse. The pregnant women were grouped into three, according to their gestational ages (1-14 weeks) – First Trimester, (15-27weeks) – Second Trimester and (28-42weeks) –Third Trimester. The third trimester pregnant women were divided into normal pregnant women and

preeclamptic women. None of the first and second trimester pregnant women was preeclamptic. Another group made up of non-pregnant women was also created.

3.3 RECRUITMENT OF PARTICIPANTS

Pregnant women who received antenatal care at the Manhyia Government Hospital, from January 2014 to March 2014, and normal non-pregnant women were enrolled in the study. The non-pregnant women were 40 in number; and the pregnant women comprised 180 normal pregnant women and 60 preeclamptic women. The normal pregnant women were put into three major groups (first trimester pregnant women, second trimester pregnant women and third trimester pregnant women), with 60 participants in each trimester. Diagnosis of preeclampsia was done by an Obstetrician/Gynaecologist and an obstetric nurse. Consent for the study was obtained from each study participant and all information was treated as confidential and used for the research alone.

3.3.1. ETHICAL ISSUES

The study was approved by the local Committee on Human Research Publications and Ethics (CHRPE/KNUST/KATH) of the Kwame Nkrumah University of Science and Technology. Informed consent was also obtained from all the participants before the study commenced.

3.3.2 INCLUSION CRITERIA

3.3.2.1 Inclusion criteria for all the Participants

All participants who satisfied the following conditions and were willing to participate were enrolled in the study.

1. Sex: Female
2. Ages Eligible for study: 18years to 40years
3. $18.5kg/m^2 \leq BMI < 30.00kg/m^2$
4. Non-smokers and teetotalers

3.3.1.2 Additional inclusion criteria for the Preeclamptic women

1. Blood Pressure equal to or higher than 140/90 mmHg
2. Proteinuria higher than 100 mg/dl by urine analysis (≥2+ positive result on a dipstick).

3.3.3. EXCLUSION CRITERIA

3.3.3.1 Exclusion criteria for all the Participants

All the participants who presented with any of the following conditions were excluded from the study.

1. Hypertension
2. Diabetes mellitus
3. Gestational diabetes mellitus
4. Renal diseases
5. Ischaemic heart disease
6. Endocrinological diseases, e.g. thyroid or adrenal diseases
7. Chronic debilitating diseases, e.g. Cancer
8. Anorexia or bulimia

9. Polycystic ovarian disease
10. Infectious diseases
11. Hyperemesis or dehydration.
12. Any pathological condition at all

3.3.2.2 Additional exclusion criteria for the Preeclamptic women

Preexisting chronic hypertension

3.3.4 CRITERIA FOR CLASSIFICATION OF PARTICIPANTS

1. Women carrying foetuses were classified as pregnant women and women not taking care of any infant or hadn't given birth within 2years after previous parturition were classified as non-pregnant women. Determination of human chorionic gonadotropin in urine and obstetric ultrasonography were used to confirm whether or not a woman was pregnant.
2. Pregnant women with gestational ages of first week of conception to the fourteenth week were classified as first trimester pregnant women; those with gestational ages from fifteenth week of conception to the twenty eighth week were classified as second trimester pregnant women; and those with gestational ages from the twenty ninth week till parturition were classified as third trimester pregnant women (Cunningham et al., 2005).
3. Pregnant women with high blood pressure (blood pressure higher than or equal to 140/90 mmHg) and significant proteinuria (proteinuria higher than 100 mg/dl by urine analysis) were classified as preeclamptic (Eiland et al., 2002).

4. Women with BMI ranging from 18.5kg/m ^{2} to 24.9kg/m^2 were classified as normal-weight and women with BMI ranging from 25.0kg/m ^{2} to 29.9 kg/m ^{2} were classified as overweight. First trimester BMI was used to categorise the pregnant women (Campbell et al., 2001, Weisell, 2002).

3.4 DATA COLLECTION

A questionnaire was privately used to obtain the relevant information about each participant, within a time frame of about thirty minutes; and was confirmed from their medical records. The information was about the age, ethnicity, occupation, educational background, marital status and medical history regarding previous pregnancies. The Body mass index (BMI) and blood pressure (BP) of each participant were measured. Blood samples were taken from each participant and were used for the assaying of adiponectin and leptin; and urine samples were also taken for the quantification of proteins.

3.4.1 Measurement of Body Mass Index

The participants stood on a Camry weight balance (Zhongshan Camry Electronic Co. Ltd, Guangdong, China) after they had removed their shoes and any other heavy clothing. Their weights were recorded to the nearest 0.1kg; and their heights were measured to the nearest whole number with a stadiometer. Each participant stood with heels, buttocks and shoulders resting lightly against the backing board so that the Frankfort plane (a line connecting the superior border of the external auditory meatus with the infraorbital rim) was horizontal. All the measurements were then converted to the SI units. That is; kilogramme (kg) for weight and metres (m) for height. BMI for each participant was calculated as weight (kg)/height (m^2) (Eknoyan, 2008).

3.4.2 Measurement of Blood Pressure

Each participant was asked to sit down comfortably, extend the left arm on a table and then relax for 10 minutes. Mercury sphygmomanometer and a stethoscope were then used by a professional nurse to measure the Systolic blood pressure and Diastolic blood pressure of each participant. Measurements were taken from the left arm (i.e. over the brachial artery) in accordance with the recommendations of the American Heart Association (Kirkendall et al., 1967). Duplicate measurements were then taken after each participant had had a 5 minute rest interval between measurements. The mean values of the duplicate measurements were then computed and recorded to the nearest 2.0mmHg.

3.4.3 Collection, Preservation and Biochemical analysis of the Blood Samples

About 5ml of venous blood samples were collected from each participant and then introduced into serum separator tubes. The blood samples were then centrifuged and aliquots of the serum pipetted into cryopure tubes, and then stored at -80 ^{0}C in a refrigerator at the Department of Serology of the Komfo Anokye Teaching Hospital.

3.4.3.1 Measurement of Serum Adiponectin Levels

Adiponectin was quantified in the serum of each participant, using the Quantikine® Human Adiponectin Immunoassay method (R&D systems, USA).

Principle and methodology

This assay employs an antibody specific for human adiponectin coated on a 96-well plate. Standards and samples were pipetted into the wells and adiponectin present in the sample were bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human adiponectin antibody was added. After washing away unbound

biotinylated antibody, horse raddish peroxidase-conjugated streptavidin was pipetted to the wells. The wells were again washed, and a 3,3,5,5-tetramethybenzidine (TMB) substrate solution, which is a chromogenic agent, was added. This compound underwent a redox reaction with the horse raddish peroxidase, leading to the development of a blue colour which was directly proportional to the quantity of adiponectin bound. A sulphuric acid stop solution was then added to terminate the enzymatic reaction, and the colour changed from blue to yellow. The absorbance was measured on a microtitre plate reader at a wavelength of 450nm. A set of standards was used to plot a standard curve from which the amount of adiponectin in the samples and the controls were directly read.

3.4.3.2 Measurement of Serum Leptin Levels

Leptin was quantified in the serum of each participant, using the Quantikine ® Human Leptin Immunoassay method (R&D systems, USA).

Principle and methodology

The principle of this test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin was immobilized onto the microwell plate and another monoclonal antibody specific for a different epitope of leptin was conjugated to biotin. During the first step, leptin present in the samples and the standards were bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibodies were removed by a washing step. In the second step, streptavidin-horse raddish peroxidase (HRP) was added, which bound specifically to

any bound biotinylated antibody. Again, unbound streptavidin-horseradish peroxidase (HRP) was removed by a washing step.

Next, the enzyme substrate was added, forming a blue coloured product that was directly proportional to the amount of leptin present. The enzymatic reaction was terminated by the addition of a "stop" solution, which converted the blue colour to a yellow colour. The absorbance was measured on a microtitre plate reader at a wavelength of 450 nm. A set of standards was used to plot a standard curve from which the amount of leptin in the samples and the controls were directly read.

3.4.4 Collection of Urine samples and Urinalysis

3.4.4.1 Urine collection

About 5ml of urine samples from each participant were collected into clean and dry plastic containers. The samples that contained visible precipitates were centrifuged to obtain clear specimens.

3.4.4.2 Urinalysis

This was about the determination of hcG and proteins.

3.4.4.2.1 Pregnancy test

Presence of human chorionic gonadotropin (hCG) in the urine was used to confirm pregnancy, especially the first trimester pregnancy; and absence of human chorionic gonadotropin in the urine was used to rule out pregnancy.

Principle and methodology

The linear hCG strip (Linear chemicals, S.L. Joaquim Costa 18 2ª planta, Barcelona, Spain) is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin in urine or serum to aid in the early detection of pregnancy. The test utilizes

a combination of antibodies including a monoclonal hCG antibody to selectively detect elevated levels of hCG.

The assay is conducted by immersing the test strip in a urine specimen and observing the formation of coloured lines. The specimen migrates via capillary action along the membrane to react with the coloured conjugate. Positive specimens react with the specific antibody-hCG-coloured conjugate to form two coloured lines, one at the test region and the other at the control region of the membrane. Absence of a coloured line at the test region suggests a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that proper volume of specimen has been added, and that membrane wicking has occurred.

In conducting the test in this study, the above procedure was used. The test strip was removed from the sealed pouch and used immediately. With the arrows pointing toward the urine specimen, the test strip was immersed vertically in the urine specimen for at least ten (10) seconds. The test strip was placed on a non-absorbent flat surface, and after about three (3) minutes, the results were read. The appearance of two distinct red lines (one at the test region and the other at the control region) indicated a positive result; and the appearance of a single line (at the control region) indicated a negative result.

3.4.4.2.2 Determination of urine protein

Principle and methodology

Urine protein was determined using the dip-stick semi-quantitative method (CYBOW™ DFI Co Ltd, Gimhae-City, Republic of Korea). The determination was carried out according to the manufacturer's description. The assay is based on the protein "error of indicators" (ability of protein to alter the colour of acid-base indicators without altering

the pH). When pH is held constant by a buffer, indicator dyes release H^+, because of the protein present, and change colour from yellow to blue green.

A fresh strip was inserted into each urine sample for about 5seconds.The edge of the strip was drawn along the brim of the vessel to remove excess urine. It was ensured that the test area of the strip did not touch the vessel. The strip was turned on its side and tapped on an absorbent paper to remove any remaining urine, since excessive urine on the strip could make the chemicals between adjacent pads interact and lead to wrong results. The test result on the strip was held horizontally and then compared with the colour chart on the vial label under good light.

3.5 STATISTICAL ANALYSIS

Percentages were used to describe the data concerning the sociodemographic characteristics of the participants. Parametric methods were used to analyse the data after Shapiro-Wilk test and Q-Q plot had indicated that the data was normally distributed.

Data regarding the levels of adiponectin and leptin, the adiponectin-leptin ratio, Body mass index, blood pressure, ages and gestational ages were presented as means ± SD. Differences in measured variables among groups were assessed with one-way analysis of variance, and differences between groups were assessed with unpaired t-test. Associations between continuous variables were described by Pearson correlation coefficients. Significance was accepted at $p<0.05$. Tukey's test was employed for all post-hoc analysis. Risk assessment was done with multivariate logistic regression model. All the tests were two-tailed. Statistical Package for the Social Sciences (SPSS, version 20) was used for the analysis.

CHAPTER 4

RESULTS

Table 4.1 Distribution of socio-demographic and obstetric parameters among the studied participants

Parameter	Non-pregnant women N=40	Normal pregnant women 1st trimester N=60	2nd trimester N=60	3rd trimester N=60	Preeclamptic women N=60
Ages (years) 18 – 23	13 (33%)	30 (50%)	15 (25%)	29(48%)	31 (52%)
24 – 29	17 (43%)	19 (32%)	25 (42%)	15 (25%)	11 (18%)
30 – 36	10 (24%)	11 (18%)	20 (33%)	16 (27%)	18 (30%)
Educational status					
University graduates	10 (25%)	15(25%)	10(17%)	21(35%)	18 (30%)
SHS graduates	16 (40%)	20(33%)	17(28%)	10(17%)	23 (38%)
JHS graduates	14 (35%)	25(42%)	33(55%)	29(48%)	19 (32%)
Occupational status					
Nurses	4 (10%)	4 (6%)	3 (5%)	0 (0%)	2 (3%)
Teachers	10(25%)	6 (10%)	2 (3%)	1 (2%)	1 (2%)
Traders	26 (65%)	50 (84%)	55(92%)	59(98%)	57(95%)
Ethnicity					
Akan	25(63%)	42(70%)	39 (65%)	35(58%)	46(77%)
Non-Akan	15(37%)	18 (30%)	21 (35%)	25(42%)	14 (23%)
Marital status Single	10(25%)	0 (0%)	0 (0%)	0 (0%)	10 (17%)
Married	25(63%)	60(100%)	60 (100%)	60(100%)	50(83%)
Divorcee	5 (12%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gravidity					
Nulligravida	10 (25%)	-	-	-	-
Primigravida	20 (50%)	45 (75%)	40 (67%)	35 (58%)	50(83%)
Multigravida	10 (25%)	15(25%)	20 (33%)	25 (42%)	10 (17%)
Parity					
Nulliparas	10 (25%)	45 (75%)	40 (66%)	35 (58%)	40 (67%)
Primiparas	20 (50%)	10 (17%)	10 (17%)	15 25%)	15(25%)
Multiparas	10 (25%)	5 (8%)	10 (17%)	10 (17%)	5 (8%)
Abortus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Results are expressed as: actual values (percentages); JHS=Junior High School; SHS=Senior High School

Table 4.1 shows the socio-demographic and obstetric characteristics of the studied participants. 58% of the non-pregnant women, 65% of the first trimester pregnant women, 58% of the second trimester pregnant women, 67% of the third trimester pregnant women, and 68% of the preeclamptic women had ages of 18 to 30. 42% of the non-pregnant women, 35% of the first trimester pregnant women, 42% of the second trimester pregnant women, 33% of the third trimester pregnant women, and 32% of the preeclamptic women had ages of 30 to 36.

All the participants had had formal education. Among the non-pregnant women, 25% were university graduates, 40% were SHS graduates and 35% were JHS graduates. Among the first trimester pregnant women, 25% were university graduates, 33% were SHS graduates and 42% were JHS graduates. Among the second trimester pregnant women, 17% were university graduates, 28% were SHS graduates and 55% were JHS graduates. Among the third trimester pregnant women, 35% were university graduates, 17% were SHS graduates and 48% were JHS graduates. And among the preeclamptic women, 30% were university graduates, 38% were SHS graduates and 32% were JHS graduates.

It is evident in the table that all the participants were employed. Among the non-pregnant women, 10% were nurses, 25% were teachers and 65% were traders. Among the first trimester pregnant women, 6% were nurses, 10% were teachers and 84% were traders. Among the second trimester pregnant women, 5% were nurses, 3% were teachers and 92% were traders. Among the third trimester pregnant women, 0% were nurses, 2% were teachers and 98% were traders. And among the preeclamptic women, 3% were nurses, 2% were teachers and 45% were traders.

Regarding the ethnicity of the participants, the table shows that most of the participants were Akans and very few were non-Akans. Among the non-pregnant women, 63% were

Akans and 37% were non-Akans. Among the first trimester pregnant women, 70% were Akans and 30% were non-Akans. Among the second trimester pregnant women, 65% were Akans and 35% were non-Akans. Among the third trimester pregnant women, 58% were Akans and 42% were non-Akans. And among the preeclamptic women, 77% were Akans and 23% were non-Akans.

With respect to the marital statuses of the participants, the table shows that most of the participants were married. Among the non-pregnant women, 25% were singles (never married), 63% were married and 12% were divorcees. And among the preeclamptic women, 17% were singles (never married) and 83% were married. All the normal pregnant women were married.

The gravidty and parity statuses of the participants are also shown in the table. Among the non-pregnant women, 25% were nulligravida, 50% were primigravida and 25% were multigravida. Among the first trimester pregnant women, 75% were primigravida and 25% were multigravida. Among the second trimester pregnant women, 67% were primigravida and 33% were multigravida. Among the third trimester pregnant women, 58% were primigravida and 42% were multigravida. And among the preeclamptic pregnant women, 83% were primigravida and 17% were multigravida.

Among the non-pregnant women, 25% were nulliparas, 50% were primiparas and 25% were multiparas. Among the first trimester pregnant women, 75% were nulliparas, 17% were primiparas and 8% were multiparas. Among the second trimester pregnant women, 66% were nulliparas, 17% were primiparas and 17% were multiparas. Among the normal third trimester pregnant women, 58% were nulliparas, 25% were primiparas and 17% were multiparas. And among the preeclamptic women, 67% were nulliparas, 25% were primiparas and 8% were multiparas.

Finally, none of the participants had a history of abortion.

Table 4.2 Maternal serum levels of Adiponectin, Leptin and the Adiponectin-leptin Ratio compared among the non-pregnant, the normal pregnant women and the preeclamptic women

Parameter	Non-pregnant women N=40	Normal pregnant women N= 180	Preeclamptic women N=60	p-value
Age(years)	29.95 ± 9.00	27.64 ± 4.36	28.11 ± 4.98	0.303
Gestational Age (weeks)	-	21.57 ± 13.03	35.15 ± 2.00	0.001
BMI(kg/m$_2$)	22.24 ± 3.52	25.25 ± 6.01	26.56 ± 3.10	0.122
Adiponectin (µg/ml)	6.75 ± 0.23	6.01 ± 0.30*	12.27 ± 3.01	0.001
Leptin (ng/ml)	5.88 ± 1.32	9.73 ± 1.15*	18.22 ± 2.01	0.001
SBP(mmHg)	101.27 ± 7.51	111.33 ± 10.34	150.21 ± 7.11	0.010
DBP(mmHg)	65.00 ± 5.07	71.28 ± 6.41	99.12 ± 9.00	0.020

N = sample size; Data = mean ± standard deviation; *statistically significant compared to the non-pregnant women (Post hoc test).

Table 4.2 shows that the ages ($p>0.05$), as well as the Body Mass Indices ($p>0.05$) of the non-pregnant women, the normal pregnant women and the preeclamptic women were comparable. But their levels of adiponectin ($p<0.05$), leptin ($p<0.05$) and the adiponectinleptin ratio ($p<0.05$), as well as their Systolic blood pressures ($p<0.05$) and Diastolic blood pressures ($p<0.05$), were different.

Table 4.3 Maternal serum levels of Adiponectin, Leptin and the Adiponectin-leptin Ratio compared among the non-pregnant women and the normal pregnant women

		Normal pregnant women			
Parameter	**Non-pregnant women N=40**	**1st trimester pregnant women N= 60**	**2nd trimester pregnant women N= 60**	**3rd trimester pregnant women N=60**	**p-value**
Age (years)	29.95±9.00	26.53± 5.35	29.28±5.19	27.12±5.92	0.313
Gestational Age (weeks)	-	8.10 ± 2.01	22. 21± 5.01	34.41±3.103	0.001
BMI(kg/m$_2$)	22.24±3.52	23.76±4.01	25.23±3.30	26.77±3.00	0.134
Adiponectin (µg/ml)	6.75 ± 0.23	7.68 ± 0.30*	6.23±0.79*	4.14 ± 0.91*	0.002
Leptin (ng/ml)	5.88 ± 1.32	6.46 ± 1.15	9.37 ±1.20*	13.36 ±3.11*	0.011
Adiponectin-leptin ratio	1.20 ± 0.22	1.20 ±0.17	0.47 ± 0.10*	0.30 ± 0.10*	0.010
SBP (mmHg)	101.27±7.51	107.15±10.34	111.00±6.79	115.85 ±8.70	0.110
DBP (mmHg)	65.00±5.07	67.57 ± 6.41	72.46 ± 7.00	73.82 ±7.95	0.121

N = sample size; Data = mean ± standard deviation; * statistically significant ($p<0.05$) compared to the non-pregnant women. Also, Post Hoc test indicated significant differences in the levels of adiponectin ($p<0.05$), leptin ($p<0.05$) and the adiponectin-leptin ratio ($p<0.05$) between the first trimester pregnant women and the second trimester pregnant women, between the first trimester pregnant women and the third trimester pregnant women, and between the second trimester pregnant women and the third trimester pregnant women.

Table 4.3 shows that the ages ($p>0.05$), the Body Mass Indices ($p>0.05$), the Systolic blood pressures ($p>0.05$), and the Diastolic blood pressures ($p>0.05$) of the non-pregnant women, the first trimester pregnant women, the second trimester pregnant women and the third trimester pregnant women were comparable.

The levels of adiponectin significantly differed among the non-pregnant women, the first trimester pregnant women, the second trimester pregnant women and the third trimester pregnant women ($p<0.05$). The levels of adiponectin were highest in the first trimester pregnant women, higher in the non-pregnant women, high in the second trimester pregnant women and low in the third trimester pregnant women ($p<0.05$).

The levels of leptin were comparable between the non-pregnant women and the first trimester pregnant women ($p>0.05$), but significantly higher in the second and third trimester pregnant women than in the non-pregnant women and the first trimester pregnant women ($p<0.05$). The third trimester pregnant women had the highest levels of leptin ($p<0.05$).

The adiponectin-leptin ratios were comparable between the non-pregnant women and the first trimester pregnant women ($p>0.05$), but significantly lower in the second and third trimester pregnant women than in the non-pregnant women and the first trimester pregnant women ($p<0.05$). The third trimester pregnant women had the highest levels of the adiponectin-leptin ratio ($p<0.05$).

Table 4.4 Maternal serum levels of Adiponectin, Leptin and the Adiponectin-leptin Ratio compared between the normal pregnant women and the preeclamptic women

Parameter	Normal pregnant women N=60	Preeclamptic women N=60	p-value
Age (years)	27.12±5.92	28.11±4.98	0.322
Gestational Age (weeks)	34.41±3.10	35.15±2.00	0.490
BMI (kg/m2)	26.77±3.00	26.56 ± 3.10	0.534
Adiponectin (µg/ml)	4.14 ± 0.91	12.27 ± 3.22	0.001
Leptin (ng/ml)	13.36 ±3.11	18.22 ± 2.01	0.010
Adiponectin-leptin ratio	0.30 ± 0.10	0.66 ± 0.10	0.001
SBP (mmHg)	115.85 ±8.70	150.21±7.11	0.001
DBP (mmHg)	73.82 ±7.95	99.12 ± 9.00	0.010

N = sample size; Data = mean ± standard deviation.

Table 4.4 shows that the normal pregnant women and the preeclamptic women had comparable ages ($p>0.05$), comparable gestational ages ($p>0.05$) and comparable Body Mass Indices ($p>0.05$). However, the preeclamptic women had higher Systolic blood pressures ($p<0.05$), higher Diastolic blood pressures ($p<0.05$), higher adiponectin levels ($P<0.05$), higher leptin levels ($p<0.05$) and higher adiponectin-leptin ratios ($p<0.05$) than the normal pregnant women.

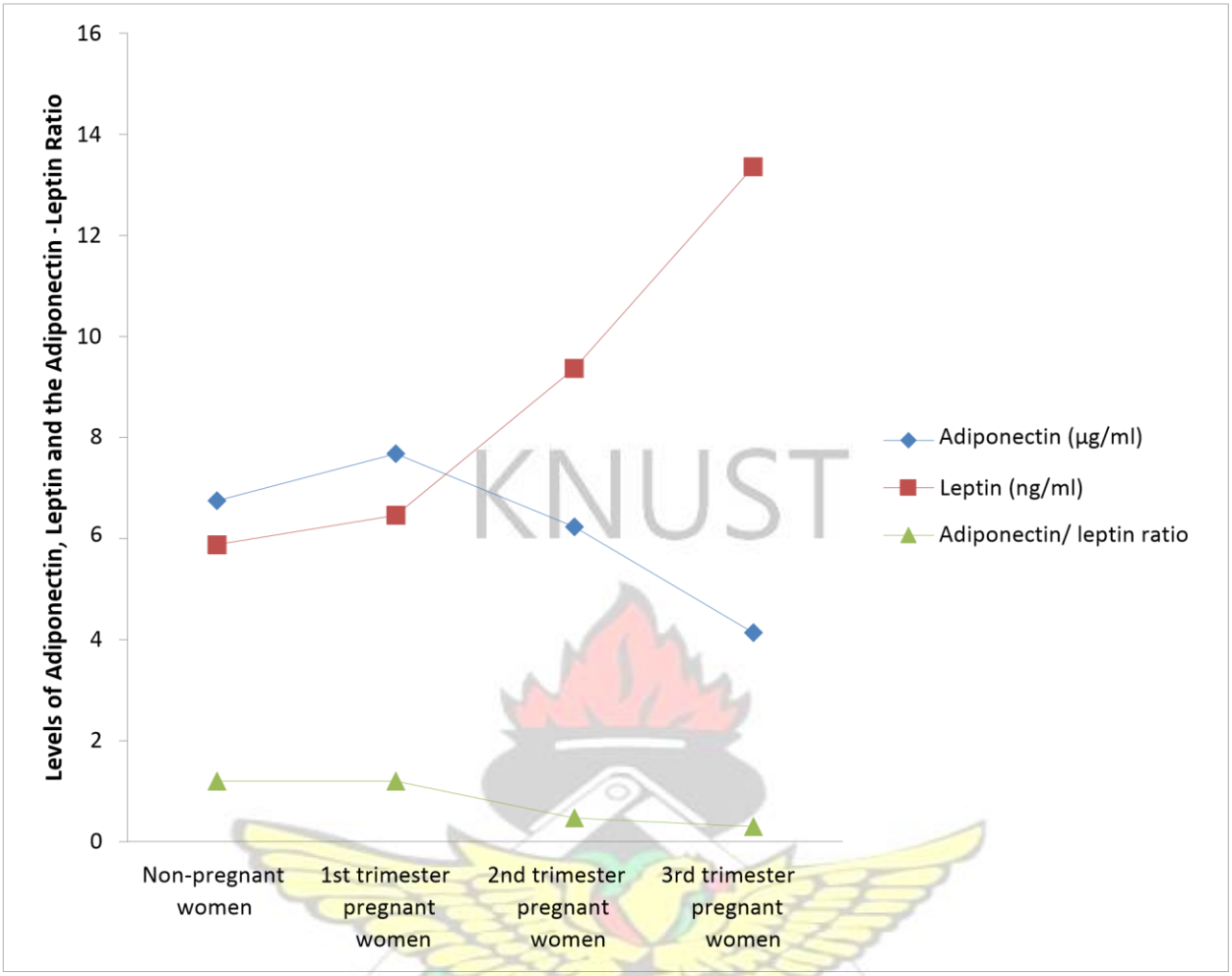

Figure 4.1 The physiological profiles of Adiponectin, Leptin and the Adiponectin-Leptin Ratio during Normal Pregnancy

Figure 4.1 shows that adiponectin levels rose slightly from the non-pregnancy state to the first trimester pregnancy, and then fell considerably from the first trimester pregnancy to

the third trimester pregnancy. Leptin levels rose very slightly from the non-pregnancy state to the first trimester pregnancy, and then rose considerably to the third trimester pregnancy. The adiponectin-leptin ratio stagnated between the non-pregnancy state and the first trimester pregnancy, but decreased considerably from the first trimester to the third trimester.

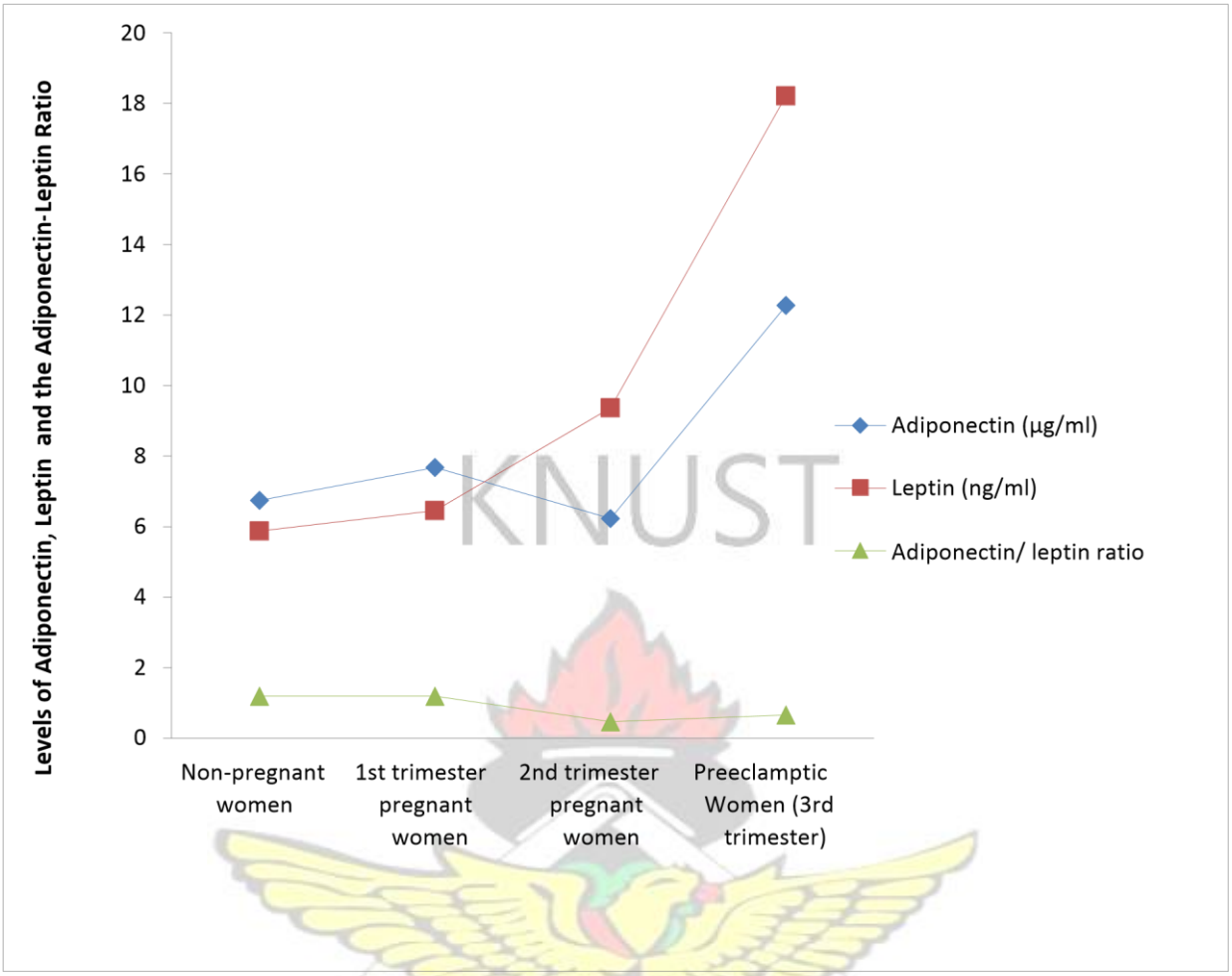

Figure 4.2 The profiles of Adiponectin, Leptin and the Adiponectin-Leptin Ratio during Pregnancy complicated by Preeclampsia in the third trimester

Figure 4.2 shows that adiponectin levels rose slightly from the non-pregnancy state to the first trimester, fell considerably from the first trimester to the second trimester and then rose considerably to the third trimester, when preeclampsia developed. Leptin levels rose very slightly from the non-pregnancy state to the first trimester, and then rose sharply to the third trimester, when preeclampsia developed. The adiponectin-leptin ratio stagnated between the non-pregnancy state and the first trimester, but decreased considerably from the first trimester to the second trimester, and then rose to the third trimester, when preeclampsia developed.

Table 4.5 Weight gain, Gravidity and Parity as risk factors of Preeclampsia

Parameter	OR (95% CI)	p-value
Age (years)		
<30	2.03 (0.32 – 10.53)	0.105
≥30		
BMI (kg/m3)		
Overweight	2.14 (0.93 – 9.84)	0.012
Normal-weight	1	
Gravidity		
Primigravida	3.57 (1.52 – 8.37)	0.005
Multigravida	1	
Parity		
Nulliparous	3.88 (1.30 – 11.62)	0.015
Multiparous	1	

OR=Odds ratio

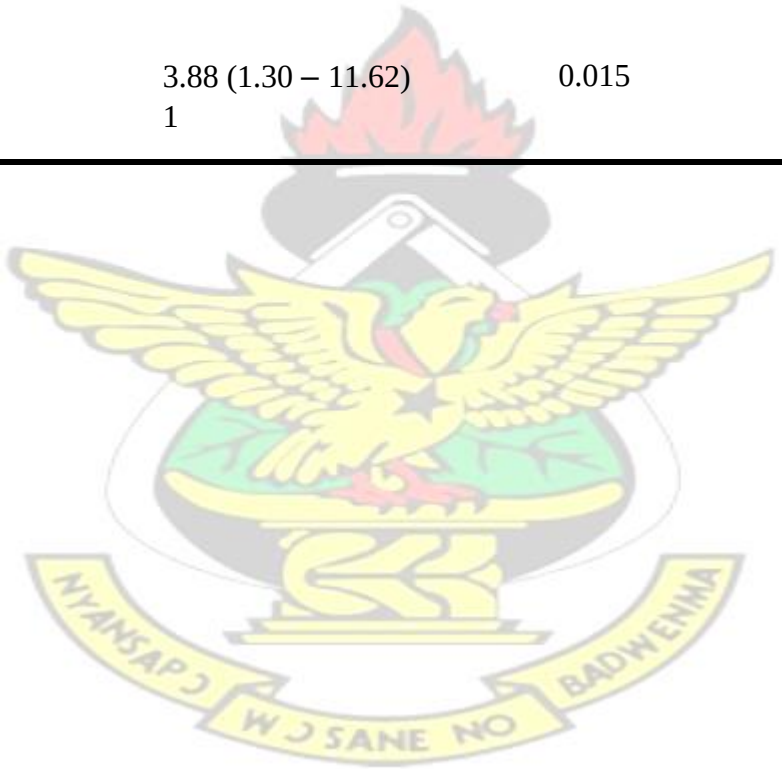

Table 4.5 shows that age is not a risk of developing preeclampsia [OR=2.03(0.32 – 10.53), p<0.05)], overweight pregnant women are at higher risk of developing preeclampsia than their normal weight counterparts [OR=2.14 (0.93 – 9.84), p<0.05], primigravida pregnant women are at higher risk of developing preeclampsia than multigravida pregnant women [OR=3.57(1.524-8.37), p<0.05]. It also shows that nulliparous pregnant women are at a higher risk of developing preeclampsia than multiparous pregnant women [OR=3.88 (1.30-11.62), p<0.05].

Table 4.6 Comparison of the levels of Adiponectin, Leptin and the Adiponectin- Leptin Ratio between the Normal-Weight and the Overweight groups of the Non- Pregnant, the Normal Pregnant and the Preeclamptic Women

Participants	Parameter	Normal-weight	Overweight	p-value
Non-pregnant Women	Age (years)	27.22±8.33	30.67± 9.67	0.332
	Adiponectin (µg/ml)	7.32 ± 0.49	6.17± 0.53	0.010
	Leptin(ng/ml)	5.36 ± 1.18	6.40 ±1.46	0.023
	Adiponectin-leptin ratio	1.40 ± 0.19	0.99 ± 0.20	0.001
1st trimester pregnant women	Age (years)	24.92±4.53	28.14±6.17	0.138
	Gestational Age (weeks)	8.03 ± 2.03	7.9 ± 1.99	0.211
	Adiponectin (µg/ml)	8.12 ±0.68	7.23± 0.50	0.010
	Leptin(ng/ml)	5.81± 0.87	7.10± 1.43	0.010
	Adiponectin-leptin ratio	1.38 ± 0.07	1.03 ± 0.09	0.002
2nd trimester pregnant women	Age (years)	29.75±4.27	28.80±6.11	0.190
	Gestational Age (weeks)	24.3 ± 2.60	20.10 ±2.50	0.120
	Adiponectin (µg/ml)	6.38± 0.90	6.01±0.68	0.030
	Leptin(ng/ml)	8.11± 0.87	9.92 ±1.52	0.022
	Adiponectin-leptin ratio	0.77 ± 0.10	0.60 ± 0.10	0.010
3rd trimester pregnant women	Age (years)	28.15±5.02	26.99±6.81	0.438
	Gestational Age (weeks)	32.90±2.01	35.92±1.90	0.220
	Adiponectin (µg/ml)	4.96± 0.88	3.91±0.94	0.029
	Leptin(ng/ml)	11.60±2.32	14.11±1.70	0.003
	Adiponectin-leptin ratio	0.34 ± 0.10	0.28 ± 0.10	0.070
Preeclamptic women	Age (years)	30.01 ± 6.02	29.03 ± 5.99	0.322
	Gestational Age (weeks)	33.92 ± 2.0	36.38 ± 2.00	0.301
	Adiponectin (µg/ml)	13.10 ± 2.99	11.44 ± 3.05	0.025
	Leptin (ng/ml)	16.88 ± 2.00	18.87 ± 2.02	0.013
	Adiponectin-leptin ratio	0.73 ± 0.20	0.53 ± 0.25	0.040
	Proteinuria (g/L)	1.41 ± 0.20	1.43 ± 0.25	0.100

Data= mean ± standard deviation; Significance = $p<0.05$.

Table 4.6 shows that the ages ($p>0.05$), as well as the Systolic blood pressures ($p>0.05$) and Diastolic blood pressures ($p>0.05$) of the participants (non-pregnant women, first trimester pregnant women, second trimester pregnant women, normal third trimester pregnant women and the preeclamptic women) within the normal-weight and the overweight categories were comparable.

The levels of adiponectin were significantly lower in the overweight participants than in their normal-weight counterparts ($p<0.05$), while the levels of leptin were significantly higher in the overweight participants than in their normal-weight counterparts ($p<0.05$). The adiponectin-leptin ratio was significantly lower in the normal-weight participants than in their overweight counterparts ($p<0.05$).

Table 4.7 Comparison of the levels of Adiponectin, Leptin and the AdiponectinLeptin Ratio in the Primigravida and the Multigravida groups of the Non-Pregnant, the Normal Pregnant and the Preeclamptic Women

Participants	Parameter	Primigravida	Multigravida	p-value
Non-pregnant Women	Age (years)	26.12±8.33	29.67± 9.67	0.132
	BMI (kg/m2)	24.21± 2.11	25.41± 1.39	0.200
	Adiponectin (µg/ml)	7.15 ± 1.59	6.27± 1.53	0.110
	Leptin(ng/ml)	5.36 ± 1.19	5.40 ±1.42	0.223
	Adiponectin-leptin ratio	1.33 ± 0.29	1.16 ± 0.30	0.312
1st trimester pregnant women	Age (years) BMI(kg/m2)	25.92±4.53 25.21± 1.99	27.14±6.17 24.52± 1.68	0.138 0.102
	Gestational Age (weeks)	8.13 ± 2.13	7.91 ±2.00	0.211
	Adiponectin (µg/ml)	7.12 ±0.68	8.23± 0.50	0.202
	Leptin(ng/ml)	6.82± 0.87	7.11± 1.44	0.111
	Adiponectin-leptin ratio	1.04 ± 0.10	1.16 ± 0.10	0.223
2nd trimester pregnant women	Age (years)	29.85±4.28	28.79±6.21	0.190
	BMI (kg/m2)	26.21± 2.11	26.41± 1.39	0.210
	Gestational Age (weeks)	24.2 ± 2.50	22.11 ± 2.5	0.200
	Adiponectin (µg/ml)	6.47± 0.81	6.38 ±0.67	0.413
	Leptin (ng/ml)	8.91± 0.87	8.82 ± 1.52	0.400
	Adiponectin-leptin ratio	0.72 ± 0.20	0.72 ± 0.11	0.333
3rd trimester pregnant women	Age (years)	28.15±5.00	26.08±6.17	0.426
	BMI(kg/m2)	27.21± 2.11	25.41± 1.39	0.131
	Gestational Age (weeks)	33.90±2.01	35.91±1.91	0.212
	Adiponectin (µg/ml)	4.28± 0.89	4.00 ± 0.96	0.159
	Leptin(ng/ml)	12.60±2.32	13.11±1.70	0.142
	Adiponectin-leptin ratio	0.34 ± 0.12	0.31 ± 0.11	0.160
Preeclamptic women	Age (years) BMI	30.01 ± 6.02	28.73 ± 5.99	
	(kg/m2)	26.21± 2.11	26.41 ± 1.39	0.322 0.221
	Gestational Age (weeks)	33.92 ± 2.00	36.38 ± 2.00	0.301
	Adiponectin (µg/ml)	12.62 ± 2.99	11.94 ± 3.05	0.211

Leptin (ng/ml)	17.87 ± 2.00	19.67 ± 2.02	0.301
Adiponectin-leptin ratio	0.71 ± 0.30	0.62 ± 0.25	0.180
Proteinuria (g/L)	1.51 ± 0.21	1.53 ±0.26	0.100

Data= mean ± standard deviation; Significance = $p<0.05$.

Table 4.7 shows that primigravida and multigravida pregnant women have comparable levels of adiponectin ($p>0.05$), comparable levels of leptin ($p>0.05$) and comparable levels of the adiponectin-leptin ratio ($p>0.05$).

Table 4.8. Comparison of the levels of Adiponectin, Leptin and the AdiponectinLeptin ratio in the Nulliparas, the Primiparas and the Multiparas of the NonPregnant, the Normal Pregnant and the Preeclamptic Women

Participants	Parameter	Nulliparas	Primiparas	Multiparas	p-value
Non-pregnant Women	Age (years)	27.12±8.34	28.67± 9.68	29.95±4.27	0.213
	BMI(kg/m2)	25.11± 1.89	25.41± 1.39	24.21±2.11	0.211
	Adiponectin(µg/ml)	6.77± 1.50	6.27± 1.44	7.15 ± 1.59	0.120
	Leptin(ng/ml)	5.45 ± 1.17	5.39 ±1.32	5.36 ± 1.19	0.123
	Adiponectin-leptin ratio	1.24 ± 0.29	1.16 ± 0.25	1.33 ± 0.11	0.112
1st trimester pregnant women	Age (years)	27.24±6.18	26.23±5.15	26.82±4.54	0.124
	BMI(kg/m2)	24.52±1.68	25.42± 1.59	26.21±1.99	0.203
	Gestational Age (weeks)	7.91 ± 2.01	8.72 ±2.11	8.23 ± 2.23	0.208
	Adiponectin(µg/ml)	8.24 ± 0.51	8.03± 0.42	7.14 ±0.69	0.291
	Leptin(ng/ml)	7.22 ± 1.44	8.15 ± 1.50	6.82± 0.87	0.300
	Adiponectin-leptin ratio	1.14 ± 0.11	0.98 ± 0.10	1.04 ± 0.10	0.121
2nd trimester pregnant women	Age (years)	29.77±6.31	28.79±6.21	29.85±4.28	0.118
	BMI(kg/m2)	26.41± 1.39	25.32±4.20	26.21±2.11	0.215
	Gestational Age(weeks)	24.20 ± 2.50	23.11±3.21	22.11±2.50	0.233
	Adiponectin(µg/ml)	6.47± 0.81	6.38±0.67	6.73±2.00	0.422
	Leptin(ng/ml)	8.85± 0.87	7.90±0.21	8.83 ±1.42	0.301
	Adiponectin-leptin ratio	0.73 ± 0.20	0.80 ±0.21	0.76 ± 0.11	0.213
3rd trimester pregnant women	Age (years)	30.11 ± 6.02	28.84±5.99	31.22±5.02	0.111
	BMI(kg/m2)	28.21± 2.11	26.41± 1.32	27.21±2.11	0.312
	Gestational Age(weeks)	32.90±2.00	35.91±1.91	33.90±2.01	0.311

	Adiponectin(µg/ml)	4.38± 0.89	4.02±0.96	4.27± 0.80	0.201
	Leptin(ng/ml)	12.70±2.32	13.11±1.70	12.66±2.33	0.311
	Adiponectin-leptin ratio	0.34 ± 0.12	0.31 ± 0.41	0.34 ± 0.44	0.109
Preeclamptic women	Age (years)	26.08±6.17	28.08±6.17	27.22±5.10	0.203
	BMI(kg/m2)	27.41± 1.39	26.42± 1.33	26.21±2.01	0.102
	Gestational Age (weeks)	36.38 ± 2.00	35.38 ±2.02	32.92±2.21	0.222
	Adiponectin (µg/ml)	12.94± 3.05	13.94± 3.15	12.62±2.76	0.339
	Leptin (ng/ml)	19.67± 2.02	18.77± 2.33	18.87±2.23	0.421
	Adiponectin-leptin ratio	0.62 ± 0.25	0.62 ± 0.25	0.81 ± 0.31	0.332
	Proteinuria (g/L)	1.48±0.26	1.54±0.26	1.53±0.21	0.309

Data= mean ± standard deviation; Significance = $p<0.05$.

Table 4.8 shows that the nulliparous, primiparous and multiparous pregnant women had comparable levels of adiponectin ($p>0.05$), comparable levels of leptin (0.05) and comparable levels of the adiponectin-leptin ratio ($p>0.05$).

Table 4.9. Pearson correlation among Adiponectin, Leptin, the Adiponectin Ratio, Body Mass Index, Systolic Blood Pressure and Diastolic Blood Pressure of the Non-Pregnant Women

Parameter	Age	SBP	DBP	BMI	Adiponectin	Leptin	Adiponectin -leptin ratio
Age		0.060	0.040	0.493*	-0.030	0.405*	-0.020
SBP			0.805**	0.120	-0.512*	0.621*	-0.421*
DBP				0.110	-0.442*	0.511*	-0.313*
BMI					-0.684**	0.571**	-0.512**
Adiponectin						-0.402**	0.569**
Leptin							-0.599**
Adiponectin-leptin ratio							

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.
* Correlation was significant at the 0.05 level; ** correlation was significant at the 0.01 level.

-Leptin

Table 4.9 shows that adiponectin correlated strongly, inversely (negatively) and significantly with body mass index (r=-0.684; p<0.01) and systolic blood pressure (r=-0.512; p<0.05) but weakly, inversely and significantly with leptin (r=-0.402; p<0.01) and diastolic blood pressure (r=-0.442; p<0.05). Leptin correlated strongly, directly (positively) and significantly with Body mass index (r=0.571; p<0.01), systolic blood pressure (r=0.621; p<0.05) and diastolic blood pressure (r=0.511; p<0.05) but weakly, inversely and significantly with adiponectin (r=-0.402; p<0.01). The adiponectin-leptin ratio correlated inversely with body mass index (r=-0.512; p<0.01), Systolic blood pressure (r=-0.421; p<0.05) and diastolic blood pressure (r=-0.313; p<0.05).

Table 4.10 Pearson correlation among Adiponectin, Leptin, the Adiponectin Ratio, Gestational Age, Body Mass Index, Systolic Blood Pressure and Diastolic Blood Pressure of the Normal Pregnant Women

Parameter	Age	GA	SBP	DBP	BMI	Adiponectin	Leptin	Adiponectin -leptin ratio
Age		0.030	0.121	0.090	0.291*	-0.112	0.202	0.060
GA			0.310	0.231	0.112	-0.824**	0.797**	-0.801**
SBP				0.566**	0.230	-0.310*	0.499*	-0.539*
DBP					0.242*	-0.490*	0.300*	-0.520*
BMI						-0.620*	0.710*	-0.510*
Adiponectin							-0.669**	0.782**
Leptin								-0.731**
Adiponectin -leptin ratio								

GA: Gestational Age; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. * Correlation was significant at the 0.05 level; ** correlation was significant at the 0.01 level.

-Leptin

Table 4.10 shows that adiponectin correlated strongly, inversely and significantly with leptin (r=-0.669; p<0.01), body mass index (r=-620, p<0.05) and gestational age (r=-0.824; p<0.01) but weakly, inversely and significantly with systolic blood pressure (r=-0.310; p<0.05) and diastolic blood pressure (r=-0.313; p<0.05). Leptin correlated strongly, directly and significantly with gestational age (r=0.797; p<0.01), weakly, directly and significantly with systolic blood pressure (r=0.499; p<0.05) and Diastolic blood pressure (r=0.300; p<0.05) but strongly, directly and significantly with Body mass index (r=0.710, p<0.05). The adiponectin-leptin ratio correlated strongly, inversely and significantly with body mass index (r=-510, p<0.05), gestational age (r=-0.801; p<0.05), systolic blood pressure (r=-0.539; p<0.05) and diastolic blood pressure (r=-0.520; p<0.05).

Table 4.11 Pearson correlation among Adiponectin, Leptin, the Adiponectin Ratio, Gestational Age, Body Mass Index, Systolic Blood Pressure and Diastolic Blood Pressure of the Preeclamptic Women

Parameter	Age	GA	SBP	DBP	BMI	PN	Adiponectin	Leptin	Adiponectin -leptin ratio
Age		0.060	0.090	0.112	0.282*	0.020	-0.050	0.020	0.100
GA			0.221	0.050	0.020	0.111	-0.121	0.040	-0.201
SBP				0.700*	0.080	0.711*	-0.320	0.578*	-0.241*
DBP					0.201	0.652*	-0.440	0.601*	-0.332*
BMI						0.213*	-0.521*	0.700*	-0.429*
PN							-0.412*	0.821*	0.500*
Adiponectin								-0.290	0.443*
Leptin									-0.321*
Adiponectin - leptin ratio									

GA: Gestational Age; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PN: Proteinuria. * Correlation was significant at the 0.05 level.

-Leptin

Table 4.11 shows that adiponectin correlated strongly, inversely and significantly with body mass index ($r=-0.521$, $p<0.05$), insignificantly with leptin ($r=-0.290$; $p>0.05$), systolic blood pressure ($r=-0.320$; $p>0.05$) and diastolic blood pressure ($r=-0.440$; $p>0.05$), but correlated weakly, inversely and significantly with proteinuria ($r=-0.412$; $p<0.05$). Leptin correlated strongly, directly and significantly with body mass index ($r=0.700$; $p>0.05$), proteinuria ($r=0.500$; $p<0.05$), systolic blood pressure ($r=0.578$; $p<0.05$) and diastolic blood pressure ($r=0.601$; $p<0.05$). The adiponectin-leptin ratio correlated weakly, inversely and significantly with body mass index ($r=-0.429$; $p<0.05$), systolic blood pressure ($r=-0.241$; $p<0.05$) and diastolic blood pressure ($r=-0.332$; $p<0.05$), but correlated strongly, directly and significantly with proteinuria ($r=0.500$; $p<0.05$).

CHAPTER FIVE

DISCUSSION

5.1 RELATIONSHIP BETWEEN ADIPONECTIN AND LEPTIN

The adipose tissue is no longer recognized as, solely, an energy storage tissue, but also a secretory tissue that synthesizes and secretes important biomolecules called adipokines, two of which are adiponectin and leptin. Several studies have found that adiponectin and leptin are secreted through distinct intracellular trafficking pathways, and so rely on different avenues for their constitutive and regulated secretion (Jürimäe et al., 2005; Xie et al., 2008).

Studies regarding the relationship between these two adipokines have yielded consistent results. It has been reported that adiponectin and leptin exhibit an inverse relationship. Among normal non-pregnant women, Matsubara et al., (2002) and Lecke et al., (2001) observed an inverse relationship between adiponectin and leptin levels; and among normal pregnant women, this relationship was also observed by Khosrowbeygi et al., (2009). The results of this present study confirm the above findings. Among the non- pregnant women, adiponectin correlated weakly and inversely with leptin (Table 4.9), and among the normal pregnant women, adiponectin correlated strongly and inversely with leptin (Table 4.10). However, among the preeclamptic women, there was no significant correlation between adiponectin and leptin (Table 4.11). The inverse relationship between adiponectin and leptin in physiological states may indicate that the synthesis and secretion of one of them, physiologically, inhibits or suppresses the synthesis and secretion of the other (Xi et al., 2008); and the alteration in the relationship between adiponectin and leptin in the

preeclamptic women may indicate that pathological conditions like preeclampsia may have an effect on the levels of adiponectin and leptin.

5.2 RELATIONSHIP BETWEEN ADIPONECTIN AND BLOOD PRESSURE

Adiponectin has been found to correlate inversely with systolic blood pressure and diastolic blood pressure. In 2003, Adamczak et al., showed for the first time that plasma adiponectin varies inversely with blood pressure. And following this, a number of clinical studies have also reported an inverse relationship between plasma adiponectin levels and high blood pressure (Adamczak et al., 2003; Iwashima et al., 2004; Chow et al., 2007). In this present study, adiponectin was found to correlate inversely with systolic blood pressure and diastolic blood pressure among the non-pregnant women (Table 4.9) and the normal pregnant women (Table 4.10) but, among the preeclamptic women, this relationship was attenuated (Table 4.11).

Adiponectin has been identified as an antagonist to the production of angiotensin II (a hormone that raises blood pressure) (Kintscher et al., 2005). Due to this, a high adiponectin level decreases blood pressure through the inhibition of the action of angiotensin II, hence the observed inverse relationship between adiponectin and blood pressure. According to Chow et al., (2007), hypoadiponectinaemia is a possible predictor of future hypertension; and according to Ohashi et al., (2006), hyperadiponectinaemia may be protective against hypertension. This information makes it clear that during hypertensive states, there could be increased secretion of adiponectin. Thus, attenuation of the relationship between

adiponectin and blood pressure in the preeclamptic women could be attributed to adiponectin resistance which resulted from increased secretion of adiponectin to offset the high blood pressure characteristic of preeclampsia (Kajantie et al., 2005).

5.3 RELATIONSHIP BETWEEN LEPTIN AND BLOOD PRESSURE

Leptin has been shown to associate positively with systolic blood pressure and diastolic blood pressure. In 1997, Kennedy et al., demonstrated a positive relationship between plasma leptin levels and elevated systolic blood pressure and diastolic blood pressure; and in the subsequent year, Suter et al., (1998) reported a significant positive relationship between plasma leptin levels and systolic blood pressure in hypertensive women (Kennedy et al., 1997; Suter et al., 1998; Patel et al., 2008).

These studies are in consonance with this present study, which has also reported a positive relationship between leptin and systolic blood pressure and diastolic blood pressure among the non-pregnant women (Table 4.9) and the normal pregnant women (Table 4.10). Among the preeclamptic women, this positive relationship between leptin and the elevated systolic blood pressure and diastolic blood pressure was similarly observed (Table 4.11). Based on the significant positive correlation observed between blood pressure and leptin level, it is suggested that a rise in leptin levels may be a regulator of blood pressure (Patel et al., 2008).This possible regulatory effect of leptin on blood pressure has been demonstrated by both intracerebroventricular and intravenous administration of leptin, indicating central as well as peripheral actions of the adipokine. Intracerebroventricular administration of leptin elicited an increase in arterial blood pressure and intravenous administration of leptin also significantly increased arterial blood pressure (Furuhashi et al., 2003). The finding that leptin stimulates the renin–

angiotensin system (through angiotensin II signaling) and the sympathetic system (Stenvinkel et al., 2000) is one possible mechanism to explain this observed relationship.

5.4 INFLUENCE OF BODY MASS INDEX ON MATERNAL SERUM ADIPONECTIN LEVELS

Following the discovery of adiponectin, many investigators have made conscious efforts to characterize the relationship adiponectin bears with body weight. It has been found that the levels of adiponectin vary inversely with the weight of an individual (Hotta et al., 2001; Ukkola et al., 2002; Nedvidkova et al., 2005). In verifying this inverse relationship adiponectin has with body weight in non-pregnant individuals, Coppola et al., (2009) found that weight reduction significantly increases circulating levels of adiponectin. In comparing the levels of adiponectin between normal-weight and overweight individuals, many investigators have found that adiponectin levels are higher in normal-weight individuals than in overweight individuals (Hotta et al., 2001; Ukkola et al., 2002; Nedvidkova et al., 2005).

The above findings are in agreement with this current study. In this study, adiponectin had an inverse correlation with body mass index among the participants (Table 4.9; Table 4:10; Table 4:11), with the normal-weight women exhibiting significantly higher levels of adiponectin than their overweight counterparts (Table 4.5). The negative correlation adiponectin has with body mass index, and the lower levels of adiponectin seen in overweight individuals are quite paradoxical. Adiponectin is known to be produced and secreted by the adipose tissue (Scherer et al., 1995), and since overweight individuals have

a larger adipose tissue than the normal-weight individuals (Cannon et al., 2008), it was expected that adiponectin levels would positively correlate with body mass index, and so assume higher levels in the overweight groups than in the normal-weight groups, but this study has found otherwise. According to Ukkola et al., (2002) and Cnop et al., (2011), increased intra-abdominal fat in overweight individuals is what accounts for the reduced circulating adiponectin levels observed in overweight individuals, since adiponectin and body fat are inversely correlated. This inverse relationship between body weight and adiponectin indicates that adiponectin may play a role in regulating body weight.

5.5 INFLUENCE OF BODY MASS INDEX ON MATERNAL SERUM LEPTIN LEVELS

Characterizing the relationship between leptin and body mass index has attracted the attention of several investigators. In 2000, for instance, Blache et al., and Delavaud et al., observed a strong positive relationship between plasma leptin and body weight (Blache et al., 2000; Delavaud et al., 2000). This finding has been supported by this present study.

In this study, there was a strong positive correlation between leptin and body mass index among the participants (Table 4.9; Table 4.10; Table 4:11), with the overweight women presenting with a significantly higher levels of leptin than the normal-weight women (Table 4.5). The higher levels of leptin in the overweight individuals than in the normal-weight individuals are not only evident in this study, but also in the studies conducted by Hamilton et al., (1995) and Considine et al., (2006). After Hamilton et al., (1995) had reported that chronically elevated leptin levels are associated with overweight, Considine et al., (2006) investigated that report, and then explained that overweight individuals exhibit an, unusually, high circulating concentration of leptin, and that these people are

resistant to the effects of leptin, in much the same way that people with type 2 diabetes are resistant to the effects of insulin. This indicates that leptin may play vital roles in regulating weight gain in humans.

The observations that leptin levels are significantly higher in overweight individuals than normal-weight individuals could be explained in three main ways. According to Zhao et al., (2004), the adipose tissue, under normal conditions, is responsible for the constant production and release of leptin; and that the level of circulating leptin is proportional to the total amount of fat in the body, since leptin levels increase exponentially with increased mass of the adipose tissue. This suggests that the higher levels of leptin in the overweight women than in the normal-weight women is due to the higher mass of adipose tissue in the overweight women than in the normal-weight women (Margetic et al., 2002).

Also, since the bones of the body are the tissues that bear the weight of the body (Bolton, 2009), and leptin affects bone metabolism via direct signalling from the brain to increase cortical bone (Hamrick et al., 2008), it has been suggested that a rise in leptin levels during weight gain may represent the body's adaptational mechanism for enlarging bone size to cope with the increased body weight (Hamrick et al., 2008).

Additionally, leptin regulates appetite. That explains why in the absence of leptin (or its receptor), a person eats a lot and becomes obese. Thus, the higher the levels of leptin in a person, the stronger her appetite regulatory mechanisms, and vice versa (Mark et al., 2002). Therefore, as a physiological mechanism to check body weight, the adipose tissue is,

probably, stimulated to synthesize and secrete a lot of leptin so as to regulate the food intake of overweight people. And the observation that during fasting, or following a verylow-calorie diet (VLCD), leptin levels are lowered, strengthens the above hypothesis (Dubuc, 1998). Nonetheless, the occurrence of leptin resistance in obese individuals attenuates this possible effect of leptin (Mark et al., 2002).

5.6 PHYSIOLOGICAL PROFILE OF ADIPONECTIN DURING PREGNANCY

Adiponectin levels were higher in the non-pregnant women than in the pregnant women (Table 4.2). However, when each trimester was treated separately and the levels of adiponectin compared among the non-pregnant women, the first, second and third trimester pregnant women, the levels of adiponectin were lower in the non-pregnant women than in the first trimester pregnant women, but higher in the non-pregnant women than in the second and third trimester pregnant women (Table 4.3).

The higher levels of adiponectin in the first trimester pregnant women than in the non-pregnant women have, similarly, been observed by Mazaki-Tovi et al., (2007). The accretion of the adipose tissue in early pregnancy, and the additional production of adiponectin by the placenta (Chen et al., 2006) are what account for the reported differences. This, however, contradicts the findings of Fuglsang et al., (2006) and Nien et al., (2007) which indicated that maternal serum adiponectin levels between non-pregnant and pregnant women were comparable.

Adiponectin levels were also significantly different among the women in the various trimesters. The first trimester women had the highest adiponectin levels, followed by the second trimester pregnant women and, finally, by the third trimester pregnant women

(Table 4.3). Thus, this study reports that, during pregnancy, there is a fall in adiponectin levels from the first trimester to the third trimester. Interestingly, some researchers have reported a similar finding. Fuglsang et al., (2006), for instance, found that adiponectin levels were lower in the third trimester pregnant women than in the first and second trimester pregnant women. These findings seem to raise some controversy. With increasing gestational age, the placenta undergoes accretion to produce a lot of adiponectin (Buchanan et al., 1990; Catalano et al., 1991) which culminates in changes in the maternal circulating adiponectin (Gavrilova et al., 1997) and with changes in the relative distribution of the isoforms (Shifman et al., 2001; Busua et al., 2003). Thus, to probably meet the needs of the foetus or play an active role in pregnancy, adiponectin levels must rise as pregnancy advances. However, in this study, adiponectin levels decreased gradually from the first trimester to the third trimester, thus contradicting the expected order. This, seemingly, contradiction has also been reported by Mazaki-Tovi et al., (2007) whose studies about the maternal serum levels of adiponectin in normal pregnant women showed that adiponectin levels do not change with advancing gestation. Incidentally, why accretion of the placenta does not result in a rise in adiponectin levels, as pregnancy advances, has been explained.

According to Lage et al., (1999), maternal fat deposition increases during pregnancy, and since adiponectin has been found to correlate inversely with body fat (Ukkola et al., 2002; Cnop et al., 2011), it could be posited that the increase in fat levels with advancing gestation

is a possible cause of the gradual decreased levels of adiponectin from the first trimester to the third trimester.

Also, as explained by Catalano et al., (1999), pregnancy is a condition of insulin resistance and this insulin resistance associates inversely with adiponectin (Berg et al., 2002; McLachlan et al., 2006; Freubis et al., 2010). Therefore, since insulin resistance varies inversely with adiponectin, increase in insulin resistance leads to a fall in adiponectin levels. This decreasing effect of insulin resistance on adiponectin is so strong that, in the presence of insulin resistance, the additional production and release of adiponectin by the placenta will not result in a rise of adiponectin levels in the plasma (Hotta et al., 2001; Hotta et al., 2003; Catalano et al., 2005). Thus, in comparing the maternal serum levels of adiponectin among pregnant women in different trimesters, the group presenting with the highest insulin resistance will present with the lowest adiponectin levels, and vice versa. As observed in this study, a gradual decrease in adiponectin levels from the first to the third trimester means that insulin resistance might have increased from the first trimester to the third trimester. And as it has been found that pregnancy-induced insulin resistance, if not corrected physiologically or therapeutically, leads to the development of gestational diabetes (Buchanan, 1990), it could be deduced that adiponectin may participate in the regulation of metabolism in pregnancy; and that the gradual decrease in adiponectin levels with advancing gestation may be part of the body's mechanisms that physiologically act to correct insulin resistance in pregnancy, so that the insulin resistance will not degenerate into metabolic disorders like gestational diabetes.

5.7 PHYSIOLOGICAL PROFILE OF LEPTIN DURING PREGNANCY

Maternal serum levels of leptin are higher in pregnant women than in non-pregnant women, and among the pregnant women, correlates positively with gestational age (Schubring et al., 1999). During normal pregnancy, leptin levels rise to about 2 to 3 fold above non-pregnancy levels, with the peak occurring around the 28th week of gestation. The levels then fall to below pre-pregnancy levels at around birth and after parturition (Moynihan et al., 2006).

The above findings are supported by the findings of this present study. This study reports a positive relationship between leptin and gestational age (Table 4.10), and that leptin levels are higher in pregnant women than in non-pregnant women (Table 4.2). It also reports that, though leptin levels are comparable between the non-pregnant and the first trimester pregnant women, the levels are significantly higher in the second and third trimester pregnant women than in the non-pregnant women and the first trimester pregnant women. In the second trimester, the levels of leptin were observed to be about 2 fold above the levels in the non-pregnant women; and in the third trimester, the levels of leptin were observed to be about 3 fold above the levels in the non-pregnant women (Table 4.3). These changes in the levels of leptin with advancing gestation indicate that leptin may play a vital role during pregnancy.

There are four main explanations for the increase in leptin towards the end of pregnancy. Firstly, during pregnancy, there is increased synthesis and secretion of leptin by specific adipose tissue depots (Tomimatsu et al., 1997), due to increased fat deposition during pregnancy, since leptin correlates positively with body fat levels (Lage et al., 1999).

Secondly, there is increased synthesis by the placenta; or there is increased release of the soluble leptin receptor (Ob-Re) by the placenta (Gavrilova et al., 1997), since the placenta has been shown to express both the leptin and leptin receptor genes (Masuzaki et al., 1997; Hoggard et al., 1998).This means that, in this present study, the observed attenuated difference in leptin levels between the non-pregnant and the first trimester pregnant women could be due to the fact that in early gestation, the placenta has not been fully formed (Girling, 2004) to produce sufficient amounts of leptin to supplement the quantities produced by the adipose tissue.

Additionally, since pregnancy is a state of physiological insulin resistance (Catalano et al., 1999), and leptin has been found to rise in insulin resistant states (Silha et al., 2003; Shimomura et al., 1999), the pregnancy-associated increases in maternal plasma leptin may result from an up-regulation of adipocyte-leptin synthesis in the presence of increasing insulin resistance and hyperinsulinaemia with advancing gestational age (Laivuori et al., 2006).

Finally, since the skeletal system provides mechanical support for the body (Guyton, 2009), and leptin increases cortical bone mass (Hamrick et al., 2008), it has been proposed that a rise in leptin levels during weight gain, as pregnancy advances, may represent a mechanism

for enlarging bone size to cope with the overall increased body weight of the mother (Hamrick et al., 2008).

5.8 ADIPONECTIN-LEPTIN RATIO DURING PREGNANCY

It was observed that the adiponectin-leptin ratio correlated inversely with gestational age (Table 4.10), and so decreased from the first trimester to the third trimester (Table 4.3). It was highest in the first trimester, higher in the second trimester and high in the third trimester; and, although this ratio was significantly lower in the second and third trimester pregnant women, as compared to the non-pregnant women, it did not differ between the non-pregnant women and the first trimester pregnant women (Table 4.4).

This observation is in line with the observation made by Skvarca et al., (2013). According to Skvarca et al., (2013), decreased adiponectin-leptin ratio is a reflection of increase in insulin resistance, and that the adiponectin-leptin ratio can be used as a marker of insulin resistance. Therefore, the observed decrease in adiponectin-leptin ratio from the first trimester to the third trimester indicates that, in normal pregnancy, there is a physiological increase in insulin resistance from the first trimester to the third trimester; and the observed blunted difference of the adiponectin-leptin ratio between the non-pregnant and the first trimester pregnant women indicates that the pregnancy-specific insulin resistance had not taken effect in the first trimester.

In comparing the normal third trimester pregnant women to the preeclamptic women, it was observed that the adiponectin-leptin ratio in the preeclamptic women was twice the value in the normal third trimester pregnant women (Table 4.4). This means that during preeclampsia, the physiological mechanisms that underlie the secretion and circulation of adiponectin and leptin becomes altered, and that a transient increase in adiponectin-leptin ratio (which is a deviation from the normal trend) with advancing gestation could be an indicator for the occurrence of preeclampsia (Steegers et al., 2010; Al-Jameil et al., 2014).

5.9. MATERNAL SERUM LEVELS OF ADIPONECTIN IN PREECLAMPSIA

As investigations of whether adiponectin levels are increased, decreased or stagnated in preeclampsia have produced conflicting results (Hendler et al., 2005; Davis et al., 2007; Khosrowbeygi et al., 2009; Mazaki-Tovi et al., 2009, Ouyang et al., 2009; Abd-Alaleem et al., 2011), this present study sought to compare the levels of adiponectin in normal pregnancy and preeclampsia and to confirm the reported increase, decrease or stagnation in the levels of adiponectin in preeclampsia. The data of this study show that adiponectin levels are significantly elevated in preeclampsia as compared to normal pregnancy, with the preeclamptic levels being approximately three times the normal pregnancy levels (Table 4.4). The studies conducted by Davis et al., (2007), Khosrowbeygi et al., (2009) and Abd-Alaleem et al., (2011) are in consonance with the findings of this present study. However, studies conducted by Hendler et al., (2005), Mazaki-Tovi et al., (2009) and Ouyang et al., (2009) oppose the findings of this present study. While Hendler et al., (2005) observed that the levels of adiponectin in normal pregnancy and preeclampsia are comparable, Mazaki-Tovi et al., (2009) and Ouyang et al., (2009) observed significantly lower levels of adiponectin in preeclampsia than in normal pregnancy. Several explanations

that possibly link higher levels of adiponectin to hypertension, proteinuria, inflammation and endothelial dysfunction, which are all characteristics of preeclampsia

(Steegers et al., 2010; Al-Jameil, 2014) could be made.

Overproduction of angiotensin II of the Renin Angiotensin System (RAS) is linked to hypertension (Stroth et al., 1999), and since it has been found that adiponectin antagonizes the production of angiotensin II (Kintscher et al., 2005), it could be stated that hyperadiponectinaemia during preeclampsia is a possible feedback mechanism to antagonize angiotensin II production so as to control hypertension (Ohashi et al., 2006). A study conducted by Sharma et al., (2008) revealed that the expression of adiponectin receptor 1(AdipoR1) in podocytes helps adiponectin to increase adenosine monophosphate kinase (AMPK) phosphorylation in podocytes to regulate kidney

function against proteinuria. These investigators then concluded that adiponectin has positive effects on the kidney, and that a fall in adiponectin levels may predispose to proteinuria, and a rise in adiponectin levels may be protective against proteinuria (Sharma et al., 2008). This indicates that elevated level of adiponectin is, probably, part of the body's mechanisms that act to check proteinuria which is characteristic of preeclampsia. This possible reason is supported by Hendler et al., (2005) who concluded that alterations in renal function and an ongoing adiponectin synthesis in adipose tissues might result in a rise in adiponectin levels.

According to Tan et al., (2004), adiponectin receptors are also expressed in human endothelial cells; so adiponectin binds to those receptors to regulate endothelial nitric

oxide synthase (eNOS) activity which results in increased NO production in human aortic endothelial cells to offset endothelial dysfunction (Ouchi et al., 2003). And since the endothelium plays a role in inflammation (Dudzinski et al., 2006), protecting it from dysfunctional effects will, in a way, control inflammatory processes. As posited by Robinson et al., (2011), adiponectin exerts an anti-inflammatory effect through activation of all of its receptors by having direct actions on inflammatory cells and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and interacting with tumour necrosis factor alpha (TNF-α) (Robinson et al., 2011). This, therefore, indicates that hyperadiponectinaemia, during preeclampsia, is a step to restoring the integrity of the endothelium, and counteracting the inflammatory processes. All these positive feedback reactions of increasing adiponectin levels to annul the pathology, then possibly result in adiponectin resistance (Kajantie et al., 2005).

The physiological profile of adiponectin shows that adiponectin levels fall as pregnancy advances (Figure 4.1). And with all the relevant roles that adiponectin has been reviewed to play against hypertension (Kintscher et al., 2005), proteinuria (Sharma et al., 2008), inflammation (Robinson et al., 2011) and endothelial dysfunction (Cheng et al., 2007), which are all characteristics of preeclampsia, it could be proposed that an abnormal decrease in the levels of adiponectin precedes the development of preeclampsia (Mazaki-Tovi et al., 2009; Ouyang et al., 2009); and so a rise in the levels of adiponectin in preeclampsia, as observed in this study (Figure 4.2), could be part of the body's reaction of antagonizing the pathophysiological progression of preeclampsia. But if this reason is true, why will the body not proactively protect itself from preeclampsia by increasing adiponectin levels, but would rather allow the condition to develop before reacting to it?

Interestingly, other investigators also have other explanations. While Ramsay et al., (2003) explained that the elevation of adiponectin levels in preeclampsia may be secondary to exaggerated non-specific adipocyte lipolysis or a physiological response to enhance fat utilization, Lu et al., (2006) believed that circulating adiponectin levels in preeclampsia might be due to a reduced degradation/elimination rather than an increased synthesis of this adipokine.

5.10. MATERNAL SERUM LEVELS OF LEPTIN IN PREECLAMPSIA

Similar to adiponectin, conflicting results have implicated leptin in the development of preeclampsia. While investigators like Kolyingit et al., (2004) and Mumtaze et al., (2008) reported that leptin levels are elevated in preeclampsia, as compared to normal pregnancy, others like Martinez-Abundis et al., (2000), Lalm et al., (2001) and Masuyama et al., (2010) reported decreased levels of leptin in preeclampsia, as compared to normal pregnancy.

The current study was carried out to ascertain the reported differences in leptin levels between normal pregnancy and preeclampsia, and to confirm whether leptin levels decrease or increase in preeclampsia. In this study, the levels of leptin were significantly higher in the preeclamptic women than in the normal pregnant women. It was also observed that leptin levels in the preeclamptic women were approximately three times the levels in the first trimester and twice the levels in the second trimester pregnant women (Table 4.3; Table 4.4). The report of this study is in concordance with the reports of Kolyingit et al., (2004) and Mumtaze et al., (2008), but in conflict with the reports of

Lalm et al., (2001) and Masuyama et al., (2010). A longitudinal study conducted by Kolyingit et al., in 2004 indicated that elevated plasma leptin levels seen in preeclampsia could be taken as a marker of placental hypoxia in severe preeclampsia. The same report was also made by Sattar et al., (1998) and Anim-Nyame et al., (2000). However, the findings of Martinez-Abundis et al., (2000) contradict the findings of this study. In their investigation, they observed that serum leptin levels are comparable between normal pregnancy and preeclampsia, and so concluded that leptin cannot be used as a biomarker for preeclampsia.

The positive correlation between leptin and blood pressure in normotensive individuals (Table 4.9; Table 4.10) and the increase in strength of this positive correlation in preeclampsia (Table 4.11) raise the thought that the pathophysiological progression of preeclampsia may involve hyperleptinaemia. The finding that angiotensin II stimulates leptin production (Kim et al., 2002), and that higher leptin levels can cause hypertension (Stenvinkel et al., 2000), proteinuria (Wolf et al., 2002), and endothelial dysfunction (Hall et al., 2010), together with the finding that leptin plays a vital role during inflammation (Fernandez-Riejos et al., 2010), may justify the higher levels of leptin seen in the preeclamptic women. Thus, unlike adiponectin, the rise of leptin levels during preeclampsia may not indicate a feedback mechanism to rectify the pathology but, rather, a pathophysiological mechanism to exacerbate the pathology.

5.11 THE PHYSIOLOGICAL PROFILES OF ADIPONECTIN AND LEPTIN, THE ADIPONECTIN-LEPTIN RATIO, AND THE DEVELOPMENT OF PREECLAMPSIA

Given the syndromic and mulitfactorial nature of preeclampsia, it is not yet possible to routinely predict the condition; and due to that, proactive medical care against the

development of preeclampsia is very difficult (Steegers et al., 2010; Al-Jameil, 2014). But since there is the need to overcome this challenge, this study sought to find out whether or not the physiological profile and ratios of adiponectin and leptin observed in normal pregnancy are also observed in preeclampsia. This will, in turn, help to understand whether or not the adiponectin-leptin ratio can serve as a biomarker for predicting preeclampsia.

This study reports that, during normal pregnancy, adiponectin levels fall from the first trimester to the third trimester while leptin levels rise from the first trimester to the third trimester, and that the adiponectin-leptin ratio decreases from the first trimester to the third trimester (Figure 4.1). Thus, in every normal pregnancy, these physiological variations are to be observed, since any large deviation in these profiles and ratios of adiponectin and leptin in pregnancy may be a disposition to a pathological condition like preeclampsia (Skvarca et al., 2013).

In the preeclamptic women, this study showed that the inverse relationship between adiponectin and leptin was altered. Rather than finding a significant inverse relationship between adiponectin and leptin in the preeclamptic women, this study found that there was no correlation between adiponectin and leptin (Table 4.11). It was observed that, as adiponectin levels in the preeclamptic women were about twice as high the levels in the first and second trimester pregnant women, and about thrice as high the levels in the normal third trimester pregnant women, leptin levels in the preeclamptic women were about thrice as high the levels in the first trimester pregnant women and about twice as high the levels

in the second trimester pregnant women (Table 4.3; Table 4.4). Also, it was observed that in the preeclamptic women, the adiponectin-leptin ratio rose to a level twice as high the level in the normal third trimester pregnant women (i.e. 100% increment) (Table 4.5). It could be inferred from these observations that a sharp rise in the adiponectin-leptin ratio at any gestational age (which is a deviation from the normal) may be predictive of preeclampsia, and about 100% rise may indicate a preeclamptic state.

5.12 MATERNAL WEIGHT GAIN DURING PREGNANCY CAN PREDISPOSE A WOMAN TO PREECLAMPSIA, THROUGH THE ALTERATIONS IN THE LEVELS OF ADIPONECTIN AND LEPTIN

It has been reported that excessive weight gain during pregnancy is a risk factor to developing preeclampsia (Owiredu et al., 2012); and even the results of this study support that report (Table 4.6), but since the pathophysiological mechanism by which weight gain results in preeclampsia has not been elucidated, this study – which has implicated abnormal alterations in the levels of adiponectin and leptin in the development of preeclampsia (Table 4.4) – sought to investigate whether or not maternal weight gain during pregnancy affects the levels of adiponectin and leptin.

Together with the studies conducted by investigators like Blache et al., (2005) and Coppola *et al.,* (2008), this study reports that body mass index correlates inversely with adiponectin and linearly with leptin (Table 4.9; Table 4.10); and that while the levels of adiponectin are higher in normal-weight women than in overweight women, the levels of leptin are lower in normal-weight women than in overweight women (Table 4.5). This means that maternal weight gain during pregnancy can decrease the maternal serum levels of adiponectin, increase the maternal serum levels of leptin, and then result in a change in

the normality of the adiponectin-leptin ratio. Since it has been observed in this study that adiponectin and leptin may play roles during preeclampsia, it could be posited that abnormal deviations in the adiponectin-leptin ratio during pregnancy can influence the occurrence of preeclampsia. Thus, the pathophysiological mechanism by which weight-gain, during pregnancy, exposes a pregnant woman to preeclampsia, may involve adiponectin and leptin.

5.13 GRAVIDITY AND PARITY; POSSIBLE RISK FACTORS OF PREECLAMPSIA

Several epidemiological studies have posited that the gravidity and parity statuses of women are possible determinants of preeclampsia. These investigators have suggested that preeclampsia commonly occurs in first pregnancies than in subsequent pregnancies (LeLorier et al., 1997; Serhal et al., 2003). According to LeLorier et al., (1997), preeclampsia is more prevalent in primiparous women than in multiparous women; and according to Serhal et al., (2003), preeclampsia is more prevalent in nulliparous women than in multiparous women, and complicates 25-30% of nulliparous pregnancies (Duckitt et al., 2005). However, in the studies conducted by Owiredu et al., (2012) and Ephraim et al., (2014), first time pregnancies did not emerge as risk factors of preeclampsia.

This study reports that first time pregnancy is a risk of developing preeclampsia. With regard to the gravidity status, it was observed, in this study, that primigravida pregnant women are at increased risk of developing preeclampsia than multigravida pregnant women; and with the parity status, it was observed that nulliparous pregnant women are at

increased risk of developing preeclampsia than multiparous pregnant women, but at comparable risk with primiparous pregnant women at developing preeclampsia. Also, it was observed that primiparous pregnant women and multiparous pregnant women are at comparable risk of developing preeclampsia (Table 4.6).

These results concur with what the previous investigators have reported. Due to these observations, the first pregnancy of every woman could expose her to preeclampsia than subsequent pregnancies (Duckitt et al., 2005). But as to why first time pregnancies are at a higher risk of developing preeclampsia, than successive pregnancies, has not been clarified, even though several hypotheses exist. In conducting this study, it was hypothesized that the bodies of women who have given birth before may be experiencing some unique biochemical changes that confer on them a protective advantage against preeclampsia. And since adiponectin and leptin have been factually implicated in preeclampsia, as observed in this study (Table 4.5; Figure 4.2), as well as studies conducted elsewhere (Masuyama et al., 2010; Abd-Alaleem et al., 2011), it became imperative to compare the levels of the two adipokines among the participants on the bases of gravidity and parity.

The comparable levels of adiponectin among the participants on the basis of gravidity and parity, the comparable levels of leptin among the participants on the basis of gravidity and parity, and the comparable levels of the adiponectin-leptin ratio among the participants on the basis of gravidity and parity (Table 4.7; Table 4.8) indicate that the alterations in the levels of adiponectin and leptin that occur during pregnancy, probably, do not persist after delivery, but return to the pre-pregnancy levels (Moynihan et al., 2006). Hence, whether or not a woman has been pregnant before does not have any effect on her serum levels of adiponectin and leptin. This indicates that the higher risk of preeclampsia associated with

first pregnancies may not be influenced by the pre- pregnancy levels of adiponectin and leptin. It is the immunological tolerance theory

(which posits that in the first pregnancy, the mother's immune system may be reacting to the partner's genes in the foetus and the placenta) that may account for the higher risk of first pregnancies to developing preeclampsia (Davis et al., 2006).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Among normal non-pregnant and normal pregnant women, adiponectin and leptin exhibit an inverse relationship, and relate differently to body mass index, systolic blood pressure and diastolic blood pressure. While adiponectin relates inversely with body mass index, systolic blood pressure and diastolic blood pressure, leptin relates linearly with these parameters.

The inverse relationship between adiponectin and body mass index, the linear relationship between leptin and body mass index, and the linear relationship between leptin and systolic blood pressure and diastolic blood pressure are not altered during preeclampsia. However, the inverse relationship between adiponectin and leptin, as well as the inverse relationship

between adiponectin and systolic blood pressure and diastolic blood pressure, is attenuated and blunted during preeclampsia.

Normal pregnancy is characterized by a progressive fall in adiponectin levels and a progressive rise in leptin levels, with the adiponectin-leptin ratio decreasing proportionally with advancing gestation. These observed physiological profiles of adiponectin, leptin and the adiponectin-leptin ratio, which denote possible roles of adiponectin and leptin in pregnancy, are to be monitored in every pregnancy, since large deviations in these profiles and ratios could be an indication of a pregnancy complication.

Adiponectin, leptin and the adiponectin-leptin ratio are elevated in preeclampsia, as compared to normal pregnancy, thus indicating that they could serve as biomarkers for preeclampsia, as well as therapeutic targets in treating preeclampsia.

Weight gain and first time pregnancies are risk factors of preeclampsia. Adiponectin and leptin levels differ between normal weight and overweight women. This indicates that the pathophysiological mechanism linking maternal weight gain to preeclampsia may involve adiponectin and leptin. Adiponectin and leptin levels are not different between women who have given birth before and women who have had successive conceptions. This means that the pre-pregnancy levels of adiponectin and leptin may not be implicated in the higher risk of preeclampsia in first time pregnancies.

6.2 Recommendations

Prospective studies are to explain the high prevalence of preeclampsia in first time pregnancies, describe the pathophysiological mechanism linking maternal weight gain to preeclampsia, investigate the possibility of adiponectin resistance in preeclampsia, and establish the baseline levels of adiponectin, leptin and the adiponectin-leptin ratio that could be used in diagnosing preeclampsia.

REFERENCES

Abd-Alaleem D, Attiaa K, Khalefa A, Ahmad R (2011). Adiponectin levels in serum of women with pre-eclampsia. EMHJ **17**(7).

Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y (2003). Decreased plasma adiponectin concentration in patients with essential hypertension. American Journal of Hypertension **16**(1)**:** 72-75.

Ahima RS (2008). Revisiting leptin's role in obesity and weight loss. The Journal of Clinical Investigation **118**(7)**:** 2380-2383.

Ahima RS, Flier JS (2000). Leptin. Annual Review of Physiology **62**(1)**:** 413-437.

Ajuwon KM, Spurlock ME (2005). Adiponectin inhibits LPS-induced NF-κB activation and IL-6 production and increases PPARγ2 expression in adipocytes. American

Journal of Physiology-Regulatory, Integrative and Comparative Physiology **288**(5)**:** R1220- R1225.

Akpinar E, Bashan I, Bozdemir N, Saatci E (2007). Which is the best anthropometric technique to identify obesity: Body mass index, waist circumference or waist-hip ratio? Collegium Antropologicum **31**(2)**:** 387-393.

Al-Jameil N, Khan FA, Khan MF, Tabassum H (2014). A brief overview of preeclampsia. Journal of Clinical Medicine Research **6**(1)**:** 123-126.

Anderson PD, Mehta NN, Wolfe ML, Hinkle CC, Pruscino L, Comiskey LL, et al. (2007). Innate immunity modulates adipokines in humans. The Journal of Clinical Endocrinology & Metabolism **92**(6)**:** 2272-2279.

Anifandis G, Koutselini E, Louridas K, Liakopoulos V, Leivaditis K, Mantzavinos T, et al. (2005). Estradiol and leptin as conditional prognostic IVF markers. Reproduction **129**(4)**:** 531-534.

Anim-Nyame S, Romero R, Vaisbuch E, Kusanovic JP, Erez O, Gotsch F, et al. (2000). Maternal serum leptin in preeclampsia. Journal of Perinatal Medicine **37**(4)**:** 349-363.

Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, et al. (2002). Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB–binding protein and regulates growth factor–induced common postreceptor signal in vascular smooth muscle cell. Circulation **105**(24)**:** 2893-2898.

Arulkumaran N, Lightstone L (2013). Severe pre-eclampsia and hypertensive crises. Best Practice & Research Clinical Obstetrics & Gynaecology **27**(6)**:** 877-884.

Baicy K, London ED, Monterosso J, Wong M-L, Delibasi T, Sharma A, et al. (2007). Leptin replacement alters brain response to food cues in genetically leptin-deficient adults. Proceedings of the National Academy of Sciences **104**(46)**:** 18276-18279.

Baker JL, Olsen LW, Sørensen TI (2007). Childhood body-mass index and the risk of coronary heart disease in adulthood. New England Journal of Medicine **357**(23)**:** 2329-2337.

Banks WA, Farr SA, Morley JE (2006). The effects of high fat diets on the blood–brain barrier transport of leptin: Failure or adaptation? Physiology & Behavior **88**(3)**:** 244-248.

Berg AH, Combs TP, Scherer PE (2002). ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in Endocrinology & Metabolism **13**(2)**:** 84-89.

Bernotiene E, Palmer G, Gabay C (2006). The role of leptin in innate and adaptive immune responses. Arthritis Research and Therapy **8**(5)**:** 217.

Bjorbaek C, Kahn BB (2004). Leptin signaling in the central nervous system and the periphery. Recent progress in Hormone Research **59:** 305-332.

Blache D, Tellam R, Chagas L, Blackberry M, Vercoe P, Martin G (2000). Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. Journal of Endocrinology **165**(3)**:** 625-637.

Bodner J, Ebenbichler C, Wolf H, Müller-Holzner E, Stanzl U, Gander R, et al. (1999). Leptin receptor in human term placenta: in situ hybridization and immunohistochemical localization. Placenta **20**(8)**:** 677-682.

Bokarewa M, Bokarew D, Hultgren O, Tarkowski A (2003). Leptin consumption in the inflamed joints of patients with rheumatoid arthritis. Annals of the Rheumatic Diseases **62**(10)**:** 952-956.

Bolton V(2009). The skeletal system in focus. Osteology **23**(6): 124-128.

Bonnard C, Durand A, Vidal H, Rieusset J (2008). Changes in adiponectin, its receptors and AMPK activity in tissues of diet-induced diabetic mice. Diabetes & Metabolism **34**(1)**:** 52-61.

Bonney EA (2007). Preeclampsia: a view through the danger model. Journal of Reproductive Immunology **76**(1)**:** 68-74.

Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrao A, Gold P, et al. (1998). Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal

rhythm in cortisol and leptin secretion. The Journal of Clinical Endocrinology & Metabolism **83**(1)**:** 280-283.

Borton, Chloe (2009). Gravidity and Parity definitions (and their implications in risk assessment. Pregnancy **10**(3): 321-333.

Boulomie A, Marumo T, Lafontan M, Busser R(1999). Leptin induces oxidative stress in human endothelial cells. The FASEB Journal **13**(10)**:** 1231-1238.

Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, et al. (2003). Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. American Journal of Physiology-Endocrinology and Metabolism **285**(3)**:** E527-E533.

Buchanan TA, Metzger BE, Freinkel N, Bergman RN (1990). Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. American journal of Obstetrics and Gynecology **162**(4)**:** 1008-1014.

Busua C, Makuta Z, Poyt B (2003). Alterations in the levels of adiponectin during pregnancy. European Journal of Endocrinology **23**(1):26-29.

Çakır B, Bozkurt A, Ercan F, Yeğen BÇ (2004). The anti-inflammatory effect of leptin on experimental colitis: involvement of endogenous glucocorticoids. Peptides **25**(1)**:** 95- 104.

Campbell LA, Klocke RA (2001). Implications for the pregnant patient. American Journal of Respiratory and Critical Care Medicine **163**(5)**:** 1051-1054.

Cannon B, Nedergaard J (2008). Developmental biology: neither fat nor flesh. Nature **454**(7207)**:** 947-948.

Catalano PM, Huston L, Amini SB, Kalhan SC (1999). Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. American Journal of Obstetrics and Gynecology **180**(4)**:** 903-916.

Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EA (1991). Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. American Journal of Obstetrics and Gynecology **165**(6)**:** 1667-1672.

Catalano P et al., (2006). Adiponectin in human pregnancy: Implications for regulation of glucose and lipid metabolism. Diabetologia **49**(7):1677-1685.

Ceddia RB, Koistinen HA, Zierath JR, Sweeney G (2002). Analysis of paradoxical observations on the association between leptin and insulin resistance. The FASEB Journal **16**(10)**:** 1163-1176.

Challacombe S (1987). Investigation of the secretory and systemic immune responses to ingested materials in animal models. Immunology of the Gastrointestinal Tract **2:** 99-124.

Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ (2003). Adiponectin stimulates production of nitric oxide in vascular endothelial cells. Journal of Biological Chemistry **278**(45)**:** 45021-45026.

Chen J, Tan B, Karteris E, Zervou S, Digby J, Hillhouse EW, et al. (2006). Secretion of adiponectin by human placenta: differential modulation of adiponectin and its receptors by cytokines. Diabetologia **49**(6)**:** 1292-1302.

Cheng KK, Lam KS, Wang Y, Huang Y, Carling D, Wu D, et al. (2007). Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. Diabetes **56**(5)**:** 1387-1394.

Chin-Chance C, Polonsky KS, Schoeller DA (2000). Twenty-Four-Hour Leptin Levels Respond to Cumulative Short-Term Energy Imbalance and Predict Subsequent Intake 1. The Journal of Clinical Endocrinology & Metabolism **85**(8)**:** 2685-2691.

Chow W-S, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, et al. (2007). Hypoadiponectinemia as a Predictor for the Development of Hypertension A 5-Year Prospective Study. Hypertension **49**(6)**:** 1455-1461.

Cnop M, Havel P, Utzschneider K, Carr D, Sinha M, Boyko E, et al. (2003). Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia **46**(4)**:** 459-469.

Conde-Agudelo A, Belizán JM (2000). Risk factors for pre-eclampsia in a large cohort of Latin American and Caribbean women. BJOG: An International Journal of Obstetrics & Gynaecology **107**(1)**:** 75-83.

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. New England Journal of Medicine **334**(5)**:** 292-295.

Coppola A, Marfella R, Coppola L, Tagliamonte E, Fontana D, Liguori E, et al. (2009). Effect of weight loss on coronary circulation and adiponectin levels in obese women. International Journal of Cardiology **134**(3)**:** 414-416.

Cox S, Werner C, Hoffman B (2005). Williams Obstetrics 22nd Edition Study Guide. edn. McGraw-Hill Professional.

Cunningham F (2005). Williams obstetrics, 22nd edition, pp 34-80, Mcgraw-hill companies.

Dalamaga M, Srinivas SK, Elovitz MA, Chamberland J, Mantzoros CS (2011). Serum adiponectin and leptin in relation to risk for preeclampsia: results from a large case- control study. Metabolism **60**(11)**:** 1539-1544.

Daniels SR, Khoury PR, Morrison JA (1997). The utility of Body mass index as a measure of body fatness in children and adolescents: differences by race and gender. Pediatrics **99**(6)**:** 804-807.

Davis JA, Gallup GG (2006). Female infidelity and paternal uncertainty. pp 191.

Delavaud C, Bocquier F, Chilliard Y, Keisler D, Gertler A, Kann G (2000). Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. Journal of Endocrinology **165**(2)**:** 519-526.

Deurenberg P, Yap M, Van Staveren WA (1998). Body mass index and percent body fat: a meta analysis among different ethnic groups. International Journal of Obesity **22:** 1164-1171.

Dubuc GR, Phinney SD, Stern JS, Havel PJ (1998). Changes of serum leptin and endocrine and metabolic parameters after 7 days of energy restriction in men and women. Metabolism **47**(4)**:** 429-434.

Duckitt K, Harrington D (2005). Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. Bmj **330**(7491)**:** 565.

Dudzinski DM, Igarashi J, Greif D, Michel T (2006). The regulation and pharmacology of endothelial nitric oxide synthase. Annu. Rev. Pharmacol. Toxicol. **46:** 235-276.

Eiland E, Nzerue C, Faulkner M (2012). Preeclampsia 2012. Journal of Pregnancy **2012**.

Einarsson JI, Sangi-Haghpeykar H, Gardner MO (2003). Sperm exposure and development of preeclampsia. American Journal of Obstetrics and Gynecology **188**(5)**:** 1241-1243.

Eknoyan G (2008). Adolphe Quetelet (1796–1874)—the average man and indices of obesity. Nephrology Dialysis Transplantation **23**(1)**:** 47-51.

Enkin M, Keirse MJ, Chalmers I, Enkin E (2000). A guide to effective care in pregnancy and childbirth. edn, vol. 525. Oxford university press Oxford.

Fang X, Sweeney G (2006). Mechanisms regulating energy metabolism by adiponectin in obesity and diabetes. Biochemical Society Transactions **34**(Pt 5)**:** 798-801.

Fantuzzi G (2005). Adipose tissue, adipokines, and inflammation. Journal of Allergy and Clinical Immunology **115**(5)**:** 911-919.

Fantuzzi G, Faggioni R (2000). Leptin in the regulation of immunity, inflammation, and hematopoiesis. Journal of Leukocyte Biology **68**(4)**:** 437-446.

Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, et al. (2007). Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. New England Journal of Medicine **356**(3)**:** 237-247.

Fernández-Riejos P, Najib S, Santos-Alvarez J, Martín-Romero C, Pérez-Pérez A, González-Yanes C, et al. (2010). Role of leptin in the activation of immune cells. Mediators of Inflammation **2010**.

Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, et al. (2010). Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice." **98**:2005–2010.

Fulsang C, Pattas C, Bofra C (2006). Impact of pregnancy on maternal serum levels of adiponectin. Nature **67**(5) 235-239.

Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, et al. (2003). Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. Hypertension **42**(1)**:** 76-81.

Galic S, Oakhill JS, Steinberg GR (2010). Adipose tissue as an endocrine organ. Molecular and cellular endocrinology **316**(2)**:** 129-139.

Gavrilova O, Barr V, Marcus-Samuels B, Reitman M (1997). Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. Journal of Biological Chemistry **272**(48)**:** 30546-30551.

Gerard KW (1993). Physiology of pregnancy. Current Opinion in Anesthesiology **6**(3)**:** 446-451.

Gilbert CA, Slingerland JM (2013). Cytokines, obesity, and cancer: new insights on mechanisms linking obesity to cancer risk and progression. Annual Review of Medicine **64:** 45-57.

Girling JC (2004). Physiology of pregnancy. Anaesthesia & Intensive Care Medicine **5**(7)**:** 215-218.

Hall JE, da Silva AA, do Carmo JM, Dubinion J, Hamza S, Munusamy S, et al. (2010). Obesity-induced hypertension: role of sympathetic nervous system, leptin, and melanocortins. Journal of Biological Chemistry **285**(23)**:** 17271-17276.

Hamilton BS, Paglia D, Kwan AY, Deitel M (1995). Increased obese mRNA expression in omental fat cells from massively obese humans. Nature Medicine **1**(9)**:** 953-956.

Hamrick M, Ferrari SL (2008). Leptin and the sympathetic connection of fat to bone. Osteoporosis International **19**(7)**:** 905-912.

Hardie L, Trayhurn P, Abramovich D, Fowler P (1997). Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clinical Endocrinology **47**(1)**:** 101-106.

Hattori Y, Suzuki M, Hattori S, Kasai K (2003). Globular adiponectin upregulates nitric oxide production in vascular endothelial cells. Diabetologia **46**(11)**:** 1543-1549.

Havel PJ (2002). Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. Current Opinion in Lipidology **13**(1)**:** 51-59.

Havel PJ, Kasim-Karakas S, Mueller W, Johnson P, Gingerich R, Stern J (1996). Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. The Journal of Clinical Endocrinology & Metabolism **81**(12)**:** 4406-4413.

Hekerman P, Zeidler J, Korfmacher S, Bamberg-Lemper S, Knobelspies H, Zabeau L, et al. (2007). Leptin induces inflammation-related genes in RINm5F insulinoma cells. BMC Molecular Biology **8**(1)**:** 41.

Hendler I, Blackwell SC, Mehta SH, Whitty JE, Russell E, Sorokin Y, et al. (2005). The levels of leptin, adiponectin, and resistin in normal weight, overweight, and obese pregnant

women with and without preeclampsia. American Journal of Obstetrics and Gynecology **193**(3)**:** 979-983.

Hernandez-Valencia M, Saldaña QL, Alvarez MM, Valdez ME (2000). [Barrier family planning methods as risk factor which predisposes to preeclampsia]. Ginecologia y Obstetricia de Mexico **68:** 333-338.

Herse F, Youpeng B, Staff AC, Yong-Meid J, Dechend R, Rong Z (2009). Circulating and uteroplacental adipocytokine concentrations in preeclampsia. Reproductive Sciences **16**(6)**:** 584-590.

Hileman SM, Tornøe J, Flier JS, Bjørbæk C (2000). Transcellular Transport of Leptin by the Short Leptin Receptor Isoform ObRa in Madin-Darby Canine Kidney Cells 1. Endocrinology **141**(6)**:** 1955-1961.

Hoggard N, Hunter L, Duncan JS, Williams LM, Trayhurn P, Mercer JG (1997). Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. Proceedings of the National Academy of Sciences **94**(20)**:** 11073-11078.

Hoggard N, Hunter L, Trayhurn P, Williams LM, Mercer JG (1998). Leptin and reproduction. Proceedings of the Nutrition Society **57**(03)**:** 421-427.

Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, et al. (2001). Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes **50**(5)**:** 1126-1133.

Hu E, Liang P, Spiegelman BM (1996). AdipoQ is a novel adipose-specific gene dysregulated in obesity. Journal of Biological Chemistry **271**(18)**:** 10697-10703.

Hug C, Wang J, Ahmad NS, Bogan JS, Tsao T-S, Lodish HF (2004). T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proceedings of the National Academy of Sciences of the United States of America **101**(28)**:** 10308-10313.

Hytten F, Chamberlain G (1980). Clinical physiology in obstetrics. edn. Blackwell Scientific Publications.

Hytten FE, Leitch I (1964). The physiology of human pregnancy. The physiology of human pregnancy.

Iikuni N, Lam QLK, Lu L, Matarese G, La Cava A (2008). Leptin and inflammation. Current Immunology Reviews **4**(2)**:** 70.

Ingalls AM, Dickie MM, Shell G (1950). Obese, a new mutation in the house mouse. Journal of Heredity **41:** 317-318.

Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, et al. (2004). Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension **43**(6)**:** 1318-1323.

Jürimäe J, Purge P, Jürimäe T (2005). Adiponectin is altered after maximal exercise in highly trained male rowers. European Journal of Applied Physiology **93**(4)**:** 502-505.

Kajantie P, Okumo D, Brafat V (2005). Serum adiponectin in hypertensive subjects. American Journal of hypertension **23**(4):123-140.

Kazumi T, Kawaguchi A, Sakai K, Hirano T, Yoshino G (2002). Young men with high-normal blood pressurehave lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. Diabetes care **25**(6)**:** 971-976.

Keller P, Møller K, Krabbe K, Pedersen B (2003). Circulating adiponectin levels during human endotoxaemia. Clinical & Experimental Immunology **134**(1)**:** 107-110.

Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, et al. (1997). The Metabolic Significance of Leptin in Humans: Gender-Based Differences in Relationship to Adiposity, Insulin Sensitivity, and Energy Expenditure 1. The Journal of Clinical Endocrinology & Metabolism **82**(4)**:** 1293-1300.

Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G (2003). Adiponectin expression from human adipose tissue relation to obesity, insulin resistance, and tumor necrosis factor-α expression. Diabetes **52**(7)**:** 1779-1785.

Khosrowbeygi A, Ahmadvand H (2009). Maternal serum levels of adiponectin in preeclampsia. J Ayub Med Coll Abbottabad **21**(3).

Kim K-y, Kim JK, Han SH, Lim J-S, Kim KI, Cho DH, et al. (2006). Adiponectin is a negative regulator of NK cell cytotoxicity. The Journal of Immunology **176**(10)**:** 5958-5964.

King JC (2000). Physiology of pregnancy and nutrient metabolism. The American Journal of clinical Nutrition **71**(5)**:** 1218s-1225s.

Kintscher U, Unger T (2005). Vascular protection in diabetes: a pharmacological view of angiotensin II type 1 receptor blockers. Acta Diabetologica **42**(1)**:** s26-s32.

Kirkendall WM, Burton AC, Epstein FH, Freis ED (1967). Recommendations for human blood pressuredetermination by sphygmomanometers. Circulation **36**(6)**:** 980-988.

Koelman CA, Coumans AB, Nijman HW, Doxiadis II, Dekker GA, Claas FH (2000). Correlation between oral sex and a low incidence of preeclampsia: a role for soluble HLA in seminal fluid? Journal of Reproductive Immunology **46**(2)**:** 155-166.

Konturek P, Jaworek J, Maniatoglou A, Bonior J, Meixner H, Konturek S, et al. (2002). Leptin modulates the inflammatory response in acute pancreatitis. Digestion **65**(3)**:** 149-160.

Kovacova Z, Vitkova M, Kovacikova M, Klimcakova E, Bajzova M, Hnevkovska Z, et al. (2009). Secretion of adiponectin multimeric complexes from adipose tissue explants is not modified by very low calorie diet. European Journal of Endocrinology **160**(4)**:** 585- 592.

Kühl C (1998). Etiology and pathogenesis of gestational diabetes. Diabetes Care **21:** B19-26.

Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, et al. (2004). Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation **109**(17)**:** 2046-2049.

Kraku Q, Bonsa R, GrafT N (2006). Guidelines to building the body. Nutrition **5**(7)**:** 215-218..

Lage M, Garcia-Mayor RV, Tomé MA, Cordido F, Valle-Inclan F, Considine RV, et al. (1999). Serum leptin levels in women throughout pregnancy and the postpartum period and in women suffering spontaneous abortion. Clinical Endocrinology **50**(2)**:** 211-216.

Laivuori H, et al., (2006). Relationships between the maternal plasma leptin, placental leptin mRNA and protein in normal pregnancy, preeclampsia and intrauterine growth restriction without preeclampsia. Moleclar Human Reproduction **12**(9):551-556.

Lara-Castro C, Fu Y, Chung BH, Garvey WT (2007). Adiponectin and the metabolic syndrome: mechanisms mediating risk for metabolic and cardiovascular disease. Current Opinion in Lipidology **18**(3)**:** 263-270.

Lea R, Howe D, Hannah L, Bonneau O, Hunter L, Hoggard N (2000). Placental leptin in normal, diabetic and fetal growth-retarded pregnancies. Molecular Human Reproduction **6**(8)**:** 763-769.

Lecke SB, Morsch DM, Spritzer PM (2011). Leptin and adiponectin in the female life course. Brazilian Journal of Medical and Biological Research **44**(5)**:** 381-387.

LeLorier, J, Gregoire, G, Benhaddad, A, Lapierre, J, Derderian, F (1997). Discrepancies between meta-analyses and subsequent large randomized, controlled trials. N Engl J Med **337**(8)**:** 536-542.

Lewandowski K, Horn R, O'Callaghan C, Dunlop D, Medley G, O'Hare P, et al. (1999). Free leptin, bound leptin, and soluble leptin receptor in normal and diabetic pregnancies. The Journal of Clinical Endocrinology & Metabolism **84**(1)**:** 300-306.

Lie RT, Rasmussen S, Brunborg H, Gjessing HK, Lie-Nielsen E, Irgens LM (1998). Fetal and maternal contributions to risk of pre-eclampsia: population based study. Bmj **316**(7141)**:** 1343.

Lieb W, Beiser AS, Vasan RS, Tan ZS, Au R, Harris TB, et al. (2009). Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. Jama **302**(23)**:** 2565-2572.

Lisonkova S, Joseph K (2013). Incidence of preeclampsia: risk factors and outcomes associated with early-versus late-onset disease. American Journal of Obstetrics and Gynecology **209**(6)**:** 544. e541-544. e512.

Low R, Puta V, Grehb X (2009). Serum adiponectin and resisting in pregnant women. European Journal of Endocrinology **24**(6):234-237.

Lu D, Yang X, Wu Y, Wang H, Huang H, Dong M (2006). Serum adiponectin, leptin and soluble leptin receptor in pre-eclampsia. International Journal of Gynecology & Obstetrics **95**(2)**:** 121-126.

Mabuchi T, Yatsuya H, Tamakoshi K, Otsuka R, Nagasawa N, Zhang H, et al. (2005). Association between serum leptin concentration and white blood cell count in middle-aged Japanese men and women. Diabetes/metabolism Research and Reviews **21**(5)**:** 441-447.

Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K (1996). cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPoseMost abundant Gene transcript 1). Biochemical and Biophysical Research Communications **221**(2)**:** 286-289.

Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. (2002). Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nature Medicine **8**(7)**:** 731-737.

Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. (2001). PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes **50**(9)**:** 2094-2099.

Magalang UJ, Rajappan R, Hunter MG, Kutala VK, Kuppusamy P, Wewers MD, et al. (2006). Adiponectin inhibits superoxide generation by human neutrophils. Antioxidants & Redox Signaling **8**(11-12)**:** 2179-2186.

Malendowicz W, Rucinski M, Macchi C, Spinazzi R, Ziolkowska A, Nussdorfer GG, et al. (2006). Leptin and leptin receptors in the prostate and seminal vesicles of the adult rat. International Journal of Molecular Medicine **18**(4)**:** 615-618.

Margetic S, Gazzola C, Pegg G, Hill R (2002). Leptin: a review of its peripheral actions and interactions. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity **26**(11)**:** 1407-1433.

Mark AL & Sivitz WI (2002). Uncoupling metabolism and coupling leptin to cardiovascular disease. Arteriosclerosis, Thrombosis and Vascular Biology **22:** 881–883

Mars M, De Graaf C, De Groot C, Van Rossum C, Kok F (2005). Fasting leptin and appetite responses induced by a 4-day 65%-energy-restricted diet. International Journal of Obesity **30**(1)**:** 122-128.

Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, et al. (1997). Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nature Medicine **3**(9)**:** 1029-1033.

Matsubara M, Maruoka S, Katayose S (2002). Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. European Journal of Endocrinology **147**: 173–180.

Matsuzawa Y, Funahashi T, Kihara S, Shimomura I (2004). Adiponectin and metabolic syndrome. Arteriosclerosis, Thrombosis, and Vascular Biology **24**(1)**:** 29-33.

Matsuzawa Y, Funahashi T, Nakamura T (1999). Molecular Mechanism of Metabolic Syndrome X: Contribution of Adipocytokines· Adipocyte-derived Bioactive Substances. Annals of the New York Academy of Sciences **892**(1)**:** 146-154.

Mazaki-Tovi S, Kanety H, Pariente C, Hemi R, Wiser A, Schiff E, et al. (2007). Maternal serum adiponectin levels during human pregnancy. Journal of Perinatology **27**(2)**:** 77-81.

Mazaki-Tovi S, Romero R, Kusanovic J, Erez O, Vaisbuch E, Gotsch F, et al. (2008). Adiponectin multimers in maternal plasma. Journal of Maternal-Fetal and Neonatal Medicine **21**(11)**:** 796-815.

Mazaki-Tovi S, Romero R, Vaisbuch E, Kusanovic JP, Erez O, Gotsch F, et al. (2009). Maternal serum adiponectin multimers in preeclampsia. Journal of Perinatal Medicine **37**(4)**:** 349-363.

McCarthy JF, Misra DN, Roberts JM (1999). Maternal plasma leptin is increased in preeclampsia and positively correlates with fetal cord concentration. American Journal of Obstetrics and Gynecology **180**(3)**:** 731-736.

McLachlan KA, O'Neal D, Jenkins A, Alford FP (2006). Do adiponectin, TNFalpha, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with and without gestational diabetes, during and after pregnancy. Clinical Endocrinology **22**:131–138.

Miehle K, Stepan H, Fasshauer M (2012). Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. Clinical Endocrinology **76**(1)**:** 2-11.

Moynihan AT, Hehir MP, Glavey SV, Smith TJ, Morrison JJ (2006). Inhibitory effect of leptin on human uterine contractility in vitro. American Journal of Obstetrics and Gynecology **195**(2)**:** 504-509.

Myers MG, Cowley MA, Münzberg H (2008). Mechanisms of leptin action and leptin resistance. Annu. Rev. Physiol. **70:** 537-556.

Nakano Y, Tobe T, Choi-Miura N-H, Mazda T, Tomita M (1996). Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. Journal of Biochemistry **120**(4)**:** 803-812.

Naruse K, Yamasaki M, Umekage H, Sado T, Sakamoto Y, Morikawa H (2005). Peripheral blood concentrations of adiponectin, an adipocyte-specific plasma protein, in normal pregnancy and preeclampsia. Journal of Reproductive Immunology **65**(1)**:** 65-75.

Nedvidkova J, Smitka K, Kopsky V, Hainer V (2005). Adiponectin, an adipocyte-derived protein. Physiol Res **54**(2)**:** 133-140.

Neumeier M, Weigert J, Schäffler A, Wehrwein G, Müller-Ladner U, Schölmerich J, et al. (2006). Different effects of adiponectin isoforms in human monocytic cells. Journal of Leukocyte Biology **79**(4)**:** 803-808.

Nien JK, Mazaki-Tovi S, Romero R, Erez O, Kusanovic JP, Gotsch F, et al. (2007a). Adiponectin in severe preeclampsia. Journal of Perinatal Medicine **35**(6)**:** 503-512.

Nien JK, Mazaki-Tovi S, Romero R, Erez O, Kusanovic JP, Gotsch F, et al. (2007b). Plasma adiponectin concentrations in non-pregnant, normal and overweight pregnant women. Journal of Perinatal Medicine **35**(6)**:** 522-531.

Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, et al. (2006). Adiponectin replenishment ameliorates obesity-related hypertension. Hypertension **47**(6)**:** 1108-1116.

Oken E, Kleinman KP, Belfort MB, Hammitt JK, Gillman MW (2009). Associations of gestational weight gain with short-and longer-term maternal and child health outcomes. American Journal of Epidemiology **170**(2)**:** 173-180.

Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH (2000). First trimester maternal serum free β human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. BJOG: An International Journal of Obstetrics & Gynaecology **107**(10)**:** 1265-1270.

Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, et al. (2001). Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation **103**(8)**:** 1057-1063.

Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, et al. (2003). Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension **42**(3)**:** 231-234.

Ouedraogo R, Gong Y, Berzins B, Wu X, Mahadev K, Hough K, et al. (2007). Adiponectin deficiency increases leukocyte-endothelium interactions via upregulation of endothelial cell adhesion molecules in vivo. The Journal of Clinical Investigation **117**(6)**:** 1718-1726.

Ouyang Y-Q, Li S-J, Zhang Q, Xiang W-P, Shen H-L, Chen H-P, et al. (2009). Plasma sFlt-1-to-PlGF ratio is correlated with inflammatory but not with oxidative stress in Chinese preeclamptic women. Archives of Gynecology and Obstetrics **280**(1)**:** 91-97.

Ouyang Y, Chen H, Chen H (2007). Reduced plasma adiponectin and elevated leptin in pre-eclampsia. International Journal of Gynecology & Obstetrics **98**(2)**:** 110-114.

Owiredu WKBA, Ahenkorah L, Turpin CA, Amidu N, Laing EF(2012). Putative risk factors of pregnancy-induced hypertension amongst Ghanaian pregnant women. Journal of Medical and Biomedical Science **1**(3): 62-76.

Pang TT, Narendran P (2008). The distribution of adiponectin receptors on human peripheral blood mononuclear cells. Annals of the New York Academy of Sciences **1150**(1)**:** 143-145.

Patel SB, Reams GP, Spear RM, Freeman RH, Villarreal D (2008). Leptin: linking obesity, the metabolic syndrome, and cardiovascular disease. Current Hypertension Reports **10**(2)**:** 131-137.

Pietrobelli A, Faith MS, Allison DB, Gallagher D, Chiumello G, Heymsfield SB (1998). Body mass index as a measure of adiposity among children and adolescents: a validation study. The Journal of Pediatrics **132**(2)**:** 204-210.

Pliato D, Praft G, Netu C, Boki Z (2010). Maternal serum adipokines among pregnant women at different stages of pregnancy. American Journal of Endocrinology **23**(4):222-227.

Poikkeus P, Hiilesmaa V, Tiitinen A (2002). Serum HCG 12 days after embryo transfer in predicting pregnancy outcome. Human Reproduction **17**(7)**:** 1901-1905.

Prins JB, O'rahilly S (1997). Regulation of adipose cell number in man. Clinical Science **92**(1)**:** 3-12.

Puhkala J, Kinnunen TI, Vasankari T, Kukkonen-Harjula K, Raitanen J, Luoto R (2013). Prevalence of metabolic syndrome one year after delivery in Finnish women at increased risk for gestational diabetes mellitus during pregnancy. Journal of Pregnancy **2013**.

Qasim SM, Callan C, Choe JK (1996). The predictive value of an initial serum β human chorionic gonadotropin level for pregnancy outcome following in vitro fertilization. Journal of Assisted Reproduction and Genetics **13**(9)**:** 705-708.

Ramsay JE, Jamieson N, Greer IA, Sattar N (2003). Paradoxical elevation in adiponectin concentrations in women with preeclampsia. Hypertension **42**(5)**:** 891-894.

Reaven GM (2005). Why Syndrome X? From Harold Himsworth to the insulin resistance syndrome. Cell Metabolism **1**(1)**:** 9-14.

Reaven GM (2007). The individual components of the metabolic syndrome: is there a raison d'etre? Journal of the American College of Nutrition **26**(3)**:** 191-195.

Rizza S, Gigli F, Galli A, Micchelini B, Lauro D, Lauro R, et al. (2010). Adiponectin isoforms in elderly patients with or without coronary artery disease. Journal of the American Geriatrics Society **58**(4)**:** 702-706.

Robinson K, Prins J, Venkatesh B (2011). Clinical review: adiponectin biology and its role in inflammation and critical illness. Crit Care **15**(2)**:** 221.

(2007). Role of placentally produced inflammatory and regulatory cytokines in pregnancy and the etiology of preeclampsia. Seminars in Immunopathology. Springer. pp 151-162.

Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, et al. (1997). Sexual Dimorphism in Plasma Leptin Concentration 1. The Journal of Clinical Endocrinology & Metabolism **82**(2)**:** 579-584.

Sargent IL, Borzychowski AM, Redman CW (2006). Immunoregulation in normal pregnancy and pre-eclampsia: an overview. Reproductive Biomedicine Online **13**(5)**:** 680-686.

Sattar, Naveed et al., (1998). Leptin levels in pregnancy, marker for fat accumulation and mobilisation. Acta Obstetricia et Gynaecologia 77(3):278-283.

Saunders TJ, Palombella A, McGuire KA, Janiszewski PM, Després J-P, Ross R (2012). Acute exercise increases adiponectin levels in abdominally obese men. Journal of Nutrition and Metabolism **2012**.

Savva S, Tornaritis M, Savva M, Kourides Y, Panagi A, Silikiotou N, et al. (2000). Waist circumference and waist-to-height ratio are better predictors of cardiovascular disease risk factors in children than Body mass index. International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity **24**(11)**:** 1453-1458.

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. Journal of Biological Chemistry **270**(45)**:** 26746-26749.

Schubring C, Englaro P, Siebler T, Blum W, Demirakca T, Kratzsch J, et al. (1999). Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to Body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. Hormone Research in Paediatrics **50**(5)**:** 276-283.

Searles CD (2006). Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression. American Journal of Physiology-Cell Physiology **291**(5)**:** C803-C816.

Serhal, PF, Craft, I (1987) Immune basis for pre-eclampsia evidence from oocyte recipients. Lancet **2**(8561)**:** 744.

Sharma K, RamachandraRao S, Qiu G, Usui HK, Zhu Y, Dunn SR, et al. (2008). Adiponectin regulates albuminuria and podocyte function in mice. The Journal of Clinical Investigation **118**(5)**:** 1645-1656.

Shell ER (2003). The hungry gene: the inside story of the obesity industry. edn. Grove Press.

Shifman O, Pett Y, Bug X, Moak B (2001). Fluctuations in adiponectin levels during pregnancy. European Journal of Endocrinology **22**(3): 123-125.

Shimizu H, Shimomura Y, Nakanishi Y, Futawatari a, Ohtani K, Sato N, et al. (1997). Estrogen increases in vivo leptin production in rats and human subjects. Journal of Endocrinology **154**(2)**:** 285-292.

Shimomura I, Funahashi T, Matsuzawa Y (2006). Metabolic syndrome, adiponectin and fat ROS. Biomedical Reviews **17:** 1-10.

Shimomura, Iichiro et al., (1999). Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature **401**(6748):73-76.

Sibai B, Dekker G, Kupferminc M (2005). Pre-eclampsia. The Lancet **365**(9461)**:** 785-799.

Siega-Riz AM, Viswanathan M, Moos M-K, Deierlein A, Mumford S, Knaack J, et al. (2009). A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. American Journal of Obstetrics and Gynecology **201**(4)**:** 339. e331-339. e314.

Silha, Josef et al., (2003). Plasma resistin, adiponectin and leptin in lean and obese subjects:Correlations with insulin resistance. European Journal of Endocrinology **149**(4):331-335.

Skcarva A et al., (2013). Adiponectin/leptin ratio and insulin resistance in pregnancy. Journal of International Medical Research. **41**(1):123-128.

Skjærven R, Wilcox AJ, Lie RT (2002). The interval between pregnancies and the risk of preeclampsia. New England Journal of Medicine **346**(1)**:** 33-38.

Spencer K, Yu CK, Cowans NJ, Otigbah C, Nicolaides KH (2005). Prediction of pregnancy complications by first-trimester maternal serum PAPP-A and free β-hCG and with second-trimester uterine artery Doppler. Prenatal Diagnosis **25**(10)**:** 949-953.

Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R (2010). Pre-eclampsia. The Lancet **376**(9741)**:** 631-644.

Stenvinkel P (2000). Leptin and blood pressure—is there a link? Nephrology Dialysis Transplantation **15**(8)**:** 1115-1117.

Stroth U, Unger T (1999). The renin-angiotensin system and its receptors. Journal of Cardiovascular Pharmacology **33:** S21-S28.

Suter PM, Locher R, Häsler E, Vetter W (1998). Is there a role for the ob gene product leptin in essential hypertension? American Journal of Hypertension **11**(11)**:** 1305-1311.

Tabák A, Brunner E, Miller MA, Karanam S, McTernan PG, Cappuccio FP, et al. (2009). Low serum adiponectin predicts 10-year risk of type 2 diabetes and HbA1c independently of obesity, lipids and inflammation–Whitehall II study. Hormone and Metabolic Research= Hormon-und Stoffwechselforschung= Hormones et metabolisme **41**(8)**:** 626.

Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, et al. (2002). Leptin regulates bone formation via the sympathetic nervous system. Cell **111**(3)**:** 305-317.

Takemura Y, Osuga Y, Koga K, Tajima T, Hirota Y, Hirata T, et al. (2007). Selective increase in high molecular weight adiponectin concentration in serum of women with preeclampsia. Journal of Reproductive Immunology **73**(1)**:** 60-65.

Taleb S, Herbin O, Ait-Oufella H, Verreth W, Gourdy P, Barateau V, et al. (2007). Defective leptin/leptin receptor signaling improves regulatory T cell immune response and protects mice from atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology **27**(12)**:** 2691-2698.

Tan K, Xu A, Chow W, Lam M, Ai V, Tam S, et al. (2004). Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. The Journal of Clinical Endocrinology & Metabolism **89**(2)**:** 765-769.

Tang C-H, Chiu Y-C, Tan T-W, Yang R-S, Fu W-M (2007). Adiponectin enhances IL-6 production in human synovial fibroblast via an AdipoR1 receptor, AMPK, p38, and NF-κB pathway. The Journal of Immunology **179**(8)**:** 5483-5492.

Tomimatsu T, Yamaguchi M, Murakami T, Ogura K, Sakata M, Mitsuda N, et al. (1997). Increase of mouse leptin production by adipose tissue after midpregnancy: gestational profile of serum leptin concentration. Biochemical and Biophysical Research Communications **240**(1)**:** 213-215.

Tomizawa A, Hattori Y, Kasai K (2009). Induction of gene expression in response to globular adiponectin in vascular endothelial cells. Life Sciences **85**(11)**:** 457-461.

Trupin LS, Simon LP, Eskenazi B (1996). Change in paternity: a risk factor for preeclampsia in multiparas. Epidemiology **7**(3)**:** 240-244.

Tsao T-S, Murrey HE, Hug C, Lee DH, Lodish HF (2002). Oligomerization state- dependent activation of NF-κB signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). Journal of Biological Chemistry **277**(33)**:** 29359-29362.

Tschöp J, Nogueiras R, Haas-Lockie S, Kasten KR, Castañeda TR, Huber N, et al. (2010). CNS leptin action modulates immune response and survival in sepsis. The Journal of Neuroscience **30**(17)**:** 6036-6047.

Uji Y, Yamamoto H, Maeda K, Tsuchihashi H, Akabori H, Shimizu T, et al. (2010). Adiponectin deficiency promotes the production of inflammatory mediators while severely exacerbating hepatic injury in mice with polymicrobial sepsis. Journal of Surgical Research **161**(2)**:** 301-311.

Ukkola O, Santaniemi M (2002). Adiponectin: a link between excess adiposity and associated comorbidities? Journal of Molecular Medicine **80**(11)**:** 696-702.

Vaisbuch E, Kusanovic JP, Erez O, Gotsch F, Chaiworapongsa T, Than NG, et al. (2009). Maternal serum adiponectin multimers in preeclampsia. European Journal of Endocrinology **23**(3):205-209.

Verhaegen J, Gallos ID, van Mello NM, Abdel-Aziz M, Takwoingi Y, Harb H, et al. (2012). Accuracy of single progesterone test to predict early pregnancy outcome in women with pain or bleeding: meta-analysis of cohort studies. BMJ: British Medical Journal **345**.

Wang M-Y, Yan TZ, Newgard CB, Unger RH (1996). A novel leptin receptor isoform in rat. FEBS letters **392**(2)**:** 87-90.

Wanninger J, Neumeier M, Weigert J, Bauer S, Weiss TS, Schäffler A, et al. (2009). Adiponectin-stimulated CXCL8 release in primary human hepatocytes is regulated by ERK1/ERK2, p38 MAPK, NF-κB, and STAT3 signaling pathways. American Journal of Physiology-Gastrointestinal and Liver Physiology **297**(3)**:** G611-G618.

Weisell RC (2002). Body mass index as an indicator of obesity. Asia Pacific Journal of Clinical Nutrition **11**(s8)**:** S681-S684.

Whitehead JP, Coch R, et al., (2006). The structure and multimerization of adiponectin. European Journal of Endocrinology **22**(3): 124-129.

Williams KW, Scott MM, Elmquist JK (2009). From observation to experimentation: leptin action in the mediobasal hypothalamus. The American Journal of Clinical Nutrition **89**(3)**:** 985S-990S.

Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME (2004). Adiponectin differentially regulates cytokines in porcine macrophages. Biochemical and Biophysical Research Communications **316**(3)**:** 924-929.

Xi W, Satoh H, Kase H, Suzuki K, Hattori Y (2005). Stimulated HSP90 binding to eNOS and activation of the PI3−Akt pathway contribute to globular adiponectin-induced NO production: Vasorelaxation in response to globular adiponectin. Biochemical and Biophysical Research Communications **332**(1)**:** 200-205.

Xie, Linglin et al., (2008). Adiponectin and leptin are secreted through distinct trafficking pathways in adipocytes. Biochimica et Biophysica Acta (BBA) Molecular Basis of Disease ***1782***(2):99-108.

Yamaguchi N, Argueta JGM, Masuhiro Y, Kagishita M, Nonaka K, Saito T, et al. (2005). Adiponectin inhibits Toll-like receptor family-induced signaling. FEBS letters **579**(30)**:** 6821-6826.

Yamauchi T, Hara K, Kubota N, Terauchi Y, Tobe K, Froguel P, et al. (2003a). Dual roles of adiponectin/Acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. Current Drug Targets-Immune, Endocrine & Metabolic Disorders **3**(4)**:** 243-253.

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. (2003b). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature **423**(6941)**:** 762-769.

Yang W-S, Lee W-J, Funahashi T, Tanaka S, Matsuzawa Y, Chao C-L, et al. (2001). Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. The Journal of Clinical Endocrinology & Metabolism **86**(8)**:** 3815- 3819.

Yilmaz MI, Sonmez A, Caglar K, Celik T, Yenicesu M, Eyileten T, et al. (2007). Effect of antihypertensive agents on plasma adiponectin levels in hypertensive patients with metabolic syndrome. Nephrology **12**(2)**:** 147-153.

Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, et al. (2000). Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood **96**(5)**:** 1723-1732.

Zhang F, Basinski MB, Beals JM, Briggs SL, Churgay LM, Clawson DK, et al. (1997). Crystal structure of the obese protein Ieptin-E100. Biochem **22**(3):123-127.

Zhang W, Telemaque S, Augustyniak R, Anderson P, Thomas G, An J, et al. (2010). Adenovirus-Mediated Leptin Expression Normalises Hypertension Associated with Diet-Induced Obesity. Journal of Neuroendocrinology **22**(3)**:** 175-180.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994). Positional cloning of the mouse obese gene and its human homologue. Nature **372**(6505)**:** 425-432.

Zhao J, Townsend K, Schulz L, Kunz T, Li C, Widmaier E (2004). Leptin receptor expression increases in placenta, but not hypothalamus, during gestation in< i> Mus musculus</i> and< i> Myotis lucifugus</i>. Placenta **25**(8)**:** 712-722.

Zhu N, Pankow JS, Ballantyne CM, Couper D, Hoogeveen RC, Pereira M, et al. (2010). High-molecular-weight adiponectin and the risk of type 2 diabetes in the ARIC study. The Journal of Clinical Endocrinology & Metabolism **95**(11)**:** 5097-5104

APPENDIX

ETHICAL CLEARANCE AND CONSENT FROM STUDY PARTICIPANTS

Title of research: Maternal serum levels of adiponectin and leptin in non-pregnant, normal pregnant and preeclamptic women

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Background. Preeclampsia is a major cause of maternal and perinatal mobidity and mortality, yet it is difficult to predict it (Eiland et al., 2012). Diagnosis of this condition is based on only blood pressure and urine protein measurement, due to unidentified biomarkers for the condition. Since this condition is believed to be of placental origin (Al-Jameil et al., 2014), it has become imperative to investigate the interplay betwen placental hormones and preeclampsia, so as to identify biomarkers for diagnosing preeclampsia. And following the discovery that adiponectin and leptin are not only produced by the adipose tissue, but also by the placenta (Lecke et al., 2011), it is obvious that a study of the levels of adiponectin and leptin in normal pregnancy and preeclampsia is warranted.

Purpose of the research: To identify biomarkers for diagnosing preeclampsia

Risks(s): During the drawing of blood samples, you may feel some sharp pains at the part of the arm where the needle will be inserted. Also, the questions in the questionnaire may require you to disclose some of your private information regarding your life history and experiences.

Benefits(s): Apart from the fact that, the utilization of the findings of the study will promote health care delivery in this country and abroad, you will not have any other benefit.

Confidentiality: You are assured of the confidentiality of the information obtained from you. Neither your name nor anything that identifies you will be recorded; hence collected data cannot be linked to you in anyway. However, as part of our responsibilty to conduct this study properly, we may allow officials of the ethics committee to have access to your records. But we assure you that those officials will never know whether you participated in the study or not, since they will not be provided with your names and identifiers.

Voluntriness: Enrolling into the study is fully voluntary.

Alternatives to participation: if you choose not to participate in the study, it will not affect your treatment in this hospital in anyway.

Withdrawal from the research: you can choose to withraw from the study at anytime, or decide not to answer questions that may force you to disclose your humiliating personal secrets.

Consequence of the withdrawal: You will suffer no consequence, loss of benefit or care if you choose to withdraw from the study.

Costs/compensation: You will neither be compensated nor benefit directly from the study, except the utilizations of the results of the study, when published.

Statement of the person obtaining consent:

I have fully explained this research to…… and have given her sufficient information, including risks and benefits, to assist her to make an informed decision.

Name: ……..…Signature:……..Date:…….

Statement of the person giving consent:

I have read the description of the research, or have had it translated into a language I understand. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research. I have received a copy of this consent form and additional information to keep for myself.

Date:……Signature:……Date……...

Contacts: Please, if you have any question concerning this study, do not hesitate to contact the researchers at the Department of Molecular Medicine, KNUST

1. E. A. Adu Gyamfi 2. Dr (Mrs.) Ahenkorah Fondjo 3. Dr. WKBA Owiredu

If you have any concern regarding this study and would like to contact someone at the blind side of the researchers, you are encouraged to contact,

The Head of Department Department of Molecular Medicine School of Medical Sciences
KNUST
Kumasi