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Pregnancy-induced Lipid Peroxidation and Oxidative Stress in Ghanaian Women with Normal Pregnancies

by

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DECLARATION

This thesis is submitted to KNUST, School of Graduate Studies through the College of Health Science, School of Medical Sciences, Department of Molecular Medicine. I hereby declare that this thesis has been composed by myself and has not been accepted in any previous application for a degree here or elsewhere. This thesis presents results of original research undertaken by me

personally. Information taken from other works has been specially and duly acknowledged.

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DEDICATION

I dedicate this thesis to God for His grace and my dear wife Mary Balkono and the entire Awinibuno family for their support. This thesis is also dedicated to Dr. Francis Agyeman- Yeboah for the encouragement and hope he gave me throughout the period of my education.

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ACRONYMS AND ABBREVIATIONS

AA: Arachidonic Acid

ANC: Antenatal Clinic

ALB : Albumin

BCG : Bromocresol Green

BMI : Body Mass Index

BP : Blood Pressure

CHRPE : Committee for Human Research and Ethics

CI : Confidence Interval

cm : Centimetre

DBP : Diastolic Blood Pressure

dH_20 : Distilled Water

DHDL : Direct High Density Lipoprotein-Cholesterol

DLDL : Direct Low Density Lipoprotein-Cholesterol

DM : Diabetes Mellitus

DNA : Deoxyribose Nucleic Acid

EFA: Esterified Fatty Acid

FBG : Fasting Blood Glucose

FR : Free Radicals

g : Gram

GDHS: Ghana Demographic Health Surveys

GLUC : Glucose

GOD : Glucose Oxidase

H_2O_2 : Hydrogen Peroxide

Hb : Haemoglobin

HDL-CHOL: High Density Lipoprotein

HPLC : High-Performanc Lliquid Chromatography

LPL: Lipoprotein Lipase

LCPUFA: Long Chain Polyunsaturated Fatty Acid

Kg/m^2 : Kilogram per Meter sqaure

MDA: Malondialdehyde

MDG : Millenium Development Goals

Mg : Milligram

Mg^{2+} : Magnesium ion

µL : Micro Litre

mmHg : Millimeter mercury

mmol/L : Millimole per Litre

µmol/L : Micromole per Litre

mmol/L : Millimole per Litre

NEFA: Non-Esterified Fatty Acids

NOS : Nitrogen Oxygen Species

O^{2-} : Super oxide

ºC : Degree celsius

OH^- : Hydroxyl ion

OS : Oxidative Stress

POD : Peroxidase

PUFA: Long Polyunsaturated Fatty Acid

RHSP : Reproductive Health Strategic Plan

ROS : Reactive Oxygen Species

Rpm : Revolution per minute

SBP : Systolic Blood Pressure

SD : Standard Deviation

SEM : Standard Error of Mean

TBA: Thio-barbituric Acid

TBARS: Thio-barbituric Acid Reactive Substances

TC : Cholesterol

TCA : Trichloroacetic Acid

TG : Triglycerides

UA : Uric Acid

VLDL: Very Low Density Lipoprotein-Cholesterol

WHO: World Health Organisation

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ABSTRACT

Introduction: Pregnancy is associated with complex physical and biochemical events which exert enormous effect on the expectant mother as well as foetus. Oxidative stress has been proposed as one of the complex biochemical events associated with disease aetiology and its concommitant effect can affect pregnancy outcomes. This however has not been elucidated thus the aim of this study was to evaluate the status of Malondialdehyde (MDA)- a lipid peroxidation biochemical marker of oxidative stress and the lipid profiles of Ghanaian women with normal pregnancy.

Methodology: This was a crossectional study of subjects in which convenience sampling technique was used to select 239 women with uncomplicated pregnancy that reported for regular antenatal care at the James Town Maternity of the Ussher Polyclinic in the Accra metroplolis of Ghana. The pregnant women were divided into three groups according to the gestational age,first trimester, second trimester and third trimester. Each semester had 81, 125 and 33 subjects respectively recruited for the purpose of this study. Forty-eight(48) age matched non-pregnant women were recruited as controls. Lipid peroxidation was assessed by the measurement of serum Malondialdehyde (MDA), thio-barbituric reactive substance (TBARS) and serum lipid profiles using standardised methods.

Results: The mean ages for the pregnant women (study group) and the non-pregnant (control group) were 24.6 and 25.8 years respectively. Both the study and control groups had similar mean age ($p = 0.1845$). The systolic (SBP) and diastolic (DBP) blood pressures were statistically significantly elevated in the study group ($p = 0.0001$). In addition, the difference in the mean body mass index (BMI) of the two groups was significant ($p < 0.007$). Serum Malondialdehyde (MDA) level, the lipid peroxidation marker was significantly raised in women with normal pregnancy (1.5 ± 0.3 μmol/L) ($p < 0.05$) compared to non-pregnant women (1.2 ± 0.2 μmol/L). Serum MDA levels were significantly increased in all the trimesters of normal pregnancy compared with nonpregnant women. A significantly positive correlation was seen between MDA levels and total Cholesterol ($p < 0.05$) , triglycerides ($p < 0.05$) and LDL Cholesterol ($p < 0.05$) in all the three trimesters of normal pregnancy.

Conclusion: Ghanaian pregnant women with normal pregnancy are prone to dyslipidemia and experience a markedly raised intensity of lipid peroxidation compared

to their nonpregnant counterparts and this rise in intensity showed a significant positive correlation with an increase in gestational age.

CHAPTER ONE
INTRODUCTION

1.1 STUDY BACKGROUND

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent (Scott W., 1994). Pregnancy is a developmental crisis in a woman's life. It places a great demand on the will be mother and requires adaptation. Changes in many of the body's biochemical function during pregnancy leads to a high demand for energy and an increased oxygen requirement (Bray *et al*., 1990). These changes result in increased intake and utilization of oxygen, which also results in increased levels of reactive oxygen species (ROS) and the consequent acceleration to Oxidative Stress (OS) (Renata *et al*., 2002). Many other free radicals are produced in the body as a result of the myriad of biochemical processes taking place in normal metabolism (Skaper *et al.*, 1997) with oxygen-derived free radicals accounting for about 95 % of all radicals (Du et al., 2003). These free radicals perform some physiologic function to some degree in the body such as, participating in xenobiotics metabolism, biosynthesis and clearance of microorganisms (Braganza *et al*., 1998). The normal homeostasis of these free radicals is maintained by anti-oxidants and anti-oxidases, leading to a balance between production and clearance (Sudha *et al*., 2003).

An imbalance between reactive oxygen species and anti-oxidant defense mechanisms of a cell leads to an excessive production of oxygen metabolites, creating a condition known as ‘Oxidative stress' (Sudha *et al*., 2001). Such free radicals can attack polyunsaturated fatty acids of membranes, leading to lipid peroxidation and disruption

of intracellular calcium homeostasis and consequent cellular apoptosis (Buttke and Sandsrom, 1994). Free radicals can also destroy key intracellular enzymes, including free radical scavenger enzymes which disrupt DNA replication and initiate the process of carcinogenesis (Wiseman and Halliwell, 1996). Free radical-induced oxidative injury have been reported to have a role in the pathogenesis of a number of diseases, including cancer, atherosclerosis, diabetes mellitus, epilepsy, radiation damage, cellular aging, reperfusion damage, inflammatory diseases and Parkinsonism (Yondim and Riederer, 1997; Ames *et al*., 1993).

The role of antioxidants and increasing superoxides is gaining importance and these have been shown to pose a threat to normal pregnancy (Wadwa., 1998). Malondialdehyde (MDA) is one of the well-known secondary products of lipid peroxidation, and it is widely used as an indicator of cellular injury and oxidative stress in pregnancy. Considerable evidence shows that maternal stress during pregnancy has profound effects on offsprings. Wadhwa (1998) showed that, in humans, prenatal oxidative stress has an effect in terms of increased spontaneous abortions and fetal malformations and decreased infant birth weight and length of gestation.

Oxidative stress has also been implicated in defective embryo development and retardation (Agarwal *et al*., 2003; Guerin *et al*., 2001). Studies have shown that prenatal oxidative stress leads to excessive neuroendocrine and behavioural responses (Takahashi L.K., 1998). Some recent studies have associated pregnancy-induced oxidative stress with hypertension and preeclampsia (Ahenkorah *et al*., 2008) both of which are leading causes of maternal and foetal mortality (Bellany *et al*., 2007). In this study, the serum concentration of malondialdehyde (MDA) and lipids were assessed in

pregnant women in three different trimesters of pregnancy with the aim of determining the effect of pregnancy on serum malondialdehyde (MDA), and lipids concentration, reactive oxygen species produced as a result of pregnancy-induced oxidative stress.

More than 200 million women become pregnant every year and in most cases the outcome of labour is successful (Diareme *et al., 2009).* An average of 850,000 pregnancies occur per annum in Ghana (GDHS, 2004). However, maternal mortality reported per annum stands at about 550/100,000 live births while infant mortality is about 65/1000 live births with neonatal death accounting for two-third of the deaths. Also, still-births account for about 2.3% and caesarean deliveries, 5.7% (GDHS, 2007). An alarming 40% of maternal deaths are as a result of hypertensive pregnancy, antepartum hemorhage and post partum hemorrhage(Osei-Nkansah, 2001). Efforts aimed at reducing these rates to meet the Millenium Development Goal (MDGs) targets over the years have met with a slowing of the pace of decline in rates (Reproductive Health Strategic Plan, 2006-2010).

In Ghana, no information is available that relates the severity of pregnancy-induced oxidative stress levels, and lipedemia with gestational age. Since the prenatal stage of a child's life seems to be very important in terms of the child's development, delivery and growth, it is important to understand the factors that adversely affect the fetal and maternal health. It is also very important to know at which stage of the pregnancy the threat to maternal and fetal life is most likely to occur. This would improve the quality of care and level of support given to pregnant women and ultimately reduce the incidence of maternal and fetal morbidity and mortality in Ghana.

1.2. PROBLEM STATEMENT AND RATIONALE FOR THE STUDY

There is growing literature on the effects of oxidative stress in female reproduction with involvement in the pathophsiology of pre-eclampsia (Tranquilla *et al*., 2004; Takagi *et al*., 2004), hydatidiform mole (Harma *et al*., 2004), free radical-induced birth defects and intrauterine growth restriction (Loeken., 2004), and other situations such as abortions (Lagod *et al*., 2004). The above pieces of evidence underscore the critical role that oxidative stress plays in fetal and maternal mortality and morbidity. It is not conclusive as to which gestational period, pregnant women experience maximum oxidative stress and which thus poses serious risk to foetus and mother's health. The trend or the changes in lipid profiles and lipid peroxidation levels in normal pregnancy among Ghanaian women has not yet been studied. Such informmation will not only clarify the debate on the actual effect and trend of lipid peroxidation in pregnancy but further provide baseline data on the course of lipid peroxidation among Ghanaian women.

The Ministry of Health (MoH), Ghana strategy on antenatal care recommends management of pregnancies with multivitamins (antioxidants) and iron-based medicines. These management interventions in pregnancies might not match the oxidative stress status of the expectant mother with the given intervention dosage. An accurate evaluation of maternal serum oxidative stress by standardized assays may help in the diagnosis, prevent fetal defects and help the management of high-risk pregnancies. Serial measurement of oxidative stress biomarkers in longitudinal studies may also help to delineate the etiology of some of the disorders in female reproduction such as preeclampsia. It was imperative therefore to undertake this study to further

elucidate the role of lipid perodixation and oxidative stress in pregnancy. Determining the stage of pregnancy when mother and fetus are likely to experience maximum oxidative stress will be a worthy tool for care givers to better manage pregnant women in Ghana thus minimizing the adverse effects of oxidative stress in pregnacy. The use of any marker requires knowledge of normal baseline levels, and this information is sparse and diffuse in the case of uncomplicated pregnancy, however most studies done in Ghana have not focused on normal pregnancies. During pregnancy, excess lipid peroxidation has been associated with preeclampsia and other reproductive complications, but there is only scattered information about baseline levels in healthy pregnant women, thus data accumulation from such a study will be invaluable reference for healthcare practioners associated with maternal antenatal care. Ghana is in the category of countries with a high-burden of maternal mortality.

The Hon. Minister of Health declared that the high maternal mortality in the country was a national emergency and highlighted the need to accord greater priority to infant and maternal health issues despite the substantial budgetary allocation they already enjoyed (Aide Memoire, 2008).

1.3 GENERAL OBJECTIVE

The aim of this study was to evaluate the lipid peroxidation status of Ghanaian women with normal prgnancy using Malondiadehyde (MDA)- a biochemical marker of oxidative stress and the lipid profiles at different gestational periods.

1.3.1 Specific objectives

1. To assess changes in lipid levels at different periods of gestation in Ghanaian women with normal uncomplicated pregnancy.
2. To assess changes in Malondiadehyde (MDA) a biomarker for oxidative stress at different periods of gestation in Ghanaian women with normal uncomplicated pregnancy
3. To compare the intensity of lipid peroxidation in normal uncomplicated pregnancy with non-pregnant women using malondialdehyde oxidative stress marker.
4. To determine correlation between maternal lipid levels and intensity of lipid peroxidation in women with normal uncomplicated pregnancy.
5. To determine at which stage in the gestational period of normal pregnancy is an expectant mother at risk of the adverse effect of oxidative stress.
6. To recommend appropriate nutritional supplements as anti-oxidants to augment the effects of pregnancy- associated oxidative stress.

1.4 PRIMARY HYPOTHESIS

Oxidative stress induced biochemical change does not play any role in the outcome of uncomplicated pregnanc

CHAPTER TWO
LITERATURE REVIEW

2.1 INTRODUCTION

Oxidative stress was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems (Sies.,1985) and by others as an elevation in the steady-state levels of various reactive oxygen species (ROS) that exceeds the body's antioxidant defenses (Agarwal *et al.,* 2003). Once this imbalance appears, cellular macromolecules may be damaged by the predominant free radicals. This leads to oxidative modifications of the genome, proteins, structural carbohydrates, and lipids; in the latter case, lipid peroxidation occurs. Lipid peroxidation is a free radical-related process, that in biologic systems may occur under enzymatic control, e.g., for the generation of lipid-derived inflammatory mediators, or nonenzymatically. This latter form, as mentioned above, is associated mostly with cellular damage as a result of oxidative stress, and a great variety of aldehydes is formed when lipid hydroperoxides break down in biological systems, among them, malondialdehyde (MDA) (Esterbauer *et al.,* 1985).

Free radicals (FR) are chemical products capable of independent existence with one or more unpaired electrons. FR participate in phagocytosis, synthesis of prostaglandins, steroid and thyroid hormones. FR such as reactive particles of oxygen are involved in the process of cell growth, division and death. Their low concentrations are beneficial for the organism. It is known that FR participate in the pathogenesis of at least 50 diseases (Kuèinskienë *et al.,* 2001 and Kazakevièius *et al.,* 2002). Activated free radical lipid peroxidation has been shown to result in an increased duration of delivery (Nakai *et al*., 2006). FR injures the endothelium of blood vessels during oxidation of

membrane lipoproteins and polyunsaturated fat acids. In case of the activation of lipid peroxidation and excess FR

production, the protective mechanisms of the antioxidative system are activated and protect the organism from the activity of free radicals.

Lipid peroxidation is a normal phenomenon that occurs continuously at low levels in all humans. These peroxidation reactions are toxic to cells and cell membranes; however, they are normally controlled by countervailing biologic mechanisms. In the opinion of numerous researchers, pregnancy invokes oxidative stress(Qanungo and Mukherjea., 2006).

Lipid peroxidation activity in the blood serum of healthy pregnant women in comparison with non-pregnant is increased. The intensity of stress during different periods of pregnancy varies. With the progression of a normal pregnancy, gradual suppression of lipid peroxidation takes place through the activated production of endogenous antioxidants to protect the fetus from toxic oxygen effects (Qanungo and Mukherjea., 2000). A marked increase of stress activity has been noted at the second and third trimesters of pregnancy and for a certain period after delivery while others have found marked activity in all three semester, in case of chronic hypertension and pre-eclampsia, gestational diabetes (Kumar., 2000 and Ohan *et al*., 2004). At delivery, due to the rapidly increasing oxygenation during intensive breathing and labor efforts, shifts of the antioxidative system are activated, which in turn are caused by reactive oxygen particles produced in the uterus and placenta tissues (Nakai *et al*., 2000).

Numerous experimental studies have demonstrated that oxidative stress is induced in the placental tissues, trophoblasts, the endothelium of maternal blood vessels

(Qanungo and Mukherjea, 2000, Poston and Raijmakers, 2004). The endothelium damaged by FR locally

releases vasoconstrictive mediators (endothelin, thromboxane) and produce pathological processes such as blood vessel inflammation, vasoconstriction, thrombosis, ischemia (Jauniaux *et al.,* 2003). Active mediators, tissue cytokines evoke degeneration on the periphery of the placenta, apoptosis and participate in the pathogenesis of miscarriage, pre-eclampsia and preterm delivery (Arikan *et al*., 2001). Placental FR, getting into general circulation, invoke the inflammatory status of the maternal organism, blood vessel spasm, and induce placental hypoxia (Poston and Raijmakers, 2004).

In Ghana some studies have been done on the oxidative stress by Ahenkora *et al* (2008) to assess lipid profile and lipid peroxiadtion among Ghanaian Pregnancy-Induced Hypertensives and found significantly raised levels of lipid peroxides in subjects compared to normal pregnancy

There is not much information available on lipid peroxidation levels among Ghanaian women with normal uncomplicated pregnancy and none available on lipids and peroxidation levels at different gestational periods in the course of pregnancy among Ghanaian women.

2.2 OXIDATIVE STRESS

2.2.1 Free Radicals and Reactive Oxygen Species

Free radicals (FR) are chemical products capable of independent existence with one or more unpaired ions. They are unstable and highly reactive. They become stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby

molecule causing a cascade of chain reactions resulting in cellular damage and disease (Pierce *et al.,* 2003 and Van Langendonckt *et al.*, 2002). There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (NOS).

The three major types of ROS are: superoxide (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl (OH^-). The superoxide radical is formed when electrons leak from the electron transport chain. Halliwell and Gutteridge, 1992). The dismutation of superoxide results in the formation of hydrogen peroxide. The hydroxyl ion is highly reactive and can modify purines and pyrimidines and cause strand breaks resulting in DNA damage (Mello *et al.*,1984) . Some oxidase enzymes can directly generate the hydrogen peroxide radical. ROS have been implicated in more than 100 diseases. (Gibson and Huang., 2004). They have a physiological and pathological role in the female reproductive tract. Numerous animal and human studies have demonstrated the presence of ROS in the female reproductive tract: ovaries, (Jozwick *et al*., 1999) fallopian tubes .(El Mouatassim *et al.*, 2001) and embryos (Guerin *et al.*, 2001). ROS is involved in the modulation of an entire spectrum of physiological reproductive functions such as oocyte maturation, ovarian steroidogenesis, corpus luteal function and luteolysis (Behrman HR., 2001). ROS-related female fertility disorders may have common etiopathogenic mechanisms. ROS may also originate from embryo metabolism and from its surroundings.

2.2.2 The lipid peroxidation chain

A FR is an atom or molecule that contains one or more unpaired electrons. The existence of an unpaired electron generally allows the radical to pair more easily with

other atoms or molecules, although there is broad variation in reactivity (Halliwell and Gutteridge, 1992). Two free radicals may combine by pairing their single electrons. Because most atoms and molecules in the body do not have unpaired electrons, the union is more likely to involve a nonradical. This new combination is in turn unbalanced for electrons, resulting in a domino effect as new radicals are formed in a chain reaction. During normal respiration, the body produces oxygen free radicals. When reactive oxygen free radicals interact with the polyunsaturated fatty acids in membranes or lipoproteins, the process of lipid peroxidation begins. In the resulting lipid peroxidation chain, the fatty acids are converted to the primary product of lipid hydroperoxides (commonly termed lipid peroxides) and to secondary metabolites such as MDA (Fig. 2.1). Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is malondialdehyde (MDA). Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation (Esterbauer *et al.*,1991).

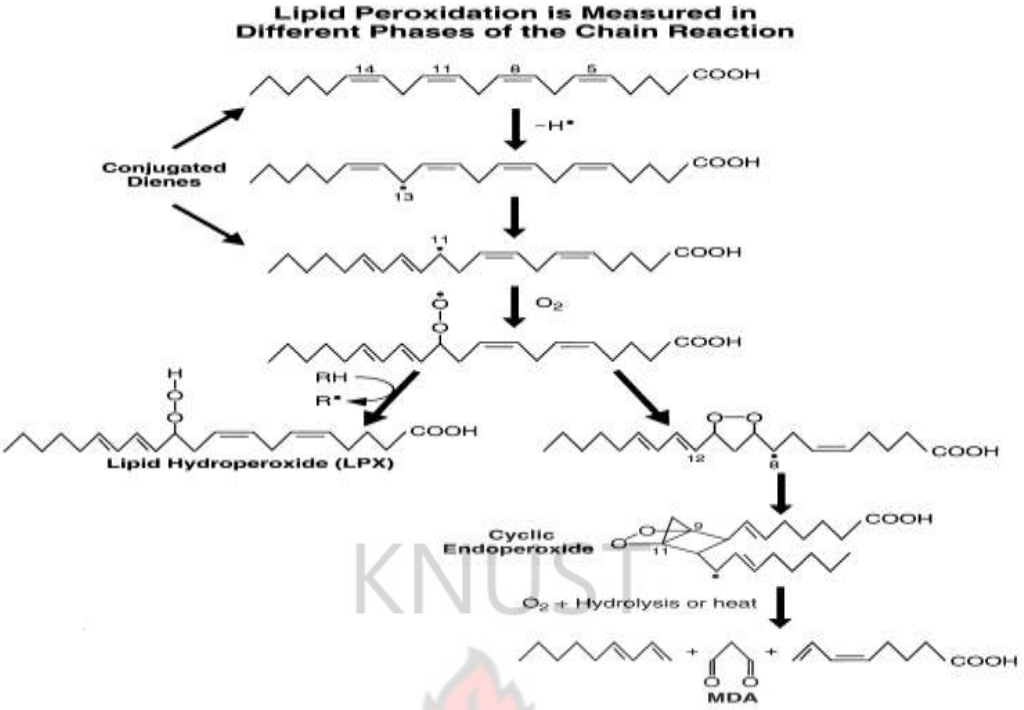

Figure 2.1: *Chain reaction of lipid illustrating the lipid peroxidation process*

2.2.3 Markers of lipid peroxidation and oxidative stress.

Different markers of oxidative stress have been reported in various studies however the sensitivity and specifity of the various biomarkers are not known. While some research is focused on studying the antioxidant capacity, others have focused on determining the levels of oxidative stress markers. Besides, there has been an assumption in those studies measuring the amount and type of antioxidants that there is an inverse correlation between oxidative stress markers and antioxidants while in such studies, others have concentrated on the measurement of the total antioxidant capacity and individual enzymes like superoxide dismutase. These observations necessitate further

studies to validate the results of the earlier studies. The biomarkers of oxidative stress that are studied should be similar across different studies to make the results comparable.

Direct measurement of oxidative stress in humans is difficult because the active oxygen species and free radicals are extremely short-lived (Pryor and Godber, 1991) and cannot be detected in humans with current techniques. Instead, products of the oxidative process are measured. In the case of human lipid peroxidation (Cheeseman.,1990 and Mason., 1996), there is no single standardized, routine reliable clinical measurement of this multistage process (Cheeseman.,1990). For example, the extent of lipid oxidation can be estimated from the losses of unsaturated fatty acid or from chemical bond rearrangement after union with a free radical (conjugated dienes), or by the amount of primary peroxidation products (lipid hypdroperoxides), or by the amount of various secondary products (such as MDA) generated in the lipid peroxidation process (Gutteridge.,1986**).** These measurements are taken at different points on the peroxidation chain and thus may produce different results (Turpeinen *et al,* 1995). Studies cited here have used one of three markers of peroxidation and have relied on the measurement of secondary metabolites by the (TBA) method. Here, thiobarbituric acid (TBA) is added to the sample and the resulting reactive substances (TBARS) are measured colorimetrically and using malondialdehyde (MDA), a secondary metabolite of lipid peroxidation as a calibrator. This method lacks specificity because many other substances in biologic samples also react with TBA(Gutteridge., 1986). Specificity of the TBARS method can be improved by screening out artifacts via high-performance liquid chromatography (HPLC) (Halliwell and Chirico.,1993). Few studies have measured conjugated dienes as a marker of

peroxidation. These structures are formed during the peroxidation of unsaturated fatty acids and absorb ultraviolet light in a specific range. Measuring this UV absorbance provides a marker for the early part of the peroxidation process. This method also lacks specificity, because there are potentially interfering substances in biologic materials that create a high background (Halliwell and Chirico.,1993). Finally, few studies measured lipid hydroperoxides, a primary product of peroxidation. The method used to determine these values can be difficult but the results are reportedly more specific and reproducible than those obtained by the TBA method (Balabaeva.,1980).

2.2.4 Role of Free Radicals in female reproduction and disease

Reactive Oxygen Species are a double-edged sword – they serve as key signal molecules in physiological processes but also have a role in pathological processes involving the female reproductive tract and pregnancy. They are key signal molecules modulating various reproductive functions and can influence how the oocytes, sperm, and embryos develop in their microenvironments, for example follicular fluid, hydrosalpingeal fluid and peritoneal fluid as depicted in Figure 2.2. These microenvironments have a direct bearing on quality of oocytes, sperm oocyte interaction, implantation, and early embryo development. Oxidative stress affects both implantation and early embryo development which determines a successful pregnancy. There is a complex interplay of cytokines, hormones, and other stressors that affects cellular generation of free radicals; these molecules act further through the modulation of many transcription factors and gene expression. The presence of oxidant and antioxidant systems in various reproductive tissues has evoked great interest on their

role in human reproduction. Oxidative stress can also be involved in the etiology of defective embryo development and miscarriage (Guerin *et al.,* 2001). Many studies have found maternal lipid peroxide levels in blood to be significantly elevated in pre-eclampsia compared to normal pregnancy (Ahenkora *et al.,* 2008) with decreased antioxidant levels. Elevated lipid peroxide levels have also been reported in hypertension of pregnancy (Aydin *et al.*,2004). The etiology of recurrent pregnancy loss remains unclear and is a scientific challenge. Oxidative stress may have a role in the etiology of recurrent pregnancy loss with no known etiology.

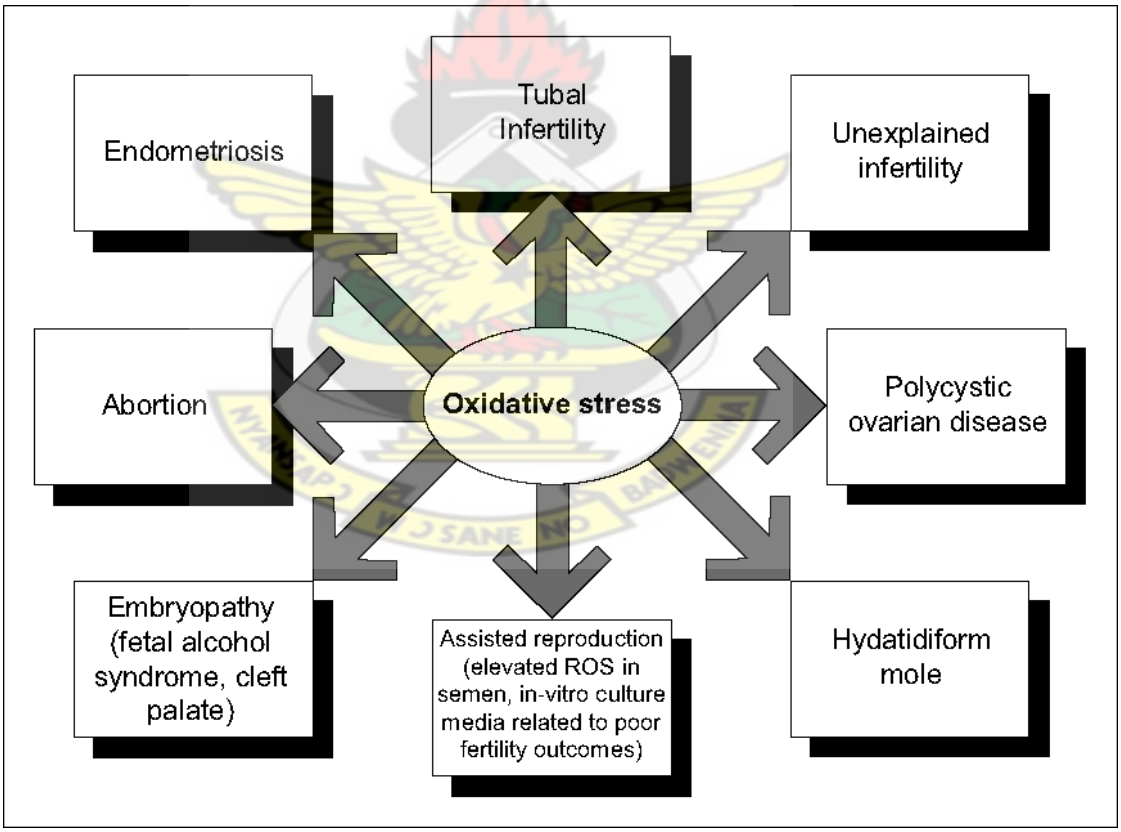

FIGURE 2. 2. illustrating the role of oxidative stress in female reproduction

2.2.5 ***Interventions to overcome oxidative stress***

Antioxidant defense systems have evolved to counterbalance the toxic actions of the free radicals by limiting the amount of lipid peroxides that can be formed. Antioxidants include; endogenously synthesized compounds such as superoxide dismutase as well as exogenous substances available from the diet such as, vitamins C and E. The antioxidant defenses are not totally effective. When the toxic peroxidation reactions are not fully counterbalanced by antioxidants, "oxidative stress" is said to result (Agarwal *et al.*,2005).

2.2.6 Lipid peroxide levels in blood

Some studies which compared levels of lipid peroxides in healthy pregnant with nonpregnant women (Morris *et al.,* 1998 and Wickens *et al.,* 1999) and found greater lipid peroxide levels in the blood of pregnant women. Similar findings were reported in previous studies (Pentieva *et al.,* 1995 and Iioka *et al.,* 1994) in which which blood lipid peroxide ratios of pregnant to non-pregnant ranged from 1.08 and 3.04 respectively. This wide range for the ratio was as result of different type of marker employed for measuring lipid peroxides.

Studies that provided data over the whole course of gestation were either conducted as longitudinal studies, in which at least part of an original cohort of pregnant women was restudied at a later date, or cross sectional studies in which the samples for the time periods were mutually exclusive. It is noteworthy that all the studies did not use more than 100 subjects.

Lipid peroxide levels in the first trimester of pregnancy were sometimes higher and sometimes lower than the level of the nonpregnant control group. By the second

trimester, increases of 10 to 50% over first trimester values were usually seen. Third trimester levels sometimes but not always declined. Only one investigation reported decreasing lipid peroxides in all trimesters of pregnancy; they used the TBA method (Uotila *et al.*, 1991). When conjugated dienes were used as biomarkers in this same study, however, values were found to be increased during the entire course of pregnancy.

Studies that provided data on lipid peroxide markers during pregnancy and post-partum showed a decrease in at least one biomarker after delivery. The decreases within the first 3 days after delivery ranged from 9% to 42% of the highest pregnancy measurement (Rebelo *et al.*, 1995 and Wu., 1996).

During pregnancy, lipid peroxidation is induced in the human placenta (Walsh and Wang.,1993). Lipid peroxides originating from both the trophoblast and the villous core compartments (Walsh and Wang.,1995) are secreted into the maternal effluent, possibly adding to levels in the maternal blood as additional peroxidation cascades are initiated. Concentration of lipid peroxides in placenta is reportedly higher than in blood (Takehara *et al* ,1990). Lipid peroxide levels in placental tissue have been reported to follow a different pattern over gestation than levels in blood, but there does not seem to be agreement about the pattern itself. One study found that the peak production in vitro is reached early in the placenta, perhaps in the third month; by the end of pregnany, there was minimal lipid peroxide production (Sekiba and Yoshioka, 1979). However, another investigation found that placental lipid peroxide formation was greater in third trimester than first trimester placentas (Damiant *et al.*, 1980). In view of the importance of pre-eclampsia for maternal and fetal morbidity, and the possible

role of the placenta in initiating the lipid peroxidation, information about placental levels of lipid peroxide is particularly needed.

The difficulty encountered in obtaining placenta from uncomplicated pregnancies over the course of gestation makes this a difficult challenge. The literature on lipid peroxide levels in uncomplicated pregnancy is limited, but some reasonable conclusions are possible. Despite the lack of precision in the predominant measure of lipid oxidation, there is agreement across a large number of studies that the level of lipid peroxides in blood is generally higher in pregnant women than in nonpregnant women. During gestation, significant elevations appear by the second trimester and may taper-off later in gestation, decreasing further after delivery. Lipid peroxides also are produced in placenta, but their pattern of change over the course of pregnancy is unclear. Lipid peroxides are important because their uncontrolled production can result in oxidative stress, with significant damage to cell integrity. There is a growing literature that associates oxidative stress with preeclampsia, a major cause of maternal and fetal morbidity. The higher levels of lipid peroxides seen in blood and the production of lipid peroxides in the placenta, even in uncomplicated pregnancies, make new baseline studies a priority. These studies should include measurements of lipids and blood volume, because they can influence marker values, with sensitive and specific measures of lipid peroxidation made at various points of gestation. From our review of the existing literature, it is evident that the importance of oxidative stress in reproduction and hence normal pregnancy has just began to be appreciated and studied.

2.3 LIPID METABOLISM (RHEOLOGY) IN PREGNANCY AND NEWBORN

2.3.1 Introduction:

Pregnancy is associated with significant variation in blood rheology (lipid metabolism), consequent mainly to changes in lipoprotein profile(Brizzi *et al.*,1999;Kuzawa and Adair., 2003). Although these changes were first described in 1845 by Bacquerel and Rodier, the exact elucidation of these changes is yet to be defined (Tonolo *et al.,* 1995). Available literature has revealed conflicting observations and implications of lipoprotien metabolism in normal and abnormal pregnancies (Sitadevi *et al.,* 1981). Because of the hormonal status changes during pregnancy, various adaptive mechanisms are initiated in order to mitigate effects of the rise in levels of sex hormones. Among some of the effects observed is that, there is alteration of lipid metabolism during pregnancy and women undergo a physiological dyslipidemia that can be measured in the laboratory by the lipid indices: total Cholesterol (T.Chol), Triglycerides (Trig), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C). The alterations of serum lipid indices are associated with the gestational age. The increase of the lipid and lipoprotein metabolism can sometimes give rise to cardiovascular risk (Lippi *et al.,* 2007) during the second trimester. Both genetic and non- genetic (hormonal) factors have also been implicated for the changes in lipid metabolism during pregnancy. Many studies in recent past have also incriminated abnormal lipid metabolism during pregnancy in the the pathogenesis of artherogenesis and ischaemic heart disease (IHD) due to changes in maternal microcirculation (Kuzawa and Adair., 2003).

Changes in carbohydrate and lipid metabolism occur to ensure a continuous supply of nutrients to the growing fetus despite intermittent maternal food intake. Changes in hepatic and adipose metabolism alter circulating concentrations of triglycerides, fatty acids, cholesterol, and phospholipids (Lesser and Carpenter., 1994). After an initial decrease in the first 8 weeks of pregnancy, there is a steady increase in triglycerides, fatty acids, cholesterol, lipoproteins, and phospholipids in normal pregnancy (Chiang *et al.,* 1995; Saarelainen *et al.,* 2006). The higher concentration of estrogen and insulin resistance is thought to be responsible for the hypertriglyceridemia of pregnancy.

Cholesterol is used by the placenta for steroid synthesis and fatty acids are used for placental oxidation and membrane formation. Changes in total cholesterol concentration reflect changes in the various lipoprotein fractions. HDL cholesterol increases by 12 weeks of gestation in response to estrogen and remains elevated throughout pregnancy (Halstead *et al.,* 1993). Total and LDL-cholesterol concentrations decrease initially, but then increase in the second and third trimesters. VLDL and triacylglycerols decrease in the first 8 weeks of gestation and then continuously increase until term. In the second half of pregnancy, VLDL clearance is altered because of the decreased activity of lipoprotein lipase (LPL) in the adipose and liver and because of the increased activity in the placenta. In the fed state, hepatic LPL is low, but increases with fasting, which increases fatty acid and ketone production for the fetus while the supply of glucose is low.

Changes in lipid metabolism promote the accumulation of maternal fat stores in early and mid pregnancies and enhance fat mobilization in late pregnancy. In early pregnancy, increased estrogen, progesterone, and insulin favor lipid deposition and

inhibit lipolysis. Lipoprotein Lipase activity in the adipose tissue from the femoral region, but not from the abdominal region, is elevated at 8–11 weeks of gestation (Rebuffé-Scrive *et al.,* 1985). Lipolysis in response to catecholamines is markedly higher in the abdominal than in the femoral region. The femoral cells are virtually unresponsive to catecholamines in pregnancy.

In late pregnancy, Human Chorionic Sommatotrophin (HCS) promotes lipolysis and fat mobilization. The increase in plasma fatty acid and glycerol concentrations is consistent with mobilization of lipid stores. This shift from an anabolic to a catabolic state promotes the use of lipids as a maternal energy source while preserving glucose and amino acids for the fetus. With prolonged fasting (48 hours), as well as shorter periods of fasting (18 hours), there is a rapid diversion of maternal metabolism to fat oxidation, with an elaboration of ketones (Metzger *et al*., 1980). Decreases in plasma glucose, insulin, and alanine, and increases in plasma fatty acid and ß-hydroxybutyrate are seen in pregnant women hours before these changes are seen in non-pregnant women (Metzger, 1991).

Another study found that in the course of normal pregnancy, plasma triglyceride and cholesterol concentrations rise and as pregnancy progresses both become normal(Desoye *et al.,* 1987) and that these trends are due to the hormonal variations observed during pregnancy(Zollner and Kirsch., 1962). The endogenous female sex hormones have significant effect on serum lipids (Patrizia *et al.,* 1999). During pregnancy, there is an increase in the hepatic lipase activity and decrease in lipoprotein lipase activity (Patrizia *et al.,* 1999). Hepatic lipase is responsible for the increased synthesis of the triglycerides at the hepatic level, whereas the decreased activity of lipoprotein lipase is responsible for the decreased catabolism at the adipose tissue level, the net effect of

which will be an increase in circulating triglycerides and the second step of uptake of the remnant chylomicrons by the liver is delayed so it leads to accumulation of triglycerides in plasma. Another hypothesis is that hypertriglyceridemia is probably consequence of competition between chylomicrons and very low-density lipoprotein cholesterol for the lipoprotein lipase. Classically, chylomicron clearance occurs in two sequential steps: (1) triglycerides hydrolysis by lipoprotein lipase, (2) uptake of the remnant by the liver. Delay in the second step leads to accumulation of remnants in plasma and is generally thought to represent the atherogenic risk of hypertriglyceridemia. The conclusion of another study also indicated that there exists a consistent positive association between elevated maternal triglyceride.

In a review, by Herrara (2002) it was stressed that during early pregnancy there is increased body fat accumulation assoaciated with both hyperphagia and increased lipogenesis. During late pregnancy, there in an accelerated breakdown of fat depots, which plays an important role in fetal development. Besisdes using placentally transferred fatty acids, the fetus also benefits from glycerol and ketone bodies. Although glycerol crosses the placental in small proportion, it is a preferrential substsrate for maternal gluconeogenesis and maternal glucose is quantitatively the main substrate crossing the placenta. Enhanced ketogenesis under fasting conditions and early transfer of ketones to the fetus allow maternal ketone bodies to reach the fetus to be used as fuel for oxidation metabolism as well as lipogenic substrate. Although maternal cholesterol is a source of cholesterol for the fetus during early gestation, it is of less importance during late pregnancy owing to the high capacity of fetal tissues to synthesize cholesterols. Maternal hypertriglyceridemia is a charisteristic feature during normal pregnancy and corresponds to an accumulation of triglyceride

not only in VLDL but also in LDL and HDL (Dabi *et al.*,2004). Although triglycerides do not cross the placenta, the presence of lipoprotein receptors in the placenta, along with lipoprotein lipase, phospholipase and intracellular lipase activities allow the release of polyunsaturated fatty acids to the fetus, transported as triglycerides in maternal plasma lipoprotein. It is well kwown that normal fetal development needs the availabilty of both essential fatty acids and long chain polyunsaturated fatty acids, thus making a persuasive case indicating a relationship between nutritional status of mother during gestation reflecting her lipid profile and fetal growth.

Fetal delopment is sustained by the metabolites crossing the placenta at the expense of those present in maternal circulation. Glucose is quantitatively the most important nutrient crossing the placenta, followed by amino acids (Knipp *et al.*,1997) and Hay *et al.*, 1994), and the development of the fetus directly depends on their continuous availability. However , although the placental transfer of lipid components is very limited (Herrera *et al.*, 1998) they also play a major role in fetal development. Changes in the availability of lipid components, like those produced by changes in dietary fatty acids, are known to have implications in fetal and postnatal development(Herrera *et al.*, 2002). In addition, adaptations of maternal lipid metabolism taking place throughout gestation also have major consequences for fetal growth. It is known that deviations in maternal hyperlipidemia, such as those caused by hypercholesterolemia, even when temporary and limited to pregnancy, trigger pathogenic events in the fetal aorta and may lead to atherosclerosis later in life (Napoli *et al.*, 2000 and Plainski *et al.*, 2001). Two consistent manifestations of altered maternal lipid metabolism normally occuring during gestation are the accumulation of lipids in maternal tissues (Hytten *et al.*, 1997) and the development of maternal hyperlipidemia (Alvarez *et al.*,

1996). Conditions known to alter any of these mannifestations by impairing maternal fat accumulation, such as hypothyroidism or overt diabetes during the first half of gestation, greatly affect fetal growth at late gestation, even if they are compensated for by appropriate hormonal treatment during the second half of gestation(Bonet *et al.,* 1991). These findings therefore emphasize the important role of maternal lipid metabolism of fetal growth and late pregnancy outcome, despite the difficulty of lipids crossing the placenta.

2. 3. 2 Adipose Tissue Metabolism and Lipolytic Activity

An accelerated breakdown of fat depots occurs in both women and rats during the last third of gestation(Sivan *et al.,* 1999 and Williams *et al.,* 1978) and higher activity and mRNA expression of the kidney enzyme for adipose tissue lipolysis, hormone-sensitive lipase, in late pregnant rats have been reported(Martin-Hidalgo *et al.,* 1994). Despite the enhanced release of lipolytic products, nonesterified fatty acids(NEFA) and glycerol into maternal circultion, their placental transfer is quantitatively low (Herrera *et al.,* 1998). The main destination of these products is the liver (Mampel *et al.,* 1985), where, after the conversion to NEFA in acetyl-CoA and glycerol into glycerol-3-phosphate, they are resterified for synthesis of triglycerides(Fig. 2.3). These are incorperated into nascent very low-density lipoprotein(VLDL), particles which are converted into the circulation, where they are converted into mature very low-density lipoproteins. Since insulin may inhibit VLDL secretion (Mason., 1998), a maternal insulin-resistant condition may contribute to the increased VLDL secretion

production. However, augumented estrogen concentration at late pregnancy seems to be the major activator for VLDL liver production and not only insulin-resistance.

Under fasting conditions during late pregnancy, maternal adipose tissue lipolytic activity becomes highly enhanced (Freinkel *et al.,* 1990). A heightened catecholamine excretion secondary to maternal hypoglycaemia even under mild dietary deprivation, together with the increased amount of gestational hormones released by the placenta and ovary, as well as the insulin- resistant conditions, seems to be responsible for higher lipolytic activity of maternal adipose tissue.

Maternal plasma concentration of glycerol and NEFA increase during late gestation (Herrera *et al.,* 1987) as a consequence of the enhanced adipose tissue lipolytic activity. Beside the use of those lipolytic products in the synthesis of triglycerides, glycerol may be used for glucose synthesis and NEFA for β-oxidation to acetyl-CoA and ketone body synthesis. These metabolic pathways become enhanced under fasting conditions at late pregnancy, when the use of glycerol for gluconeogenesis is even higher than other more gluconeogenic substrates, such as alanine and pyruvate (Zorzano *et al.,* 1984). Under this condition of food deprivation, ketogenesis is also greatly heightened in maternal liver(Herrera *et al.,* 1969). Increased gluconeogenesis from glycerol and ketogenesis from NEFA may benefit the fetus, which at late gestation is at its maximum accretion rate and its requirements for substrates and metabolic fuels are greatly augmented. The preferential use of glycerol for gluconeogenesis and efficient placental transfer of the newly formed glucose may be of major importance to the fetus under these fasting conditions (Fig.2.4), in which the availablity of other essential substrate such as amino acids is reduced (Herrera *et al.,*

1992). Placental transfer of ketone bodies is highly efficient, reaching fetal plasma at the same level as in maternal circulation (Herrera *et al.*, 1987).

Ketones may be used by the fetus as fuels and substrate for brain lipid synthesis (Shambaugh *et al.*, 1992).

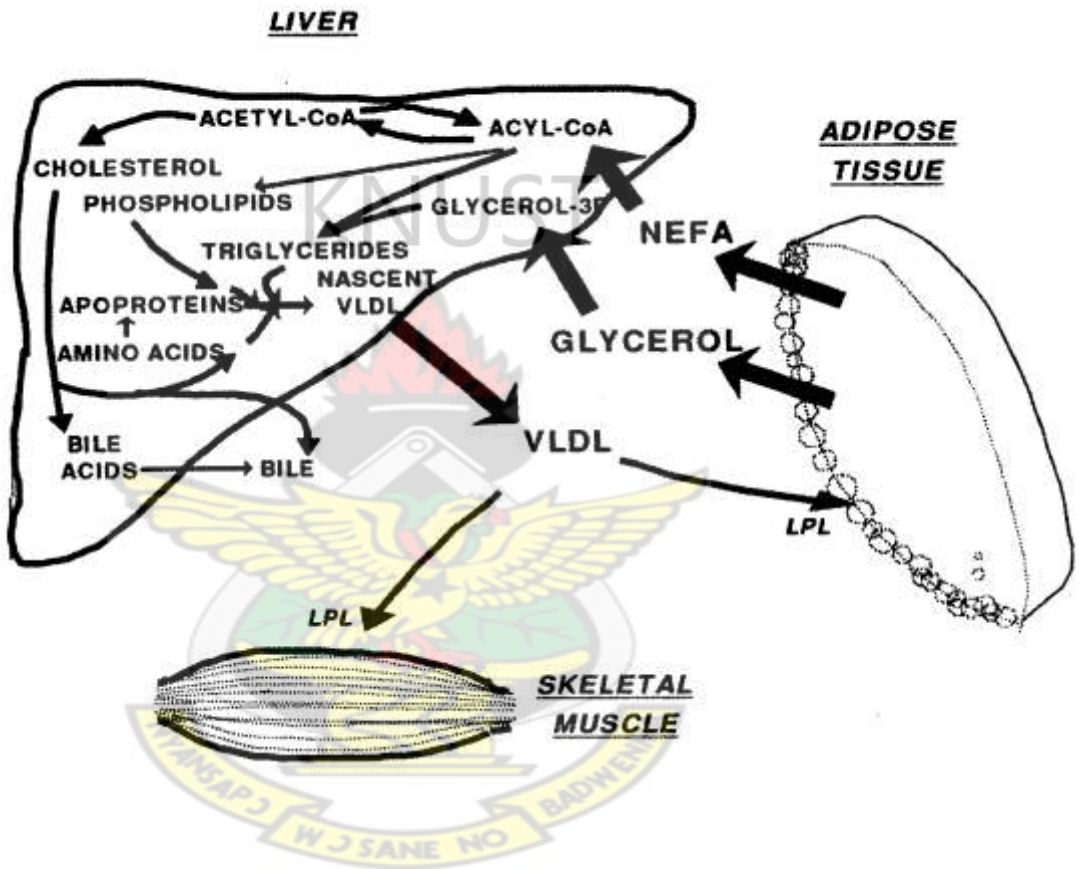

Figure: 2.3 Schematic Representation Of Utilization By Liver Of Adipose Tissue Lipolytic Products, nonesterified fatty acids and glycerol as substrates for triglyceride synthesis and very low density lipoprotein production for their catabolism by extrahepatic tissues.

2.3.3 Accumulation of Body fat in pregnancy

Accumulation of fat is one of the most common characteristics of pregnancy, occuring in both women (Villar *et al.,* 1992 and King *et al.,* 1994) and experimetal animals (Lopez *et al.,* 1991). Fat accumulation accounts for the conceptus-free increase in maternal body weight during gestation (Lederman *et al.,* 1999). The increase in maternal fat deposits occurs during the first two-thirds of gestation, and declines or during the last third (Hytten and Leitch., 1997), which corresponds to the phase of most accelerated lipolytic activity of adipose tissue.

Accummulation of body fat during early pregnancy has been associated with both hyperphagia and increased lipogenesis. Hyperphagia is present in both pregnant women (Piers *et al.,* 1995) and rats (Lederman and Rossso., 1980), and increases as gestational time advances. This progressive increases in the availability of exogenous substrates actively contributes to maternal accumulation of fat depots. A study showed that total energy cost of fat deposition in women from poor countries is lower than those observed among well-nourisehed populations(Prentice and Goldberg, 2000).

Fatty acid and glycerol synthesis from glucose by rats peri-uterine adipose tissue in situ, progressively increases until day-20 of gestation and sharply decline on day-21 (Herrera *et al.,* 1991)**.** Enhanced fatty acids synthesis was also found in pregnant rats when studied in vivo (Fain and Scow., 1966), and this increased lipid synthesis seems to actively contribute to the fat accumulation occuring during gestation.

The accumulation of fat which is usually observed during early pregnancy has been associated with a proportional increase in adipose tissue lipoprotein lipase (LPL) activity. This enzyme is normally bound in its active form to the capillary endothelium of extrahepatic tissues(Braun and Severson., 1992) and hydrolyses triglycerides

circulating in plasma in the form of triglyceride-rich lipoproteins such as chylomicrons, and VLDLs which are respectively converted to remnant particles and intermediate-density lipoproteins(figure 1). The hydrolytic products, Non-esterified Fatty acids (NEFA) and glycerol, are partially taken up by the subjacent tissue thereby controlling the fat uptake in adipose tissue. Although a few reports have shown that at day-12 of gestation in pregnancy rats experience an increase in the LPL activity of adipose tissue which is small and not always reproduced (Martin-Hidalgo *et al.,* 1994). No significant change has been in the post heparin LPL activity in pregnant women at mid-gestation(Alvarez *et al.,* 1996). Thus , enhanced LPL activity of adipose tissue does not seem to contribute substantially to the accumulation of body fat taking place during the first part of gestion.

During late pregnancy, adipose tissue LPL consistently decreases in rats (Ramirez *et al.,* 1983) and postheparin LPL also decreases in women at third trimester of pregnancy (Kinnunen *et. al.*, 1980). These findings indicate that fat uptake by adipose tissue decreases during late pregnancy, which together with enhanced lipolytic activity mentioned earlier, result in a net accelerated breakdown of fat deposits. It seems to reason that, the anabolic condition of adipose tissue present during early pregnancy switches to net catabolic condition during the the last trimester of pregnancy, coinciding with the phsae of maximal fetal growth. These changes contribute to the development of maternal hyperlipidemia , which besides other lipids, includes increments in plasma levels of NEFA, sparing glucose, which are essential not only to certain maternal tissues, such as brain, but also to sustain fetal development.

Enhanced maternal insulin levels and changes in insulin sensitivity which occurs throughout pregnancy may be directly responsible or indirectly responsible for the early anabolism and late cabolism present in maternal adipose tissue in pregnancy. During early pregancy, heightened pancreatic β- cell is developed and shown by the augmented insulinotropic effect of glucose seen in both women and rats (Munoz *et al.,* 1995). At this stage, insulin sensitivity is either unchanged or even augmented. Since both glycerolgenesis and lipogenesis from glucose are pathways sensitve to insulin, maternal hyperinsulinemia whenever the mother eats, ought to contribute to her active deposition of fats depots. The situation changes drastically during the last third of gestation, when, despite maternal hyperinsulinemia, a major insulin-resistant condition developes(Girard *et al.,* 1995). Studies in isolated adiposites from pregnant women have shown that insulin resistance is responsible for both enhanced and adipose tissue lipolytic activity and decreased LPL activity of adipose tissue(Sivan *et al.,* 1999). The transition from anabolic to catabolic condition in maternal adipose tissue metabolism concides with the maximal fetal growth phase (Herrera *et al.,* 1994), i.e when the mother needs to progressively increase the supply of nutrients to the fetus. This situation causes the development of maternal hyperlipidemia, which spares glucose and other essential metabolites, such as amino acids, for the fetus. This is of major importance not only to maternal metabolic economy but fetal development.

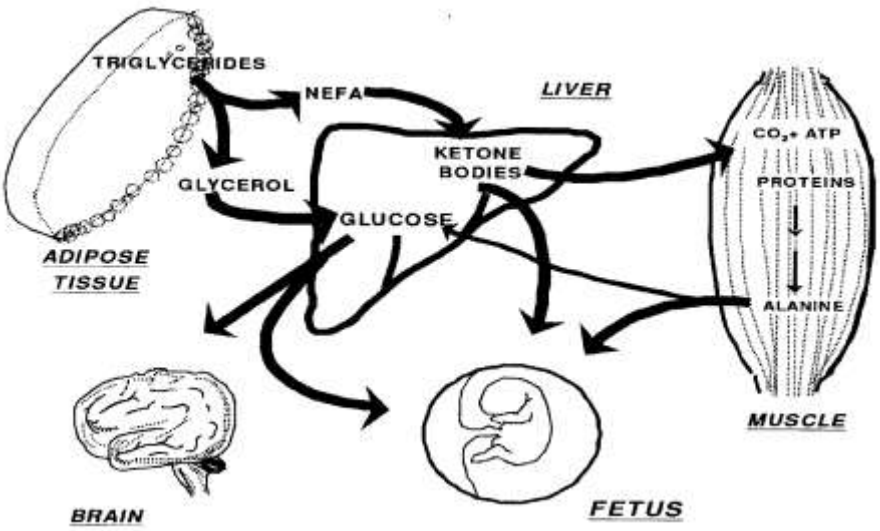

Figure 2.4*:* Schematic Representative Of The Role Of Adipose Tissue Triglyceride Stores as source of nonesterified fatty acids and glycerol for liver ketogenesis and gluconeogenesis during late pregnancy in fasting condition, to sustain availability of substrate for fetal and maternal tissues.

2. 3. 4 Hyperlipidemia in pregnancy

Maternal hypertriglyceridemia is also a characteristic feature during pregnancy and usually appears in marked amounts, whereas rises in phospholipids and cholesterol are smaller (Knopp *et al.*, 1992). This change corresponds not only to an increment in VLDLs but also to an enrichment of triglycerides in other lipoprotein fractions that normally do not transport them, such as low density lipoproteins (LDLs) and high density lipoproteins (HDLs). Even within the HDL sub-fractions, there is a specific increment in the proportion of triglycerides in the HDL_{2b} sub-fractions at the expense of the HDL_{2a} or HDL_3, which are rich in cholesterol **(**Alvarez *et al.*, 1996). The greatest increase in plasma triglycerides corresponds to the VLDL triglycerides (Montelongo *et al.,* 1992), synthesized in the liver. The main factors inducing this

increase in plasma VLDL triglycerides during gestation are the enhanced production by the liver and their decrease removal from the circulation as a consequence of reduced LPL activity of adipose tissue (Martin-Hidalgo *et al.,* 1994).

An increase in cholesterol ester transferase protein activity taking place at mid gestation (Iglesias *et al.,* 1994) together with the abundance of VLDL triglycerides seems to contribute to the accumulation of triglycerides in the other lipoproteins, LDL and HDL (Montelongo *et al.,* 1992), which under non-pregnant conditions are normally poor in this lipid moiety. Another factor contributing to this same effect is the decrease in the hepatic lipase activity, which also occurs during late pregnancy. The decrease in this enzyme activity decreases the conversion of buoyant HDL_2 triglyceride-rich particles into small HDL_3 triglyceride-poor particles, allowing a proportional accumulation of the former (Alvarez *et al.,* 1996).

Both the insulin-resistant condition and the increase in plasma oestrogen levels occurring during late pregnancy are the main hormonal factors responsible for these metabolic changes addressing to the development of maternal hypertriglyceridemia. The insulin-resistant condition constantly present during late gestation is known to contribute to both enhanced lipolytic activity of adipose tissue, which, as discussed, augments the arrival of glycerol and NEFA to the liver and their subsequent conversion into triglycerides, which are released back into the circulation in the form of VLDL and the concommittant decrease in LPL activity (Herrera *et al.,* 1990). The progressive increase in plasma oestrogen levels during gestation (Ramos *et al.,* 1995) also actively contributes to maternal hypertriglyceridemia; this has been shown to enhance liver production of VLDL (Knopp *et al.,* 1997) and decreases the expression and activity of hepatic lipase in liver (Brinton, 1996).

2.3. 5 Role of maternal hypertriglyceridemia as a source of fatty acid for the fetus

Although triglycerides do not directly cross the placental barrier (Herrera *et al.*, 1998), essential fatty acids (EFA) derived from maternal diet, which are transported as triglycerides in triglyceride-rich lipoproteins in maternal plasma (Herrera, E., 2002), must become available to the fetus. Placental trophoblast cells have been shown to express both very low-density apo-E receptors as well as LDL receptor-related proteins. In addition, they also express LPL activity, phospholipase A_2 and intracellular lipase activity (Rice *et al.*, 1998).

Maternal triglycerides in plasma lipoproteins are therefore hydrolyzed and taken up by the placenta, where there are re-esterified to provide a reservoir of fatty acids. After the intracellular hydrolysis of triglycerides, released fatty acids diffuse into fetal plasma; where they bind to a specific onco-fetal protein, the α-fetoprotein (Benassayag *et al.*, 1998). Fatty acids are then rapidly transported to fetal liver, where they are re-esterified and released back into circulation in the form of triglycerides.

Because the amount of polyunsaturated fatty acids present in plasma in the form of non-esterified fatty acids (NEFA) represents a minor proportion compared to those carried in the form of lipoproteins (Herrera *et al.*, 2002), the mechanism described indicates that maternal hyperlipoproteinemia plays a key role in the availability of EFA to the fetus. In fact, a linear correlation has been found between maternal and fetal plasma triglycerides in the rat (Herrera *et al.*, 2002), and a direct relationship between maternal triglycerides and newborn weight has also been found in humans (Kitajima *et al.*, 2001). Furthermore, a reduction in maternal hypertriglyceridemia, such as that

caused by treatments with hypolipidemic drugs, has negative effect on fetal development (Soria *et al.*, 2002).

2.3. 6 Transfer of lipid metabolites to fetus

2. 3.6. 1 Nonesterified fatty acids

The net flux of fatty acids crossing to the placenta differs among species. In species with a placenta having both maternal and fetal layers, such as sheep, pigs, and cats, the maternal fetal fatty acids transfer is small (Soria *et al.*, 2002). In species in which the placenta is formed by layers of fetal origin, such as rabbit (Elphick and Hudson., 1984), the amount of fatty acids crossing the placenta exceeds even that needed to fulfill lipid storage requirements. In humans, although in smaller proportions than lipoprotein triglycerides, maternal plasma NEFA are important source of polyunsaturated fatty acids for the fetus (Kuhn and Crawford., 1986).

The plasma membrane fatty acid-binding protein present in human placental membrane (Campbell et al., 2000) is responsible for the preferential uptake of long chain polyunsaturated fatty acids (LCPUFA). A selective cellular metabolism of certain fatty acids may also contribute to the placental transfer process, as would the conversion of a certain proportion of arachidonic acid to prostaglandins, the incorporation of some fatty acids into phospholipids, and the oxidation of placental fatty acid, and the synthesis of fatty acids.

The combination of all these processes determines the actual rate of placental fatty acid transfer and its selectivity, resulting in the proportional enrichment of certain LCPUFA, such as decosahexaenoic (DHA) in fetal as compared with maternal compartments (Crawford *et al*., 1976).

2.3.6. 2 Role of Cholesterol in the offspring

Cholesterol plays a key role in embryonic and fetal development. It is an essential component of cell membranes, where it contributes to the membrane fluidity and passive permeability by interacting with phospholipids and sphingolipids (Ohvo-Rekila *et al.*, 2002). Cholesterol is precursor of bile acids and steroids, and the fetus at late pregnancy there is an intense synthesis of glucocorticoids in the adrenals. Cholesterol is also required for cell proliferation (Suarez *et al.*, 2002) and plays important role in cell differentiation and cell-to-cell communication (Mauch *et al.*, 2002). In addition, cholesterol and its additives, oxysterols, are key regulators of different metabolic processes (Schroepfer *et al.*, 2000).The demand for cholesterol in the embryo and fetus are therefore relatively high. The fetus may obtain cholesterol from endogenous synthesis as well as from the yolk sac and the placenta.

Placental transfer of maternal cholesterol has been shown to be effective in different species, such as rat, guinea pig, and rhesus monkey (Pitkin *et al.*, 1992) although the estimated contribution of maternal cholesterol to fetus were quite variable, mainly owing to differences in methodology. Cholesterol synthesis in fetal tissues, and especially in fetal brain, is highly active in different species, even higher than in maternal tissues when expressed per mass unit (Haave and Innis., 2001). These findings are consistent with the high level of mRNA expression of different enzymes involved in cholesterol synthesis (Levin *et al.*, 1989) and the high activity of 3-hydroxy-3-methyl glutaryl coenzyme A reductase, the rate-limiting enzyme of cholesterol synthesis, in fetal tissue.

In the rat, it has been shown that the fetus receives little or no cholesterol from the mother, satisfying its need for cholesterol through endogenous synthesis ((Haave and Innis., 2001). Feeding pregnant rat with cholesterol, which resulted in increased plasma cholesterol concentration and reduced maternal cholesterol synthesis, did not affect any of these parameters in the fetus or fetal development (Munilla and Herrera., 1997). All of these findings led to the conclusion that in the rat, during late gestation, fetal cholesterol originates mainly from endogenous de novo synthesis rather than from placental transfer. These findings indicate that during early pregnancy in the rat, maternal cholesterol reaches the fetus and plays important physiologic role.

In humans, it has been found that umbilical venous level of HDL-, LDL-, and total-cholesterol concentrations were higher than in the umbilical arterial plasma at term, indicating the delivery of cholesterol from placenta to fetus, but the contribution of this cholesterol to the fetal plasma cholesterol pool was very small (Parker *et al.*, 1983). Comparison of maternal lipoprotein cholesterol levels and those in mixed umbilical cord blood cholesterol gave either a positive correlation (Ortega *et al.,* 1996) or no correlation between these values (Parker *et. al.*, 1983). Gestational age could influence these comparisons since plasma cholesterol levels have been found to be higher in 5- than in 7-month-old foetuses (Napoli *et. al.*, 1997). In foetuses younger than 6 month, plasma cholesterol are significantly correlated to the maternal ones (Napoli *et al.,* 1997), suggesting that maternal cholesterol actively contributes to fetal cholesterol in early gestation.

The presence of several lipoprotein receptors in the placenta (Gaoua *et al.,* 2000 and Suarez *et al.,* 2002) and, to a lesser extent in the yolk sac (Wyne and Woollette., 1998) allowing these tissues to take up cholesterol from

maternal lipoproteins, although the contribution of this process to the export of cholesterol to the fetus remains to be clarified.

2.3.6.3 Role of dietry fatty acids in the offspring

Normal fetal development needs not only the esterified fatty acids (EFA) but also their long-chain polyunsaturated fatty acids (LCPUFA) derivatives to support the synthesis of structural lipids (Neuringer ad Connor., 1996). Both term and preterm infants seem to be able to form arachidonic acid (AA) from α-linoleic acid and decosahexaenoic (DHA) from α-linoleic acid, which are their respective EFA precursors (Uauy *et al.,* 2000) however the degree to which the fetus is capable of fatty acid desaturation and elongation is not clear. Although fetal baboons have been shown to effectively synthesize both AA and DHA from their respective EFA precursors (Su *et al.,* 2001), in the newborn infant during the first week of life, the endogenous synthesis of AA seem to contribute very little to the plasma AA pool (Szitany *et al.,* 1999), the limiting factor being a low desaturation activity.

During gestation, a reduced nutritional status with respect to EFA has been correlated to reduce neonatal growth (Jumpsen *et al.*,1997), and plasma levels of LCPUFA has been consistently correlated between the mother and the fetus or newborn in untreated healthy woman (Matorras *et al.,* 1999). After fish oil supplementation during pregnancy, increment in DHA levels has been found in mother and newborns. Since these findings show the important role of dietary fatty acids during pregnancy in controlling the supply of LCPUFA to the fetus and newborn, supplementation with oils

rich in these fatty acids has been advised during the last trimester of pregnancy (Connor *et al.,* 1996).

The inhibitory effects of an excess of certain dietary fatty acid on LCPUFA synthetic pathways may acquire major relevance during the perinatal period, where the AA has been correlated to body weight in preterm infants (Wolti *et al.,* 1998). Adverse effects of low AA concentration in blood on growth during infancy have also been reported (Carlson *et al.,* 1993). When polyunsaturated fatty acids (PUFA) intake is increased, the PUFA content of the LDL particles increases concordantly (Abbey *et al.,* 1993). The in vitro susceptibility of LDLs to undergo oxidative modification was reported to increase with diets rich in ω-6 PUFA (Abbey *et al.,* 1993). An increase in plasma thiobarbituric acid reactive substances (TBARs) was also found after dietary periods of ω-6 PUFA enrichments (Berry *et al.,* 1991). Whether a diet high in ω-3 PUFA increases lipid peroxidation is controversial (Mori and Beilin., 2001). Whereas several studies in humans have shown that dietary supplementation with fish oil rich in ω-3 PUFA does not increase in vivo lipid peroxidation (Wander and Du., 2000), other studies in rats and in cell culture have shown that this same treatment reduces the antioxidant capacity (Anusquivar *et al.,* 2000) and enhances susceptibility to oxidative damage (Song *et al.,* 2000). Increased reactive oxygen species and lipid peroxidation resulting in fetal damage, as well as its reversion by vitamin E treatment, have been experimentally shown to take place in diabetic pregnancy (Cederberg and Eriksson., 2001). However, it is not yet clear whether oxidative stress plays any role in the development of complications in diabetic patients (Jain., 1999). In addition, it has been shown that treating diabetic children with high doses of antioxidative agents, including vitamin E, has no effect on the preservation of β-cell function or on metabolic balance

(Ludvigsson *et. al.,* 2001). Some studies (Claycombe and Meydani., 2001) suggest a potential usefulness of vitamin E in the prevention of mutagenic effects cave used by genotoxic free radicals, whereas other studies report none.

The negative effect of high density fish oil intake during pregnancy on offspring could be mediated either by the decreased AA levels (Bourre *et al.,* 1990) or by an enhanced consumption of α-tocopherol owing to high LCPUFA content in fish oil. In contrast with fish oil, dietary olive oil protects the ω-3PUFA series (241), does not affect AA concentrations (Rao *et al.,* 1993), and is much more resistant to lipid peroxidation (Oztezcan *et al.,* 1996). A comparative study of these variables in rats fed a diet supplement with either 10% fish oil or olive oil as the only nonvitamin lipid during pregnancy and lactation was carried out. A decrease in AA and α-tocopherol concentration as well as a delay in postnatal development was found in the offspring of rats fed the oil-rich diet (Annusquivar *et al.,* 2000). The study was extended to determine whether dietary supplementation with either vitamin E or γ-linolenic acid as a precursor of AA, could ameliorate these changes. Both AA concentration and postnatal development indexes, although not α-tocopherol concentration, were recovered when the fish oil diet was supplement with γ-linolenic acid. However, postnatal development indexes were not recovered when the fish oil rich diet was supplemented with sufficient exogenous vitamin E to normalize α-tocopherol levels (Anusquivar *et al.,* 2000). It was therefore concluded that low AA acid rather than α-tocopherol was responsible for the delayed postnatal development in the offspring of rats receiving a diet supplement with fish oil instead of olive during pregnancy and lactation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 RESEARCH DESIGN

The study was an analytical one which was conducted amongst pregnant women in James Town Maternity of the Ussher Polyclinic of Ashiedu Keteke Sub Metropolis of the Accra Metropolitan area in the Greater Accra Region of Ghana between 2006 and 2009. Pregnant women attending the Polyclinic were examined irrespective of the gestational age by an Obstetrician/ Gynaecologist and Obstetric nurse for inflammatory diseases. The pregnant women were grouped into three, according to their gestational age (1-14 weeks) –First Trimester, (15-27weeks)-Second Trimester and (28-42weeks) –Third Trimester. A fourth group made up of non-pregnant women was recruited as controls.

3.2 STUDY SITE/ AREA

Ashiedu Keteke Sub Metropolis of the Accra Metropolitan area was the study area. The study site however, was James Town Maternity of the Ussher Polyclinic which serves as the the main antenatal/maternity facility for the Ashiedu Keteke Sub metropolis of the Accra metropolitan area. The sub metro has an estimated population of 142,467 as at 2011. The population was extracted from the 2000 National Population census.

The population of the Sub-metro is comprised of traditional indigenous Gas that live in communities along the coast and migrant population from various parts of the country who have come to settle in these communities further away from the coast. The

traditional indigenous Gas form the majority of population and live in James Town, Bukom, Sempe, Ngleshie whilst the migrants reside in Agbogbloshie, Yam market, Kokomba, Sodom and Gomorrah. Majority of the migrants' population are people from the Northern part of Ghana and Ewes from the Volta region and Akans from the other Regions of Ghana.

The Ashiedu Keteke sub-metro is the smallest but the most populated in the Accra Metropolis. Although it is situated in an urban area, it is deprived in respect of many social amenities. Ashiedu Keteke is at the centre of Accra Metropolitis and the centre of commercial activities in Accra. The major occupation within the traditional areas is fishing among the men and fish mongers and petty traders among the women population. The migrants are mostly petty traders; hawkers and 'Kayayei' in Agbogbloshie and Yam markets. There are also civil servants, private organizations and companies. Participants for the study were virtually made up of individuals from all the Regions of Ghana living in these areas.

3.3 STUDY POPULATION

The study population (239) was made up of pregnant women receiving antenatal care at James Town Maternity of the Ussher Polyclinic. Irrespective of the gestational age, pregnant women receiving antenatal care (ANC) services at this health facility were randomly selected for the study as well as nonpregnant women as controls.

3.4 RECRUITMENT OF STUDY WOMEN

Prospective participants were Ghanaians attending the antenatal clinic who agreed to participate in the study by signing the consent form.The purpose of the study was explained to all participants attending the antenatal clinic serivces. The risks and benefits associated with the study were also explained to the participants.

Inclusion criteria

All pregnant women without any history of pregnancy –associated complications were eligible for inclusion in the study.

Exclusion criteria

All pregnant women with history of pregnancy–associated complications and some other complications such pre-eclampsia and eclampsia, diabetes mellitus, kidney and liver diseases, any other systemic disorder, anemia (Hb < 10.0 g/dL), chronic disease and history of alcoholism was excluded from the study.

3.5 DATA COLLECTION

Primary data was collected using structured questionnaire administered to pregnant women by Research Assistants. Information regarding the demographic characteristics, disease history and any other known risks associated with pregnancy were captured using the questionnaire. Anthropometric measurements were used to determine Body Mass Index (BMI) and blood pressure. Blood sample was taken for the determination of lipid peroxidation, lipid profiles and other biochemical assays.

3.5.1 Sampling and Allocation of study women

Convenience sampling technique was used to select pregnant women based on inclusion and exclusion criteria

Pregnant women reporting regular to antenatal clinic at the James Town Maternity of the Ussher Polyclinic were recruited and allocated into three categories based on the age of the pregnancy. The study participants were grouped into three gestational ages: First Trimester (1-14 weeks), Second Trimester (15-27weeks) and Third Trimester (28-42weeks).

3.5.2 Anthropometric Assessment of Subjects:

All women were clinically examined by an Obstetrician/Gynaecologist and Obstetric nurse after which anthropometric measrements taken. Weight (Kg) of subjects were taken using standardised scales and Height(m) using a standardised measuring tape. Body Mass Index(BMI) BMI= [Weight (Kg)/ (Height $(m)^2$]) was the derived using the equation above.

3.5.3 Measurement of Blood Pressure

Diastolic and Systolic blood pressure measurements were taken by a qualified nurse using a sphygmomanometer and stethescope from the left upper arm of subjects after sitting for at least five minutes.

3.5.4 Blood Sampling

Each recruited pregnant woman had to fast 10-14hours overnight and fasting blood sample (10 millilitres) asceptical collected between the hours of 6.00 am-9.30 am each day by a phlebotomist into plain tubes. Blood samples were allowed to clot, centrifuged and serum collected for analysis of laboratory parameters. Samples that were meant for glucose analysis were collected into fluoride oxalate tubes before centrifugation and separation. Serum and plasma samples that could not be analyzed immediately were stored at –20ºC until required for use. Samples that were required for glucose determination were collected into gray top tubes.

3.5.5 Urine Sampling

About 10mls of early morning urine sample was collected from each of the study participants for protein and glucose analysis.

3.6 SAMPLE SIZE DETERMINATION

Sample size of 200 was estimated for the study, however, a total of 239 pregnant women were recruited in order to increase the power of the study. Thus, a power of 90% at 0.05 α significance level was assumed. Sample size was estimated using the equation:

$N = Z^2 (P)(1-P) /(Error)^2$,Where N = minimum sample size.

P = Prevalence level of 50% of oxidative stress was assumed

Z= 1.96 (the standard score for the confidence interval of 95%).

The allowable error margin was 7 % (alpha 0.05)

$N= 1.96^2(0.50)(0.50) / (0.07)^2$

N=196

A total of 81 first trimester, 125 second trimester and 33 third trimester pregnant women were respectively recruited for the study. Also, a total of 48 non-pregnant women were recruited as controls to match age for the study women.

3.7 LABORATORY METHODS

The following laboratory methods were used to determine various laboratory parameters using both blood and urine samples.

3.7.1 Serum Malondialdehyde (MDA)

The determination of oxidative stress indice was analysed by the method described by Kamal *et al.,* (1989) and Shlafer and Shepard (1984). The thiobarbituric acid (TBA) reacting substances (TBARS) assay has been used as an indicator of lipid peroxidation and levels of free radicals in serum samples. The assay was based upon the reaction of TBA with Malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation at the optimum pH and temperature conditions and the amount of MDA – TBA adduct produced was then measured. To increase sensitivity, the complex was extracted into an organic solvent such as butanol and measured spectrophotometrically at 535 nm.

A volume of 0.5ml of serum was treated with 2.5ml of 20% Trichloacetic acid and then thoroughly mixed with 1.0ml of 0.67% Thio-Barbituric Acid . The mixture was incubated at 100 ºC for 30 minutes. After cooling , the sample was extracted with 4.0ml n-butanol, vortexed for 30seconds before it was centrifuged at 500g for 10

minutes. The absorbance of the supernatant was measured at 535nm wavelength and the results expressed in umol/L using the extinction coefficient of 1.56 $X10^5$ $Lmmol^{-1}cm$. ***(Kamal et al., 1989***).

Biochemical Assessments:

Biochemical assays carried-out on serum samples included Triglycerides(TG),Total Cholesterol(TC), High Density Lipoprotein(D-HDL),Low Density Lipoprotein (D-LDL), Albumin(ALB), Uric Acid(UA), Magnesium(Mg^{2+}) and Glucose(GLUC). Assays were performed using Randox reagents which were used on DAYTONA Chemistry Analyser by Randox Laboratories Systems,USA. Prinicples and methods utilised on this analyser for each test are given below.

Magnesium (Mg): Principle is based on Magnesium ions reacting with xylidyl blue in an alkaline medium to form a water soluble purple-red chelate, the colour intensity of which is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexing with Ethylenediaminetetra Acetic Acid.

Total Cholesterol (T.Chol): Total Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase and measured at 500nm spectrophotometrically.

Albumin(Alb):The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromocresol sulphonphthalein (bromocresol green). The albumin –BCG-complex absorbs maximally at 578 nm.

Glucose (Gluc):The method is totally enzymatic utilizing both hexokinase and glucose-6-phosphate dehydrogenase enzymes. The absorbance of final colour developed is measured in the UV at 340nm.

Direct LDL-Cholesterol (DLDL-C): The assay consists of two distinct reaction steps: The elimination of chylomicron, VLDL-Cholesterol and HDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase. This is follwed by specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by detergents. The intensity of the quinoneimine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm.

Uric Acid (UA): Uric Acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet, quinoneimine compound.

Direct HDL Cholesterol (DHDL-C): The assay consists of two distinct reaction steps: The elimination of chylomicron, VLDL-Cholesterol and LDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase. The specific measurement of HDL-Cholesterol is done after release of HDL-Cholesterol by detergents. The intensity of the quinoneimine dye produced is directly proportional to the cholesterol concentration when measured at 600 nm.

3.8 STATISTICAL ANALYSIS

Continuous variables between groups were compared using general linear modelling, whilst categorical variables were compared using exact logistic regression. The rates of change were estimated for each patient separately by linear regression. General linear modelling, was used to compare the mean rate of change in outcome measurements between groups, unadjusted and adjusted for actual and potential confounding variables. Mean differences, 95% confidence intervals and *p*-values were corrected for repeated measures, and *p*-values corrected for multiple comparisons. A level of $p \leq 0.05$ was considered statistically significant.

3.9 ETHICAL ISSUES

The Committee for Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, KNUST ethical and administrative gave clearance for the study. Consent for study was obtained from each study participant and all information was treated as confidential and used for research purposes only.

CHAPTER FOUR

RESULTS

4.1. DEMOGRAPHICS AND CLINICAL ASSESSMENTS OF STUDY PARTICIPANTS

The total number of volunteers that participated in the research was two hundred and eighty-seven (287). The subjects comprised two hundred and thirty-nine (239) women with uncomplicated pregnancy. They were matched for age with forty-eight (48) apparently healthy non-pregnant women. The mean age for the pregnant women (study group) and the non-pregnant (control group) was 24.6 and 25.8 years respectively and this difference in means was not statistically significant (p = 0.1845) as shown in (Table 4. 1). The systolic (SBP) and diastolic (DBP) blood pressures were both significantly elevated in the study group (p = 0.0001) compared to control group. In addition, the difference in the mean body mass index (BMI) of the two groups was significant ($p < 0.007$).

TABLE 4.1: DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF STUDY PARTICIPANTS

Parameter	**Pregnant Women (N = 239)**	**Non-pregnant women (N=48)**	***p*-value**
Age (yrs)	24.6 ± 5.6	25.8 ± 6.2	0.1845
SBP (mmHg)	108. 6 ± 10.6	100.1 ± 5.8	0.0001*
DBP(mmHg)	66.3 ± 9.3	60.5 ± 6.1	0.0001*
BMI (Kg/m^2)	25.8 ± 4.7	23.8 ± 4.4	0.0070*
Gestation period	18.4 ± 6.5		

N = Sample size, * = Mean is statistically significant, ± = standard deviation.

4.2. COMPARISON OF DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF VARIOUS TRIMESTERS TO NON-PREGNANT WOMEN

Ssignificantly elevated results were obtained for both ssystolic ($p < 0.05$) and diastolic ($p < 0.05$) blood pressures in the second and third trimesters of the study group compared to control. However, no significant difference was observed in the first trimester ($p > 0.05$) when compared to contol goup. The significant difference in the mean body mass index (BMI) was seen only in the third trimester ($p < 0.05$) of pregnancy when compared to the control group (table 4.2).

TABLE 4.2: COMPARISON OF DEMOGRAPHIC AND CLINICAL ASSESSMENTS OF VARIOUS TRIMESTERS TO NON-PREGNANT WOMEN

Parameter	Non-pregnant	First Trimester	Second Trimester	Third Trimester
Age (yrs)	25.8 ± 6.2	25.6 ± 5.8	24.4 ± 5.3	23.8 ± 5.5
SBP (mmHg)	100.1 ± 5.8	104.1 ± 10.4	107.2 ±	112.3 ± 9.7***
DBP(mmHg)	60.5 ± 6.1	62.7 ± 8.2	66.4 ± 9.8***	71.9 ± 8.8***
BMI (Kg/m^2)	23.8 ± 4.4	24.5 ± 2.6	24.9 ± 4.1	27.9 ± 4.4***
Gestational		11.6 ± 2.2	19.9 ± 3.2	29.6 ± 2.3

***** =** $p < 0.001$ compared with non-pregnant women; ± = mean standard deviation.

4.3. BIOCHEMICAL ASSESSMENT FIDINGS IN THE SERUM OF UNCOMPLICATED PREGNANT AND NON-PREGNANT WOMEN

The lipid profile including Total Cholesterol(T.C), Triglycerides(TG), Low density lipoprotein(LDL-C) and Very low density lipoprotein cholesterol (VLDL-C), LDL–C /HDL–C ratio, T. C / HDL–C ratio, atherogenic index all show significantly elevated levels in studied subjects ($p < 0.05$) when compared with the control group. There was significant reduction in the levels of High density lipoprotein (HDL-C) and HDL-C/ VLDL-C ratio ($p < 0.05$) are significantly reduced when compared with the

control group. The liperoxidation marker, Malondialdehyde(MDA) was significantly raised higher in the study subjects ($p < 0.05$) compared with control group(Table 4.3). No significant difference however, were found in the levels of Uric Acid(UA), Albumin(Alb), Glusose(Gluc) and Magnesium(Mg^{2+}) of the studied subjects ($p > 0.05$) compared to the control group.

TABLE 4.3: COMPARISON OF SERUM BIOCHEMICAL PARAMETERS OF NORMAL PREGNANT AND NON-PREGNANT WOMEN

Parameters	Pregnant Women (N =239)	Non- Pregnant Women (N =48)	*p*-value
T. C (mmol/L)	4.9 ± 1.2	4.3 ± 0.8	0.0010*
HDL – C (mmol/L)	1.0 ± 0.6	1.3 ± 0.2	0.0007*
TG (mmol/L)	1.5 ± 0.5	1.2 ± 0.3	0.0001*
LDL – C (mmol/L)	2.1 ± 0.9	1.7 ± 0.5	0.0031*
VLDL-C (mmol/L)	0.7 ± 0.2	0.6 ± 0.2	0.0017*
LDL – C /HDL – C ratio	2.4 ± 1.8	1.4 ± 0.4	0.0002*
T. C / HDL – C ratio	5.8 ± 0.6	3.3 ± 0.8	0.0001*
HDL-C/ VLDL-C ratio	1.8 ± 1.3	2.4 ± 1.1	0.0030*
Atherogenic index	3.7 ± 1.2	3.3 ± 0.8	0.0278*
Albumin (mmol/L)	36.1 ± 4.7	37.2 ± 4.7	0.1401
Uric Acid (mmol/L)	209.8 ± 63.9	211.6 ± 50.6	0.8543
Mg^{+2} (mmol/L)	0.9 ± 0.1	0.9 ± 0.2	1.0000
Glucose (mmol/L)	4.7 ± 0.6	4.8 ± 0.5	0.2805
MDA (µmol/L)	1.5 ± 0.3	1.3 ± 0.2	0.0001*

* Mean difference is significant. Atherogenic index = Total chol. -HDL cholesterol / HDL chol., malondialdehye (MDA) is oxidative stress indicator. Cardiovascular risk= total cholesterol (T.C) / high density lipoprotein (HDL). Values are given as mean ± standard deviation.

4.4. COMPARISON OF SERUM BIOCHEMICAL INDICES OF VARIOUS TRIMESTERS OF SUBJECTS TO NON-PREGNANT WOMEN

Table 4.4 shows each trimester of pregnancy compared to the non-pregnant woman was clearly shows that malondialdehyde (MDA) levels were significantly higher in all trimesters ($p< 0.05$) compared to non-pregnant women. Most parameters also showed significant ($p < 0.05$) change in levels their in second and third trimesters. No significant changes were however found in the levels of Uric Acid (UA), Albumin

(Alb), Glucose (Gluc) and Magnesium ($Mg^{2+)}$ ($p > 0.05$) in the three timesters compared to the control group

TABLE 4.4: COMPARISON OF BIOCHEMICAL ASSESSMENTS OF VARIOUS TRIMESTERS OF SUBJECTS TO NON-PREGNANT WOMEN

Parameter	**Non-Pregnant Women (N =48)**	**First Trimester Pregnancy (n= 81)**	**Second Trimester Pregnancy (n= 125)**	**Third Trimester Pregnancy (n= 33)**
T. C (mmol/L)	4.3 ± 0.8	4.5 ± 1.0	4.9 ± 1.3*****	5.2 ± 1.0******
HDL–C (mmol/L)	1.3 ± 0.2	0.9 ±0.4**	1.0 ± 0.6**	1.1 ± 0.3
TG (mmol/L)	1.2 ± 0.3	1.3 ±0.4	1.5 ± 0.5*******	1.7 ± 0.3*******
LDL–C (mmol/L)	1.7 ± 0.5	1.8 ± 0.7	2.0 ± 0.9*	2.8 ± 0.9*******
VLDL-(mmol/L)	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.2***	0.9 ± 0.1*******
LDL – C /HDL – C ratio	1.4 ± 0.4	1.6 ± 1.3	2.0 ± 1.2***	2.6 ± 0.9***
T. C / HDL – C ratio	3.3 ± 0.8	3.8 ± 0.8**	4.9 ± 1.0***	4.8 ± 1.1***
HDL-C/ VLDL-C ratio	2.4 ± 1.1	1.8 ± 0.6***	1.4 ± 0.7***	1.4 ± 0.4***
Atherogenic index	3.3 ± 0.8	3.3 ± 1.0	3.9 ± 1.3***	4.2 ± 1.0***
Albumin (mmol/L)	37.2 ± 4.7	37.4 ± 4.5	35.7 ± 4.9	34.5 ± 3.3*
Uric Acid (mmol/L)	211.6 ± 50.6	218.5 ± 61.0	202.1 ± 61.6	217.9 ± 76.5
Mg^{+2} (mmol/L)	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
Glucose (mmol/L)	4.8 ± 0.5	4.8 ± 0.5	4.7 ± 0.6	4.4 ± 0.7
MDA (µmol/L)	1.3 ± 0.2	1.4 ± 0.2**	1.5 ± 0.5***	1.7 ± 0.3***

*p < 0.05, ** p < 0.01, ***p < 0.001, *** compared with non-pregnant women.

Table 4.5 : Pearson correlation between clinical and biochemical indices for all trimesters. *Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.01 level(2-tailed), ***Correlation is significant at 0.001 level(2-tailed). SBP: Systolic Blood Pressure,

	GES.AGE	*BMI*	*SBP*	*DSP*	TC	*HDL-C*	*TG*	*LDL-C*	*TC/HDL*	*VLDL*	*LDL/HDL*	*HDL/VLDL*	*TC-HDL/HDL*	*ALB*	*U*
0															
	1.000														
*	.177*	1.000													
*	-.056	.191*	1.000												
*	-.033	.113	.504***	1.000											
3	.255*	.148*	.098	.117	1.000										
0	.119	.035	.007	-.015	.116	1.000									
*	.194*	.089	.127*	.134*	.676***	-.008	1.000								
7	.331**	.113	.085	.060	.861***	.158*	.438**	1.000							
3	-.117	-.016	-.052	-.040	-.014	-.388**	.049	-.074	1.000						
*	.194*	.089	.127*	.134*	.676***	-.008	1.000	.438 **	.049	1.000					
3	-.033	.008	-.042	-.047	.161*	-.392**	.116	.188*	.946***	.116	1.000				
5	-.048	-.019	-.068	-.044	-.269**	.750***	-.497**	-.138 *	-.303**	-.497**	-.346**	1.000			
3	.255*	.148*	.098	.117	1.000	.116	.676***	.861***	-.014	.676***	.161*	-.269*	1.000		
2	-.198*	-.068	-.032	-.039	.062	-.054	.136*	-.056	.142**	.136*	.107	-.112	.062	1.000	
3	.009	-.043	.015	-.033	-.011	.118	-.059	.009	-.070	-.059	-.096	.094	-.011	-.008	1.00
1	-.030	-.013	-.090	.036	-.115	-.012	-.225*	-.049	-.086	-.225	-.099	.121	-.115	.029	-.1
*	-.195*	.040	.090	-.030	.115	.057	.033	.055	.053	.033	.051	.033	.115	.177*	.02
2	.354**	.151*	.083	.116	.905***	.107	.594***	.826 ***	.028	.594***	.200*	-.231 *	.905***	.026	.00

Diastolic Blood Pressure, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density lipoprotein, VLDL: Very Low Density Lipoprotein, ALB: Albumin, UA: Uric Acid, : Mg^{2+} : Magnesium, GLUC:Glucose, MDA: Malondialdehyde.

Table 4.6 : Pearson correlation between clinical and biochemical indices for second trimester.

	AGE	GES.AGE	BMI	SBP	DSP	TC	HDL-CHOL	TG	LDL-CHOL	TC/HDL	LDL/HDL
AGE	1.000										
GES.AGE	-.043	1.000									
WEIGHT	.412**	.051									
HEIGHT	.075	.064									
BMI	.406**	.019	1.000								
SBP	.247*	.118	.315*	1.000							
DSP	.175	-.013	.171	.454**	1.000						
CHOL	.293*	.043	.143	.095	.173	1.000					
HDL-CHOL	-.007	.183*	.023	.089	.064	.269*	1.000				
TG	.377*	.035	.146	.073	.183	.709***	.123	1.000			
LDL-CHOL	.217*	.098	.043	.104	.080	.893***	.291*	.505**	1.000		
TC/HDL	.119	-.177*	.004	-.051	-.035	.191*	-.742***	.220	.107	1.000	
LDL/HDL	.152	-.099	.006	-.013	-.054	.404**	-.594**	.302*	.444**	.909***	1.000
Hdl/ VLDL	-.270*	-.026	-.095	.037	-.002	-.349*	.331*	-.615***	-.211	-.494*	-.484*
VLDL	.377*	.035	.146	.073	.183*	.709***	.123	1.000	.505**	.220	.302*
ALB	.001	-.084	-.081	-.046	.006	.066	-.158	.191	-.071	.233*	.127
UA	.008	.191*	.013	-.016	-.042	.080	.208*	.047	.067	-.151	-.152
Mg^{2+}	.004	-.013	-.013	-.027	.088	-.082	.171	-.233*	-.053	-.195*	-.215*
GLU	.168	-.027	.062	.038	-.123	.146	.168	.073	.080	-.008	-.027
MDA	.259*	.063	.132	.117	.176*	.917***	.109	.625 ***	.825***	.263*	.449**

*Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.01 level(2-tailed), ***Correlation is significant at 0.001 level(2-tailed). SBP: Systolic Blood Pressure, Diastolic Blood Pressure, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density lipoprotein, VLDL: Very Low Density Lipoprotein, ALB: Albumin, UA: Uric Acid, : Mg^{2+} : Magnesium, GLUC:Glucose, MDA: Malondialdehyde. (-): Negative correlation.

Table 4.7: Pearson correlation between clinical and biochemical indices for third trimester.

	AGE	BMI	HDL-C	TG	LDL-C	VLDL	TC/HDL	LDL/HDL	HDl/VLDL	TC-HDL/HDL
AGE	1.000									
BMI	.206	1.000								
SBP	-.102	.043								
DSP	.037	.127								
TC	.004	.071								
HDL-C	-.224	-.103	1.000							
TG	-.136	.058	.242	1.000						
LDL-C	.005	.070	.397*	.213	1.000					
VLDL	-.136	.058	.242	1.000	.213	1.000				
TC/HDL	.200	.146	-.519**	.346*	.317*	.346*	1.000			
LDL/HDL	.158	.138	-.264	.112	.762***	.112	.732***	1.000		
HDl/VLDL	.039	-.163	.126	-.810**	-.137	-.810***	-.541**	-.304*	1.000	
TC-HDL/HDL	.004	.071	.316*	.621***	.711***	.621***	.623***	.547**	-.487**	1.000
ALB	-.177	-.038	.325*	.192	.284	.192	-.044	.041	-.005	.322*

UA	.003	-.213	.348*	-.149	-.115	-.149	-.336*	-.330 *	.260	-.068	
Mg2+	-.036	.005	.066	-.276	.009	-.276	-.249	-.071	.417**	-.207	

AGE	1.000											
BMI	.206	1.000										
SBP	-.102	.043	1.000									
DSP	.037	.127	.616***	1.000								
TC	.004	.071	-.114	.217	1.000							
HDL-C	-.224	-.103	.104	.129	.316*	1.000						
TG	-.136	.058	-.091	.061	.621***	.242	1.000					
LDL-C	.005	.070	-.065	.117	.711***	.397 *	.213	1.000				
VLDL	-.136	.058	-.091	.061	.621***	.242	1.000	.213	1.000			
TC/HDL	.200	.146	-.244	.073	.623***	-.519**	.346*	.317 *	.346*	1.000		
LDL/HDL	.158	.138	-.186	.040	.547**	-.264	.112	.762***	.112	.732***	1.000	
HDl/VLDL	.039	-.163	.123	-.156	-.487**	.126	-.810***	-.137	-.810	-.541**	-.304*	1.000
TC HDL/	.004	.071	-.114	.217	.689***	.316*	.621***	.711***	.621***	.623***	.547**	.487**

GLU	.042	.147	.012	.183	.189	.183	.288	.179	-.185	.364*
MDA	-.026	.014	.334*	.616***	.572**	.616***	.519**	.395**	-.465**	.907***

*Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.01 level(2-tailed), ***Correlation is significant at 0.001 level(2-tailed). SBP: Systolic Blood Pressure, Diastolic Blood Pressure, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density lipoprotein, VLDL: Very Low Density Lipoprotein, ALB: Albumin, UA: Uric Acid, : Mg^{2+} : Magnesium, GLUC:Glucose, MDA: Malondialdehyde.

HDL												
ALB	-.177	-.038	-.003	.040	.322	.325*	.192	.284	.192	-.044	.041	-.005
UA	.003	-.213	.024	.069	-.068	.348*	-.149	-.115	-.149	-.336	-.330 *	.260
Mg^{2+}	-.036	.005	.023	-.112	-.207	.066	-.276	.009	-.276	-.249	-.071	.417**
GLU	.042	.147	-.071	.139	.364 **	.012	.183	.189	.183	.288*	.179	-.185
MDA	-.026	.014	-.125	.223	.907***	.334*	.616***	.572**	.616***	.519**	.395*	.465**

Table 4.8: Pearson correlation between clinical and biochemical indices for control group. *Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.01 level(2-tailed), ***Correlation is significant at *Correlation is significant at 0.05 level (2-tailed), **Correlation is significant at 0.001 level (2-tailed). SBP: Systolic Blood Pressure, Diastolic Blood Pressure, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density lipoprotein, VLDL: Very Low Density Lipoprotein, ALB: Albumin, UA: Uric Acid, : Mg^{2+} : Magnesium, GLUC:Glucose, MDA: Malondialdehyde.

Generally there was no significant correlation between the biochemical variables Uric acid, Magnesium and Glucose in relation to MDA within the control group, the second and third trimesters of pregnancy (Table 4.5- Table 4.8). There was a significant positive correlation between BMI and MDA when mean values of all three trimesters were considered (Table 4.5). There was generally also a significant positive correlation between most constituents of the lipid profile and MDA in the control group, second, third trimesters as well as when all three trimesters were combined. The HDL-C/VLDL-C ratio consistently showed a negative but significant correlation with MDA, lipids and their derivatives. However HDL-C showed a positive and insignificant correlation with MDA (Table 4.5- Table 4.8).

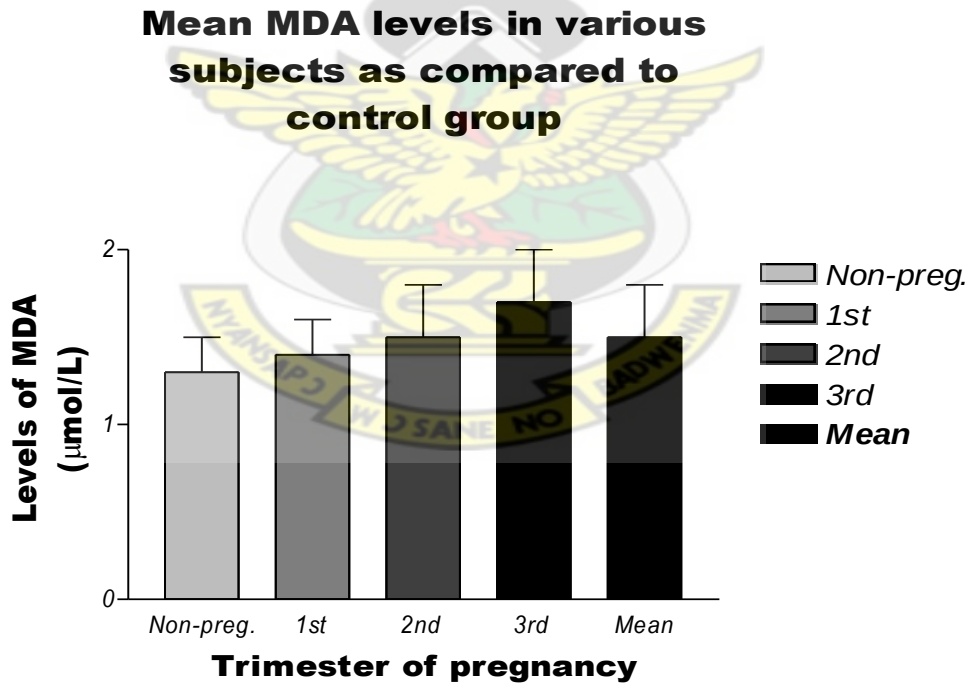

Figure 4.1: *Box- plot of mean MDA levels in various trimesters of pregnancy as compared to non-pregnant women.*

There was consistent increment in the MDA levels throughout the gestation as indicated by the means in trimester 1, 2 and 3 values. MDA levels (Fig. 4.1) in three

trimesters were also greater than the non- pregnant subjects. The increment was statistically significant.

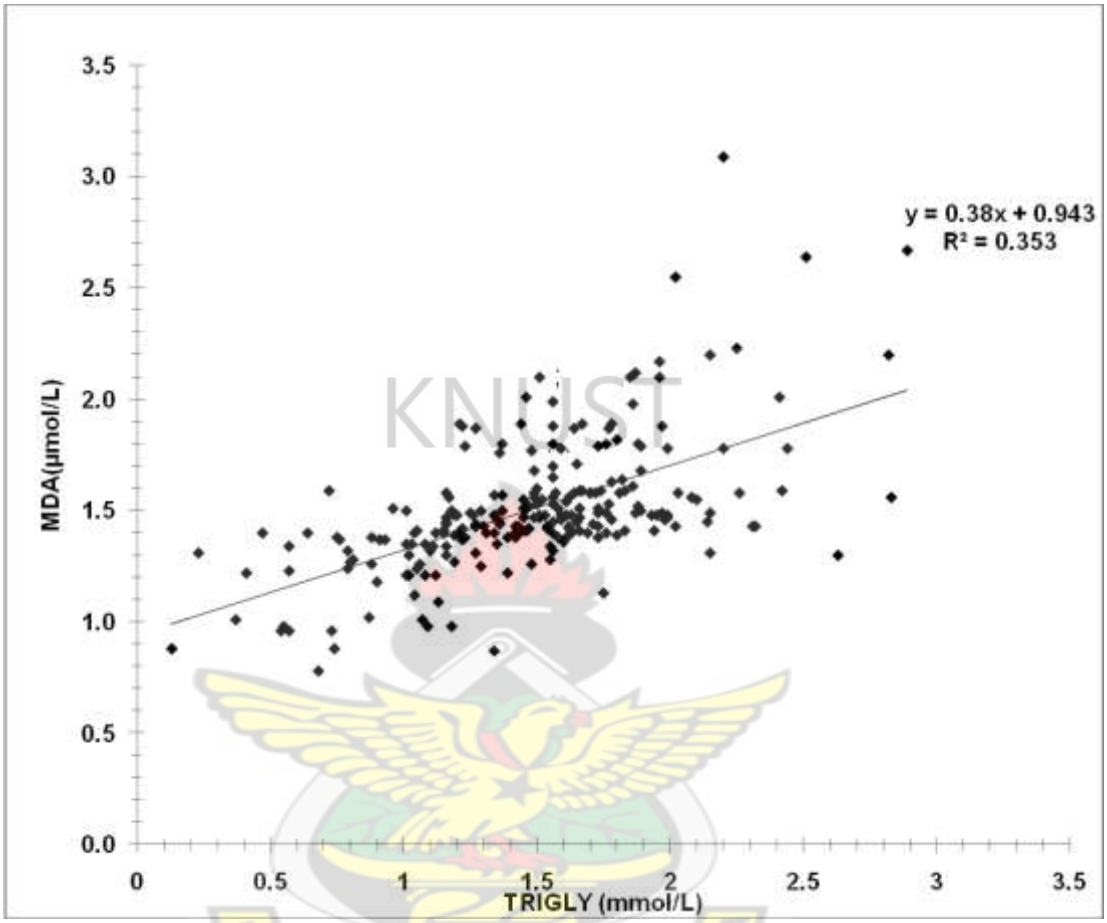

Figure 4.2: *Regression of Triglycerides on MDA showing the significant contribution of trigycerides to the production of MDA with a regression coefficient of* $R^2 = 0.353$ *and very Significant p-value =* $3.37\ E^{-24}$.

There was a strong positive correlation between Gestational age and MDA levels which was very significant with a *p*- value < 0.001. The contribution of triglycerides to the generation of MDA and hence oxidative stress was very significant(Fig 4.2).

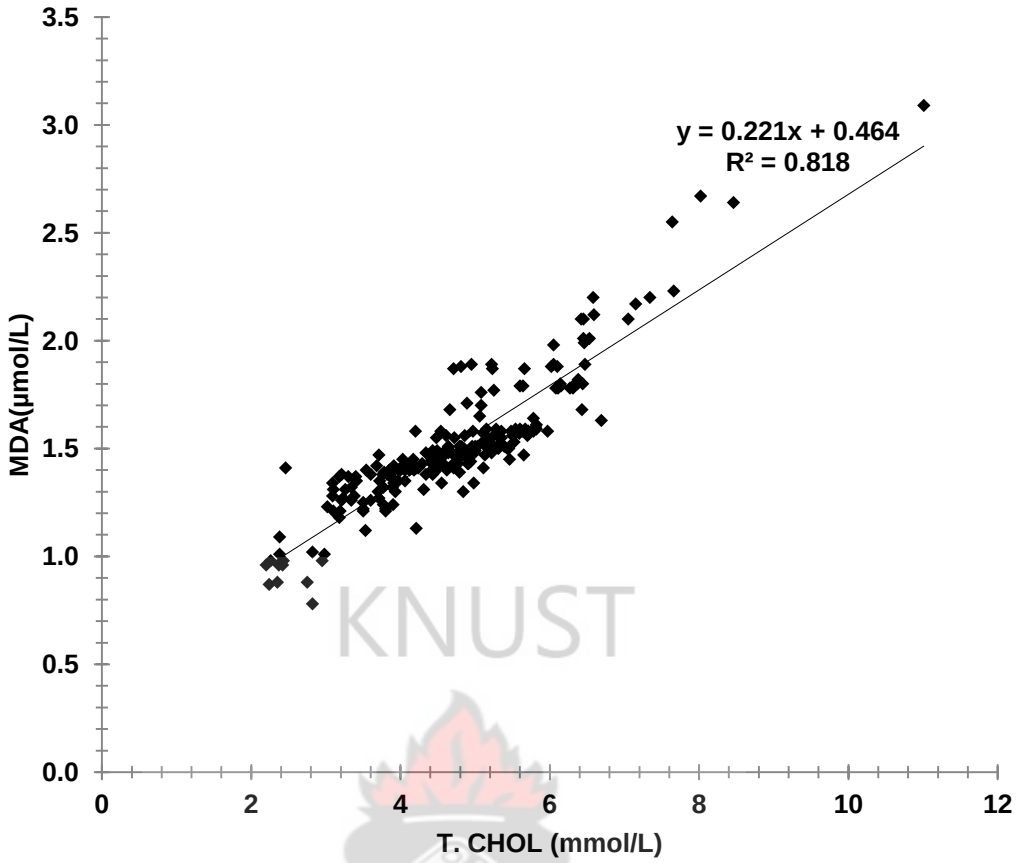

Figure 4.3: *Regression of Total Cholesterol on MDA levels in normal pregnancy Regression coefficient, $R^2 = 0.818$ and very Significant p-value = $8.77E^{-90}$.*

There was a strong positive correlation between Gestational age and MDA levels which was very significant with a *p*- value < 0.001. The contribution of Total Cholesterol to the generation of MDA and hence oxidative stress was very significant.

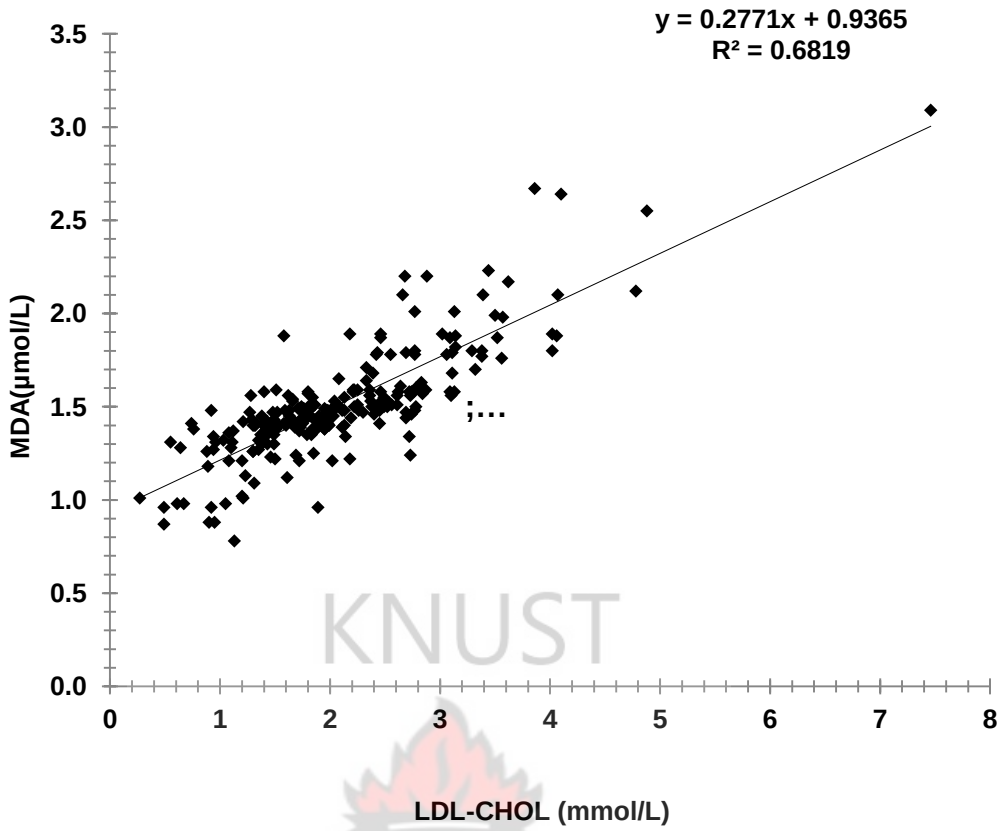

Figure 4.4: *Regression of LDL Cholesterol on MDA levels in normal pregnancy Regression coefficient, $R^2 = 0.768$ and very Significant p-value = $7.19\ E^{-61}$.*

There was a strong positive correlation between Gestational age and MDA levels which was very significant with a *p*- value < 0.001. The contribution of LDL Cholesterol therefore to the generation of MDA and hence oxidative stress was very significant.

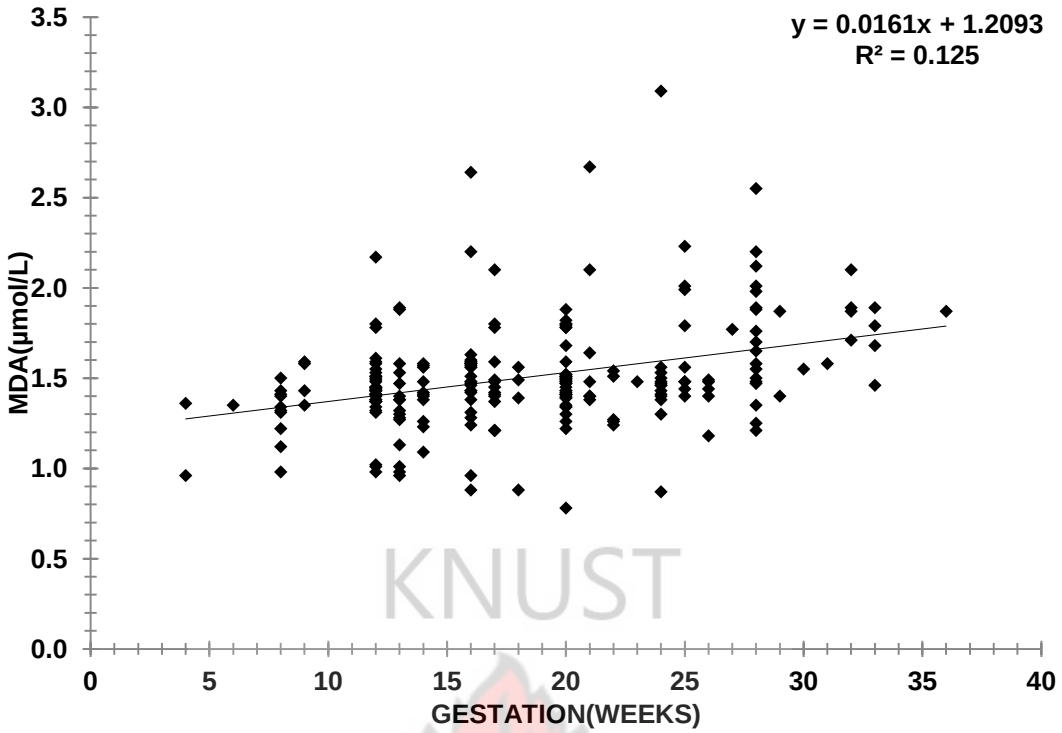

Figure 4.5: *Correlation between Gestational Age and levels of MDA; Regression co-efficient of* $R^2 = 0.125$*; p-value =*$1.90E^{-8}$**.**

There was a strong positive and significant relationship between Gestational age and MDA levels which is very significant with a *p*- value < 0.001. There was also consistent increment in the MDA levels and hence oxidative stress levels as the gestation in weeks advances.

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION OF THE KEY FINDINGS

Pregnancy induces significant changes in lipid metabolism due to rise in levels of insulin, progesterone, 17-β estradiol and high levels oestrogen which induce hepatic biosynthesis of endogenous triglycerides and Human Placental Lactogen. Normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen, which results in increased oxidative stress and antioxidant defenses (Knapen *et al*., 1999). Arikan *et al.,* (2001) reported significant increase in the level of thiobarbituric acid during normal pregnancy. Lipid peroxidation products are formed when lipids interact with oxygen radicals. The increased lipid peroxidation is known to be potentially harmful because its uncontrolled, self-enhancing process causes disruption of membrane lipids and other cell components (Mahboob *et al*., 2005). This could have undesirable effects on foetal development and maternal health. Oxidative stress has also been associated with reproductive problems such as pre-eclampsia and abortion (Poston and Raijmakers. 2004).

In rats and humans, high concentrations of lipid peroxidation products have been measured in normal pregnancy, but controversial information has been available on the oxidative and antioxidative status in normal pregnancy (Arikan *et al.,* 2001; Little and Gladen., 1999; Djordjevic *et al.*, 2004; Nakai *et al.,* 2000; Yoshioka *et al.,* 1987). An elevation of various indices of oxidative stress during the physiological course of pregnancy has been shown (Arikan *et al.*, 2001)

Numerous studies compared healthy pregnant and non-pregnant women (Arikan *et al.*, 2001; Djordjevic *et al.,* 2004; Nakai *et al.*, 2000); all found greater lipid peroxide concentrations in the pregnant women. The above findings support our study

findings which showed that physiological state of pregnancy causes an increase in the amount of lipid peroxidation products (MDA levels) in the blood of pregnant women when compred to their non- pregnant conterparts($p< 0.001$) (Table 4.3 and 4.4).

In this study there was however no significant increase in MDA levels when second trimester values were compared with first trimester ($p> 0.05$. Pregnancy is characterized by increased lipid peroxidation. Peroxidation in the third trimester was significantly higher than in the first (Appendix V) and second trimester (Appendix IV), $p< 0.05$. These findings were also supported by Carine *et al,* (1993) and Djordjevic *et al.,* 2004 who found that peroxidation in the third trimester was significantly higher than in the first and second trimesters. On the other hand, Balal *et al.,* (2004) have demonstrated that the plasma MDA concentrations in pregnant women in the third trimester were similar to the non-pregnant. Our study also shows that plasma concentration of malondialdehyde (MDA) and hence oxidative stress levels increases with progressing gestational age (table 4.4). This is consistent with an earlier study by Patil *et al* (2006) . Two investigations however reported decreasing serum lipid peroxides concentrations during pregnancy (Uotila *et al.*, 1991;Naziroglu *et al.*, 2004). Another interesting finding from our study was the consistent and significant increase in lipid peroxidation marker (MDA) during the physiological course of pregnancy (Fig.4. and 4.5). The association of lipid peroxidadtion marker (MDA) in advancing gestation indicates a state of increased oxidative stress as normal pregnancy advances. This finding is consistent with previous findings made by Wickens D (1981). Patil *et al.,* (2006) also observed from their study that lipid peroxide levels of normal pregnant women in the three trimesters showed significant increases in all the three trimesters when compared to levels in non-pregnant women. Other studies by Ishihara *et al.,(*1979) and Kodliwadmath *et al.,* (1989) found remarkable increases in MDA levels

only in the second and third trimester. Even though the first trimester also showed an increase when compared to the non-pregnant subjects it was not statistically significant. In a study carried out in Nigeria, Luqman *et al.,* (2008) to evaluate the effects of vitamin C on lipid peroxidation and non-enzymatic antioxidant status during second trimester in normal pregnancy it was found that normal pregnancy was associated with increased malondialdehyde production. This finding is also consistent with the findings of this study. A probable physiological explanation is hyperestrogenemia which promotes hepatic lipase activity. Some earlier studies showed that the most dramatic change in the lipid profile in normal pregnancy is serum hypertriglyceridemia, which may be as high as two to three folds in the third trimester over the levels in non-pregnant women (Chiang *et al.,* 1995). In our study however, triglyceride levels in the third trimester was 1.5 fold compared to the levels in non-pregnant women possibly due to the the fact that many of the pregnant women engage in manual work which utilizes significant levels of the fat deposits including cholesterol. The mean triglycerides values showed a consistent increase in each trimester over the previous ones (Table 4.4) and each increase was statistically significant (Table 4.4 and Appendix III), $p< 0.05$. The mean of each trimester's values was also significantly higher than the mean of the non-pregnant women, $p< 0.05$ which is consistent with findings of Chiang *et al.,(*1995) as stated above. These observations may probably be explained by the consistent increase in the level of reproductive and placental hormones during the course of pregnancy, that cause the body to initiate various adaptive mechanisms which alter carbohydrate and lipid metabolism resulting in physiological dyslipidemia (Merabishvili *et al.,* 2006) in pregnancy. The principal modulator of this hypertriglyceridemia is oestrogen as pregnancy is associated with hyperoestrogenaemia. Oestrogen induces hepatic

biosynthesis of endogenous triglycerides, which is carried by VLDL-C (Glueck *et. al.,* 1975). This process may be modulated by hyperinsulinism found in pregnancy (Adegoke *et al.,* 2003). The above observation along with increased endothelial triglyceride accumulation may result in endothelial cell dysfunction in gestation (Mikhail *et al.,* 1995).

However in the second and third trimester there was significant increase in cholesterol levels of pregnant subjects ($p < 0.05$) when compared to the non-pregnant subjects. It is worth noting that TG, T.C, LDL, and VLDL levels were all higher in pregnant than the non-pregnant women even though the differences were not statistically significant in the first trimester ($p > 0.05$). This phenomenom may be explained by nausea which usually characterizes early stages of pregnancy and the fact that rise in reproductive hormones which are the main cause of hyperlipidemia will not have reached significantly high levels in the first trimester. Significantly higher levels of these lipids however were seen in the second and third trimesters when compared to the non-pregnant women. Mean value of total cholesterol increased along trimesters (Table 4.4).

There was no significant statistical difference between cholesterol levels of pregnant subjects in the second and third trimester ($p > 0.05$). These findings are similar to findings of Hubel *et al.,* (1998) and Glueck *et al., (*1975) but at variance with findings of Satter *et al. (*1999). Low density lipoprotein had similar patern with total Cholesterol (Table 4.4). The mean of the first trimester was not statistically different when compared with the mean of the non-pregnant women ($p > 0.05$). The values in the other two trimesters were statistically different from those of non-pregnant women ($p < 0.001$). These findings are consistent with findings of Martin *et al.,* 1999. The LDL-C correlates positvely and strongly with the Serum MDA levels and could partly

explain the significant increase in MDA levels in pregnant women compared to the non-pregnant women.The increased LDL-C levels provides a higher concentration of polyunsaturated fats for the lipid peroxidation process which generates more of secondary lipid peroxidation product MDA.

In our study, the mean value of HDL-C was about 15% lower in the third trimester of normal pregnancy over the non-pregnant women, but statistically the alteration was not significant ($p>0.05$). It was however significantly reduced ($p<0.05$) in the first and second trimesters when compared to the non-pregnant women. This is clearly at variance with previous findings of Jayante *e.t al.,* (2006) who found a significant increase of 30% in HDL-C levels in the third trimester using a similar comparison.

Serum VLDL-C level rose significantly ($p <0.001$) in the third trimester of pregnancy in comparison to non-pregnant women, which is perhaps due to hypertriglyceridemia leading to enhanced entry of VLDL that carries endogenous triglyceride into circulation. The VLDLC level, as reported by some researchers, might increase to 2.5 fold at term over the pre-pregnancy level (Knopp *et al.,* 1986 and Teichmann *et al.,* 1988).

Generally there was no significant correlation between the biochemical variables uric acid, magnesium and glucose in relation to MDA within the control group, the second and third trimesters (Table 4.5- Table 4.8). There was a significant positive correlation between BMI and MDA when mean values of all three trimesters were considered(Table 4.5). There was generally also a significant positive correlation between most lipid profile and MDA in the control group, second, third and when all three trimesters were combined. The HDL/VLDL ratio consistently showed a negative but significant correlation with MDA, lipids and their derivatives except HDL-C where the correlation was positive but not significant (Table 4.5- Table 4.8). The

increased BMI as shown in Table 4.5 could partly explain the rise in triglycerides, low-density lipoprotein and total cholesterol and the significant positive correlation between these measurements and BMI because increased body weight/BMI is associated with increased body fat levels.

The significantly raised levels of lipid fractions especially T. Cholesterol, LDL-C and TGs as seen from our work could partly explain the increased state of oxidative stress as indicated by the significantly raised levels of MDA present in normal pregnancy compared to the non-pregnant women. This is because hyperlipidemia promotes the increased generation of ROS in the presence of increased oxygen demand during pregnancy (Halliwell and Gutteridge, 1990), reduction in the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Walsh and Wang, 1993b), and reduction in plasma vitamin C and vitamin E concentration during pregnancy (Kumar and Das, 2002; Kharb *et al*., 2000).

In a study carried out in Nigeria, Luqman *et al.*, (2008), to evaluate the effects of vitamin C on lipid peroxidation and non-enzymatic antioxidant status during second trimester in normal pregnancy among Nigerian women, it was found that normal pregnancy was associated with increased malondialdehyde production, and ingestion of vitamin C reduced its production. This finding of significantly increased MDA production and hence increased intensity of lipid peroxidation in the second trimester is also consintent with our findings.

Vitamin C supplementation is particularly important in pregnant women as its deficiency has been shown to affect placenta structure and facilitates placenta infection both of which results in increased risk of premature rupture of placenta membranes and premature births (Casanueva and Viteri., 2002). Supplementation also helps to

prevent the development of such complications of pregnancy (Rumbold and Crowther., 2005).

CHAPTER SIX

6.1 CONCLUSION

The study has clearly established that pregnant women undergo marked physiological dyslipidemia as result of alteration in hormones and that there is significant and consistent rise in lipid levels with advancing course of pregnancy or gestational age.

We have demonstrated that normal pregnancy is associated with increased lipid peroxidation and that pregnant women experience an increased intensity of oxidative stress compared to their non-pregnant counterpart.

The intensity of the oxidative stress increases as pregnancy advances and peaks at the third trimester. There is significant and strong correlation between maternal lipid levels and intensity of lipid peroxidation both in normal pregnant and non pregnant women.

It stands to reason from these observations that antenatal caregivers should consistently increase antioxidant supplementation levels as part of routine antenatal care as pregnancy advances to mitigate the undesirable effects of increased intensity of oxidative stress.

6.2 RECOMMENDATION

Lipid profiles should be included as part of routine antenatal laboratory testing for pregnant women being managed by antenatal care centres in Ghana. Alteration in serum lipid levels could serve as good prognostic tool for poor oxidative stress management in pregnancy especially in the seond and third trimesters when the oxidative stress begins to peak. Simple, affordable accurate and reproducible markers of lipid peroxidation and hence Oxidative stress should be included as part of laboratory testing for pregnant women especially in the second and third trimesters to

ensure timely detection and management of oxidative stress before undesirable effects occur.

A uniform method with comprehensive assessment of the OS biomarkers should be used so that the results can be compared with reference values for MDA, antioxidants, minimum safe levels or physiologically beneficial concentrations have yet not been defined. Pregnant subjects should be assessed according to the etiological factors and analyzed separately. Further studies should be designed with national representive numbers so as determine reference values. Measurement of biomarkers of OS is subject to inter-laboratory variations, and inter-observer differences. Further studies should be carried out to determine the antioxidant status of pregnant women to aide our understanding of oxidative status in Ghanaian women

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APPENDIX 1: CLIENT CONSENT FORM

CLIENT CONSENT FORM

STUDY TITLE: Pregnancy-induced Lipid Peroxidation and Oxidative Stress in Ghanaian Women with Normal Pregnancies.

Pregnancy is a stressful condition which places a great demand on a womans body. There are changes in the way the body works during pregnancy which leads to a high demand for energy and oxygen.The increased intake of oxygen results in increased chemical reactions in the body to form other products called Reactive Oxygen Species. These Reactive Oxygen Species and their activiies can be harmful to the health of the prospective mother and also the baby if not well controlled or managed.

I have been told that the purpose of this research is to assess the amount of these harmful products in my body and also find out at which point of pregnancy these products could be most harmful to me and my baby. I have also been told that results of this research will provide useful information that will help caregivers better manage Ghanaian pregnant women and their babies during the pregnancy period and at the point of delivery.

As part of the study about 10mls(about the size of my middle finger) of my blood would be collected by a qualified health professional with minimal pain, minimal discomfort and in a safe manner. I would also be required to answer a questionaire related to the study. My height, weight and blood pressure would also be checked by qualified health professional. The data would be used solely for purpose of the study and all information will be handled confidentially.

I have read the foregoing information, or it has been read to me. I have had the chance to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject and understand the risks and dangers and my right to withdraw from the study any time.

If I have further questions about this I can contact the persons below.

Name/Sign./Date:

……../……/……..

Name/ Thumbprint/ Date: ……..

/……/……

Witness/Sign./Date:…… /……/….....

Interviewer/Sign./Date: ……/……/……

Clinician/Sign./Date:……../……/…….

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APPENDIX II: STANDARD PROJECT QUESTIONAIRE

STUDY TITLE: **Pregnancy-induced Lipid Peroxidation and Oxidative Stress in Ghanaian Women with Normal Pregnancies**

Section A: Personal /Demographic Data

Identification number: ……. Date of visit: …/……/…….

Name : ……..... Age :……..... (yrs).

Gestational Age:.....(Weeks). Sub-metro: /Health Facility: ……....

Addres…….....

H/No:…….....

Mobile No: /Tel. No…….....

Parity: ……..... Gravidity:.....

Expected Date of Delivery: …….

Weight: .....(Kgs) Height: …….....(M)

Body Mass Index:..... [Weight kg/ (Height m)2]).

Blood Pressure:.....(mmHg)

Section B: Historical Data

HISTORY OF PREVIOUS PREGNANCY COMPLICATIONS:

1. Eclampsia/ Preeclampsia 2. Hypertension 3. Heamorrhage 4. Others

DO YOU HAVE ANY HISTORY OF DIABETES? …….

DO YOU HAVE ANY HISTORY OF ALCOHOLISM ?.....

DO YOU HAVE ANY HISTORY OF THYROID DISORDER?:.....

ANY OTHER SYSTEMIC DISORDER?.....

ANEMIA (HB < 10.0 G/DL).....

Do you have any further queries?

Signature/Thumbprint of subject: ……

Name/Signature of Clinician: ……

Name/ Signature of Interviewer: …….

APPENDIX III: COMPARISON OF BIOCHEMICAL INDICES OF FIRST AND SECOND TRIMESTERS

Comparison of biochemical indices of first and second trimesters in uncomplicated pregnant women.

Parameters	First Trimester Pregnant Women (N =82)	Second Trimester Pregnant Women (N =125)	95 % CI of mean difference	p-value
T. C (mmol/L)	4.5 ± 1.0	4.9 ± 1.3	-0.734 – 0.066	0.0190*
HDL – C (mmol/L)	0.9 ±0.4	1.0 ± 0.6	-0.249 – 0.049	0.1858
TG (mmol/L)	1.3 ±0.4	1.5 ± 0.5	-0330 – (- 0.070)	0.0027*
LDL – C (mmol/L)	1.8 ± 0.7	2.0 ± 0.9	-0.902 – 0.032	0.0902
VLDL-C (mmol/L)	0.6 ± 0.2	0.8 ± 0.2	-0.256 – (- 0.144)	0.0001*
LDL – C /HDL – C ratio	1.6 ± 1.3	2.0 ± 1.2	-0.748 – (-0.052)	0.0243*
T. C / HDL – C ratio	3.8 ± 0.8	4.9 ± 1.0	-1.359 – (- 0.841)	0.0001*
HDL-C/ VLDL-C ratio	1.8 ± 0.6	1.4 ± 0.7	0.214 - 0.586	0.0001*
Atherogenic index	3.3 ± 1.0	3.9 ± 1.3	-0.934 – (- 0.266)	0.0005*
Albumin (mmol/L)	37.4 ± 4.5	35.7 ± 4.9	0.370 - 3.030	0.0125*
Uric Acid (mmol/L)	218.5 ± 61.0	202.1 ± 61.6	-0.793 - 33.593	0.0614
Mg^{+2} (mmol/L)	0.9 ± 0.2	0.8 ± 0.1	0.059 - 0.141	0.0001*
Glucose (mmol/L)	4.8 ± 0.5	4.7 ± 0.6	-0.058 - 0.258	0.2125
MDA (mmo/mL)	1.4 ± 0.2	1.5 ± 0.5	-0.215 - 0.015	0.0866

* Mean difference is significant. Atherogenic index = total cholesterol HDL cholesterol / HDL cholesterol, malondialdehye (MDA) is oxidative stress indicator. Cardiovascular risk is total cholesterol (T.C) / high density lipoprotein (HDL). Values are given as mean ± standard deviation.

APPENDIX IV: COMPARISON OF BIOCHEMICAL INDICES OF SECOND AND THIRD TRIMESTERS

Comparison of biochemical indices of second and third trimester uncomplicated pregnant women

Parameters	Second Trimester Pregnant Women (N = 125)	Third Trimester Pregnant Women (N = 33)	95 % CI of mean difference	p-value
T. C (mmol/L)	4.9 ± 1.3	5.2 ± 1.0	-0.781- 0.181	0.2199
HDL (mmol/L)	1.0 ± 0.6	1.2 ± 0.3	-0.413 – 0.013	0.0660
TG (mmol/L)	1.5 ± 0.5	1.7 ± 0.3	-0.380– (-0.020)	0.0298*
LDL – C (mmol/L)	2.0 ± 0.9	2.8 ± 0.9	-1.148 – (-0.452)	0.0001*
VLDL-C (mmol/L)	0.8 ± 0.2	0.9 ± 0.1	-0.171 – (-0. 029)	0.0062*
LDL – C /HDL – C ratio	2.0 ± 1.2	2.6 ± 0.9	-1.043 – (-0.157)	0.0082*
T. C / HDL – C ratio	4.9 ± 1.0	4.8 ± 1.1	-0.295 – 0.495	0.6176
HDL-C/ VLDL-C ratio	1.4 ± 0.7	1.4 ± 0.4	-0.251 – 0.251	1.0000
Atherogenic index	3.9 ± 1.3	4.2 ± 1.0	-0.781 – 0.181	0.2199
Albumin (mmol/L)	35.7 ± 4.9	34.5 ± 3.3	-0.585 – 2.985	0.1861
Uric Acid (mmol/L)	202.1 ± 61.6	217.9 ± 76.5	-40.903 – 9.303	0.2156
Mg^{2+} (mmol/L)	0.8 ± 0.1	0.9 ± 0.1	-0139 – (-0.061)	0.0001*
Glucose (mmol/L)	4.7 ± 0.6	4.4 ± 0.7	0. 060 - 0.540	0.0148*
MDA (mmo/mL)	1.5 ± 0.5	1.7 ± 0.3	-0.380 – (-0.020)	0.0298*

* Mean difference is significant. Atherogenic index = total cholesterol HDL cholesterol / HDL cholesterol, malondialdehye (MDA) is oxidative stress indicator. Cardiovascular risk is total cholesterol (T.C) / high density lipoprotein (HDL). Values are given as mean ± standard deviation.

APPENDIX V: COMPARISON OF BIOCHEMICAL INDICES OF FIRST AND THIRD TRIMESTERS

Comparison of biochemical indices of first and third trimesters of uncomplicated pregnant women.

Parameters	First Trimester Pregnant Women (N =82)	Third Trimester Pregnant Women (N = 33)	95 % CI of mean difference	p-value
T. C (mmol/L)	4.5 ± 1.0	5.2 ± 1.0	10.741 – (-0.659)	0.0001*
HDL – C (mmol/L)	0.9 ±0.4	1.3 ± 0.3	-0.353 – (-0.047)	0.0108*
TG (mmol/L)	1.3 ±0.4	1.7 ± 0.3	-0.553 – (-0.247)	0.0001*
LDL – C (mmol/L)	1.8 ± 0.7	2.8 ± 0.9	-1.311 – (-0.689)	0.0001*
VLDL-C (mmol/L)	0.6 ± 0.2	0.7 ± 0.1	-0.172 – (-0.028)	0.0070*
LDL – C /HDL – C ratio	1.6 ± 1.3	2.6 ± 0.9	-1.490 – (-0.510)	0.0001*
T. C / HDL – C ratio	3.8 ± 0.8	4.8 ± 1.1	-1.366 – (-0.634)	0.0001*
HDL-C/ VLDL-C ratio	1.8 ± 0.6	1.4 ± 0.4	0.175 – 0.625	0.0006*
Atherogenic index	3.3 ± 1.0	4.2 ± 1.0	-1.308 – (-0.492)	0.0001*
Albumin (mmol/L)	37.4 ± 4.5	34.5 ± 3.3	1.187 – 4.613	0.0011*
Uric Acid (mmol/L)	218.5 ± 61.0	217.9 ± 76.5	-26.258 – 27.458	0.9648
Mg^{+2} (mmol/L)	0.9 ± 0.2	0.9 ± 0.1	-0.072 – 0.072	1.0000
Glucose (mmol/L)	4.8 ± 0.5	4.4 ± 0.7	0.170 – 0.630	0.0008*
MDA (mmo/mL)	1.4 ± 0.2	1.7 ± 0.3	-0.395 – (-0.205)	0.0001*

*Mean difference is significant. Atherogenic index = total cholesterol HDL cholesterol / HDL cholesterol, malondialdehye (MDA) is oxidative stress indicator. Cardiovascular risk is total cholesterol (T.C) / high density lipoprotein (HDL). Values are given as mean ± standard deviation.

APPENDIX VI: SHOWING THE CONSISTENT PERCENTAGE INCREASE IN LEVELS OF MDA

Showing the consistent percentage increase in the levels of MDA by 10 % as gestation advances and a decrease of 10 % in the non-pregnant subjects using the first trimester as a denominator.

Parameter	**First trimester (N_1= 82)**	**Second trimester (N=125)**	**Third trimester (N=33)**	**Mean in pregnant women (N=239)**	**Non-pregnant (N=48)**
Mean MDA levels (µmol/L)	1.4	1.5	1.7	1.5	1.3
Ratio (N/N_1)	1.0	1.1	1.2	1.1	0.9
% change of MDA	-	+ 10	+ 20	+ 10	-10

(+) Means there is a percentage increase in in the mean levels of MDA while (-) means a decrease in mean MDA levels.

APPENDIX VII: SOLUTIONS AND REAGENT PREPARATION

Solutions and Reagent preparation

MAGNESIUM (Mg^{2+})

Principle: Magnesium ions react with xylidyl blue in an alkaline medium to form a water soluble purple-red chelate, the colour intensity of which is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexing with EDTA

REAGENT COMPOSITION

CONTENTS	INITIAL CONCENTRATIONS OF SOLUTIONS
R1.COLOUR REAGENT	
Xylidyl blue	0.1mmol/l
Tris buffer	0.2mmol/l
Potassium Carbonate	77mmol/l
EDTA	0.04mmol/l

URIC ACID (UA)

Principle: Uric Acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidise, oxidizes 3,5– Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound. Uric acid + O_2 + $2H_2O$ $\xrightarrow{\text{uricase}}$ Allantoin + CO_2 + H_2O_2

$2H_2O_2$ + 3, 5-dichloro-2-hydroxybenzenesulfonic acid + 4-aminophenzone $\xrightarrow{\text{peroxydase}}$ N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzo-quinoneimine.

REAGENT COMPOSITION

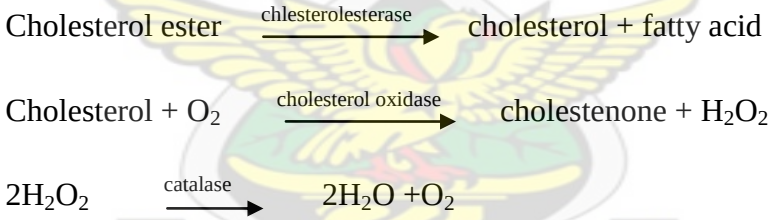
CONTENTS	CONCENTRATIONS IN THE TEST
R1.ENZYME REAGENT	
HEPES Buffer	200mmol/l, pH 7.5
4-Aminophenazone	0.25mmol/l
3,5 DCHBS	4.0mol/l
Uricase	≥200μ/l
Peroxidase	≥1000μ/l

DIRECT HDL-CHOLESTROL (HDL-C)

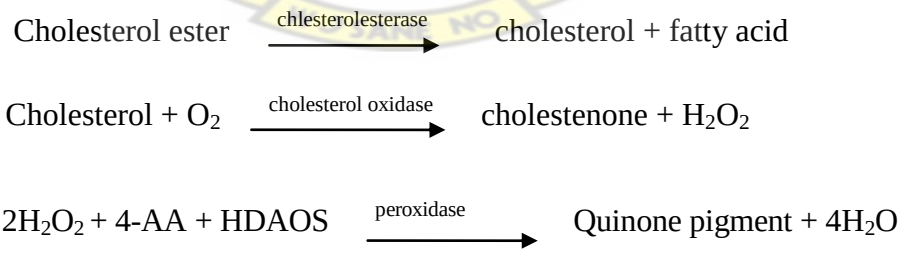
Principle

The assay consists of two reaction steps:

1. Elimination of chylomicron, VLDL- cholesterol and LDL- cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase.



2. Specific measurement of HDL-Cholesterol after release of HDL-cholesterol by detergents in Reagent 2.



The intensity of the quinone imine dye produced is directly proportional to the cholesterol concentration when measured at 600nm.

In the second reaction catalase is inhibited by sodium azide in Enzyme Reagent 2.

Key: 4-AA-4- Aminoantipyrine

HDAOS –N- (2-hydroxy – 3 – solfopropyl) – 3, 5 – dimethoxyaniline.

REAGENT COMPOSITION

CONTENTS	INITIAL CONCENTRATION OF SOLUTION
R1. Enzyme Reagents 1	
N,N-Bis(2-hydroxyethyl)	100mM, pH 6.6 (25^0C)
2-aminoethanesulfonic acid N-(2-hydroxy-3-sulfopropyl)- 3, 5- dimethoxyaniline, sodium salt (HDAOS)	0.7 mM
Cholesterol Esterase [E.C.3.1.1.13. Microorganism]	≥800μ/l
Cholesterol Oxidase [E.C.1.1.3.6. Streptomyces sp]	≥500μ/l
Catalase [E.C.1.11.1.6. Microbial]	≥300Kμ/l
Ascorbate Oxidase [E.C.1.1o.3.3. Acremonium sp]	≥3000μ/l

R2. Enzyme Reagent 2	
N,N-Bis(2-hydroxyethyl)- 2-aminoethanesulfonic acid	100mM, pH 6.6(25^0C)
4-Aminoantipyrine	4.0mM
Peroxidase [E.C.1.11.1.7, Horse Radish, 25^0C]	≥3500µ/l
Sodium Azide	0.05w/v%
Surfactants	1.4w/v%

GLUCOSE (GLUC-HK)

UV METHOD

The method is totally enzymatic utilising both hexokinase and glucose-6-phosphate dehydrogenase enzyme.

REACTION PRINCIPLE

Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ G-6-P + ADP

G-6-P + NAD^+ $\xrightarrow{\text{Glucose-6-phosphate Dehydrogenase}}$ gluconate-6-P + NADH + H^+

REAGENT COMPOSITION

CONTENTS	CONCENTRATIONS IN THE TEST
R1. Buffer	
Pipes	100mmol/l, pH 7.6
ATP	4mmol/l
NAD^+	3mmol/l
Magnesium ions	15mmol/l
Sodium Azide	0.07%w/v
R2 Enzymatic Reagent	
Hexokinase	≥25μ/ml
G-6-PDH	≥75μ/ml
Sodium Azide	0.01%w/v

TOTAL PROTEIN 2 (TP 2)

Principle

A coloured complex is formed between protein and cupric ions in an alkaline medium.

REAGENT COMPOSITION

CONTENTS	CONCENTRATIONS IN THE TEST
R1. BLANK REAGENT	
Sodium Hydroxide	400mmol/l
Na-K-tartrate	64mmol/l
R2. BIURET REAGENT	
Sodium Hydroxide	400mmol/l
Na-K-tartrate	64mmol/l
Potassium Iodide	30mmol/l
Cupric Sulfate	12mmol/l

DIRECT LDL-CHOLESTEROL (LDL)

Principle

The assay consists of two reaction steps:

1. Elimination of chylomicron, VLDL- cholesterol and HDL- cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase.

   Cholesterol ester $\xrightarrow{\text{chlesterolesterase}}$ cholesterol + fatty acid

   Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholestenone + H_2O_2

   $2H_2O_2$ $\xrightarrow{\text{catalase}}$ $2H_2O + O_2$

2. Specific measurement of HDL-Cholesterol after release of HDL-cholesterol by detergents in Reagent 2.

Cholesterol ester $\xrightarrow{\text{chlesterolesterase}}$ cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholestenone + H_2O_2

$2H_2O_2$ + 4-AA + TOOS $\xrightarrow{\text{peroxidase}}$ Quinone pigment + $4H_2O$

The intensity of the quinone imine dye produced is directly proportional to the cholesterol concentration when measured at 600nm.

In the second reaction catalase is inhibited by sodium azide in Enzyme Reagent 2.

4-AA-4- Aminoantipyrine

TOOS –N- (2-hydroxy – 3 – solfopropyl) – 3 – methyl aniline.

REAGENT COMPOSITION

CONTENTS	CONCENTRATION OF SOLUTION
PIPES Buffer Piperazine-1,4-bis(2-ethanesulfonic acid)	50mmol/l, pH 7.0
TOOS N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylanine	2.0mmol/l
Cholesterol Esterase [E.C.3.1.1.13. Pseudomonas, 37^0C)	≥600μ/l
Cholesterol Oxidase [E.C.1.1.3.6. Nocardia, 37^0C]	≥500μ/l
Catalase [E.C.1.11.1.6. Microbial]	≥600Kμ/l
R2. ENZYME REAGENT 2	
PIPES Buffer Piperazine-1,4-bis(2-ethanesulfonic acid)	50mmol/l, pH 7.0
4-Aminoantipyrine	4.0mmol/l
Peroxidase [E.C.1.11.1.7, Horse Radish, 25^0C]	≥4Kμ/l
Sodium Azide	0.05w/v%