

# Relationship between lipid and lipoprotein metabolism in trimesters of pregnancy in Nigerian women: Is pregnancy a risk factor?

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## Abstract

**Background:** Changes in lipid metabolism have been shown to occur during pregnancy, to ensure a continuous supply of nutrients to the growing fetus, despite intermittent maternal food intake. Abnormal lipid metabolism has also been linked to atherosclerosis. **Objective:** To investigate the effect of pregnancy on the lipid profile and possible predisposition of pregnant Nigerian women to atherosclerosis. **Settings and Design:** Serum lipid and lipoprotein levels of 60 apparently healthy pregnant women aged between 25 and 45 years, attending the antenatal clinic of the U.N.T.H, Enugu and 60 apparently healthy non-pregnant, age-matched females (controls) were estimated. The test samples were collected from each subject at each of the trimesters. **Materials and Methods:** Total cholesterol (TC), high-density lipoprotein (HDL) and triglyceride (TG) were analyzed using enzymatic/spectrophotometric methods while low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated using Friedewald's formula. **Statistical analysis used:** The data obtained were analyzed with Students' t-test and Pearson's Product Moment Correlation, using graph pad prism software program and results expressed as mean  $\pm$  SD. The level of significance was determined at 95% confidence level. **Results and Conclusion:** The serum lipid levels were significantly higher ( $P < 0.05$ ) in all the trimesters of the pregnant women than in the controls. There was a steady increase in the serum lipid levels with increasing gestational age. A significant positive correlation ( $P < 0.05$ ) was observed between the lipid fractions and the different trimesters of pregnancy. TC/HDL was decreased significantly ( $P < 0.05$ ) in pregnant women, with increasing gestational age. Cardiac risk factor, however, decreased with gestational age, signifying possible protection from atherosclerosis. A comparison of two age groups of pregnant women (25-34 years and 35-45 years) showed no significant differences ( $P > 0.05$ ) in all the lipid fractions studied, suggesting no possible age-related effect on lipid metabolism in the women in their first trimester. Even with significant increase in plasma lipid during pregnancy, normal pregnancy in Nigerian women does not appear to increase the risk.

**Key words:** Lipid, lipoprotein, metabolism, pregnancy, relationship

## INTRODUCTION

Lipid profile is used to detect primary and secondary lipid

disorders (hyper- or hypolipemias) and usually includes the total cholesterol (TC), high-density lipoprotein (HDL), triglyceride (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL). The measured parameters are cholesterol, TGs and HDL, whereas the calculated values are the LDL, VLDL and a cardiac risk factor TC/HDL.<sup>[1]</sup> The ratio TC/HDL is one of the determinants of the predisposition to the risk of atherosclerosis, with an accepted value of  $< 4.5$  and  $< 5$  in males and females, respectively, beyond which they become at risk of atherosclerosis.<sup>[1]</sup> The TC/HDL ratio has also been shown to be high in pregnancy-induced hypertension

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and pre-eclampsia, compared to normal pregnancy.<sup>[2]</sup> Hyperlipemia refers to the increase in the plasma concentrations of TGs or cholesterol, both exposing the body to dangers of formation of atherosclerotic plaques, with hypertriglyceridemia being more common.<sup>[3]</sup> Part of the body's cholesterol is derived from dietary intake, but majority is synthesized by the liver and other tissues.<sup>[3]</sup> A study by Brites *et al*<sup>[1]</sup> has shown that the risk of heart disease drops 2-3% for every 1% drop in cholesterol level. The female hormonal cycle is an exquisitely controlled system that includes the hypothalamus, pituitary, adrenal, thyroid and gonadal tissues; involving both positive and negative feedback loops.<sup>[4]</sup> Changes in ovarian hormone concentrations during the menstrual cycle have been assumed to account for the within-month variations in serum lipids noted in menstruating women.<sup>[4]</sup> Thyroid hormones affect sex steroid hormone levels and sex hormones themselves contribute to regulation of lipid metabolism.<sup>[3]</sup> Estrogen and progesterone are known to affect plasma volume and an expansion in plasma volume contributes to the decrease in TC.<sup>[5]</sup>

Because estrogen stimulates the synthesis of LDL receptors and lowers plasma level of LDL, it might reduce the incidence of coronary heart disease (CHD).<sup>[6]</sup> The study by Ikekpeazu *et al*<sup>[7]</sup> showed an association between early and late menopause and lipid profile of postmenopausal Nigerian women and also reveals a possible predisposition to unfavorable lipid profile and risk of CHD with progress from early to late menopausal age. During pregnancy, maternal metabolism must satisfy the demands of the developing fetus in addition to the energy requirements of the mother.<sup>[8]</sup> Early pregnancy is considered the anabolic phase, characterized by increased hepatic production of triglycerides (TG) and enhanced removal of TGs from the circulation, resulting in an increased deposition of fat in maternal adipose tissue. In contrast, late pregnancy is referred to as the catabolic phase; the release of free fatty acids from the adipocytes is enhanced due to both relative insulin resistance and stimulation of hormone-sensitive lipase by placental hormones.<sup>[6]</sup> As a consequence, the maternal lipid metabolism is specifically altered during pregnancy. Thus, elevated TGs and accumulation of LDL during pregnancy are thought to increase the risk of endothelial damage,<sup>[9]</sup> despite the fact that there is a preponderance of buoyant HDL in late gestation. Weight gain and dietary habits have an effect on the lipid metabolism of pregnant women,<sup>[10]</sup> while observations in guinea pigs and rats suggest that manipulations of maternal dietary intake during gestation permanently alters cholesterol synthesis and plasma cholesterol concentrations.<sup>[11]</sup> Small body size at birth has been reported to be associated with an atherogenic lipid profile (high plasma LDL-cholesterol and low plasma HDL-cholesterol concentration).<sup>[12]</sup>

Some investigators found associations between low birth weight and low HDL-cholesterol or high plasma TG concentrations,<sup>[13]</sup> whereas others found an association between short body length at birth or reduced abdominal circumference and elevated TC, LDL-cholesterol and apolipoprotein B concentrations.<sup>[14]</sup>

These observations usually come from studies involving Caucasian subjects, whereas little or no information has been given concerning Negro women.

The general objective of the study is to determine the possible predisposition of Nigerian pregnant women to atherogenic lipids. The specific objectives are to study the lipid and lipoprotein profile in pregnant Nigerian women in the three trimesters of pregnancy and compare changes in the lipids with the values in (nonpregnant) control subjects.

## MATERIALS AND METHODS

### Study setting/population

Enugu is the capital of Enugu State, South-East Nigeria and has a population of about 464,514 inhabitants. The population is predominantly Ibos and the city has a Nigerian Federal Government-sponsored Teaching Hospital (UNTH) and a State Government-sponsored Specialist and Teaching Hospital; Enugu State University Teaching Hospital (ESUTH) in addition to privately owned hospitals.

### Subjects

The study test subjects consist of 60 apparently healthy pregnant Nigeria women (parturients) in their first trimester of pregnancy. Subjects were drawn from parturients attending antenatal care clinic at the University of Nigerian Teaching Hospital (UNTH), Enugu, Nigeria.

Also 60 apparently healthy nonpregnant age matched females of child-bearing age from Enugu Metropolis served as control subjects. All the subjects were within the age range of 25 - 45 years. The parturients were assured of their confidentiality and were given the option to opt out of the study at any stage they desired without attracting any form of penalty or denial of benefits. Written consent was obtained from each of the women and ethical clearance for the study was obtained from the Ethical Committee of the University of Nigeria Teaching Hospital (UNTH) Enugu.

### Clinical studies

Parturients were studied while waiting to be attended to in the antenatal clinic. Consecutively consenting parturients and control subjects were recruited until the required sample size was completed. The parity of the women as well as the date of last conception was checked.

Pretested, self-administered, semistructured questionnaires were administered immediately after recruitment. The questionnaires contained sections on socioeconomic status, diet, level of physical activity, age, occupation and history of pregnancy complications social status of the women as well as weight gain in pregnancy was also checked.

### Exclusion criteria

Only multiparous women were recruited in the study. Subjects with pregnancy complications such as hypertension, hypothyroidism, gestational diabetes, renal failure, nephrotic syndrome and obesity were excluded from the study.

### Sample collection and preparation

Fasting blood samples were collected from the test subjects during each of the three trimester of pregnancy. The samples from the control subjects were collected only once at a recruitment center outside the hospital. The samples were collected by clean venepuncture from the antecubital vein under aseptic conditions, without undue pressure to the arm, while the subjects were in a sitting position. About 3 ml of blood was collected into sterile plain tubes and allowed to clot, and the clotted samples were centrifuged at 3000 rpm for 5 minutes to obtain the sera.

The sera were separated into sterile tubes and were used for TC, TG and HDL assay. Hemolysis of samples was carefully avoided and the separated sera were stored frozen where immediate analysis was not possible and analysis was carried out within 1 week of samples collection.

### Analytical methods

Enzymatic estimation (TC) was done by the method of Allain *et al.*,<sup>[15]</sup> whereas the method of Buccolo and David<sup>[16]</sup> was used for enzymatic estimation of TG.

Precipitation/ enzymatic method of Allain *et al.*,<sup>[15]</sup> Burstein *et al.*<sup>[17]</sup> and Groove<sup>[18]</sup> was used for HDL-Cholesterol estimation. All the kit reagents were produced by Biosystem S.A Barcelona (Spain).

The LDL and VLDL were both estimated in the serum using the Friedewald formula.<sup>[19,20]</sup>

### Statistical analysis

Data entry and statistical analysis utilized the graph pad prism computer soft ware. Descriptive statistics was done using percentages and expressing mean as mean  $\pm$  standard deviation (mean  $\pm$  SD).<sup>[21]</sup> Statistical analysis utilized the Student's t-test to test for statistical significance and Pearson's Product Moment Correlation test for association. *P*-values of  $<0.05$  were considered to be statistically significant.

## RESULTS

Serial serum lipid and lipoprotein levels of 60 parturients were checked in the first, second and third trimesters of pregnancy. The lipid and lipoprotein levels in a control matched for maternal age was done in nonpregnant women concurrently between June 2008 and March 2009. The age range of the women studied was 25-45 years with a mean age of 32.4 years. Most of the parturients (68%) were house wives, artisans (18%), junior workers (10%), and senior civil servants and business executives (4%). The outcome of the life styles showed that most of them were of the low socioeconomic class and mostly predisposed to increased physical activities as a result of their occupation. Most of them also showed preference for diets containing fish and enjoyed boiled walnut as snacks. Weight gain in pregnancy varied from 10.2 kg to 14.1 kg, with a mean weight of 12.1 kg.

Table 1 shows the different lipid fractions (mmol/L) in the first, second and third trimesters respectively, compared to the control subjects. From the table, there were highly statistically significant differences ( $P<0.05$ ) in the values (mean  $\pm$  SD) between the three trimesters and the control subjects in TC, HDL, TG, LDL and

**Table 1: Variations (Mean  $\pm$  SD) in the lipid and lipoprotein levels (mmol/l) of control subjects and pregnant women in the three trimesters**

	Test subjects (n = 60)	Control subjects (n = 60)	<i>P</i> -value
First trimester			
TC	4.44 $\pm$ 0.11	4.01 $\pm$ 0.08	<0.05*
HDL	1.07 $\pm$ 0.04	0.91 $\pm$ 0.02	<0.05*
TG	1.05 $\pm$ 0.04	0.86 $\pm$ 0.06	<0.05*
LDL	2.91 $\pm$ 0.11	2.69 $\pm$ 0.10	<0.05*
VLDL	0.47 $\pm$ 0.18	0.39 $\pm$ 0.26	<0.05*
TC/HDL	4.20 $\pm$ 0.16	4.44 $\pm$ 0.17	<0.05*
Second trimester			
	n = 60	n = 60	
TC	5.24 $\pm$ 0.11	4.01 $\pm$ 0.08	<0.05*
HDL	1.33 $\pm$ 0.04	0.91 $\pm$ 0.02	<0.05*
TG	1.45 $\pm$ 0.07	0.86 $\pm$ 0.06	<0.05*
LDL	3.26 $\pm$ 0.09	2.69 $\pm$ 0.10	<0.05*
VLDL	0.66 $\pm$ 0.28	0.39 $\pm$ 0.26	<0.05*
TC/HDL	3.93 $\pm$ 0.08	4.44 $\pm$ 0.17	<0.05*
Third trimester			
	n = 60	n = 60	
TC	6.01 $\pm$ 0.11	4.01 $\pm$ 0.08	<0.05*
HDL	1.63 $\pm$ 0.37	0.91 $\pm$ 0.02	<0.05*
TG	1.93 $\pm$ 0.06	0.86 $\pm$ 0.06	<0.05*
LDL	3.53 $\pm$ 0.08	2.69 $\pm$ 0.10	<0.05*
VLDL	0.88 $\pm$ 0.27	0.39 $\pm$ 0.26	<0.05*
TC/HDL	3.64 $\pm$ 0.12	4.44 $\pm$ 0.17	<0.05*

\* = Statistically Significant; n= number of subjects; P = values of significance with difference of each group at 95% confidence level, TC: Total cholesterol, HDL: High-density lipoprotein, TG: triglyceride, LDL: Low-density lipoprotein VLDL: Very low-density lipoprotein

VLDL, respectively, whereas TC/HDL showed significant difference ( $P < 0.05$ ) in the second and third trimesters, respectively, in comparison with the controls. The results of the Pearson's product moment correlation showed a statistically significant positive correlation ( $r = 0.71-0.87$ ) ( $P < 0.05$ ) in the lipid fractions studied (TC, HDL, TG, LDL, VLDL and TC/HDL) as pregnancy progresses [Table 2]. Further classification of the parturients in the first trimester into two age groups (25-34 years) and (35-45 years) showed no significant difference ( $P > 0.05$ ) in the lipid fractions in the two age groups. However, statistically significant increases ( $P < 0.05$ ) were observed in the lipid fractions in the two groups compared with the age-matched controls. However, there were examples as suggested in Tables 3-5.

## DISCUSSION

Changes in lipid metabolism have been shown to occur during pregnancy to ensure a continuous supply of nutrients to the growing fetus, despite the intermittent maternal food intake.<sup>[22]</sup> Some previous studies showed

that the most dramatic damage in the lipid and lipoprotein profile in normal pregnancy is serum triglyceridemia, which may be as high as two or three folds in the third trimester over levels in non pregnant women.<sup>[2]</sup>

The observation, to a great extent, holds true in this present study. Here the serum TG concentration showed very significant increases ( $P < 0.0001$ ) in the third trimester of pregnancy than in the nonpregnant women, the mean value being raised almost two folds. The principle modulator of this hypertriglyceridemia is estrogen, as pregnancy is associated with hyperestrogenemia.

Serum TC, HDL, LDL and VLDL also increased significantly ( $P < 0.0001$ ) as pregnancy progressed toward the third trimester. This corroborates with the previous discovery of Udoh *et al.*,<sup>[23]</sup> who observed a progressive increase in serum TC, HDL, LDL, VLDL and TG at various stages of pregnancy.

The continuous increase recorded in this study, however,

**Table 2: Correlation analysis of the lipid fractions in pregnant women between the different trimesters**

Parameter	r-value	P-value
TC	0.71	< 0.05 *
HDL	0.82	< 0.05 *
TG	0.77	< 0.05 *
LDL	0.74	< 0.05 *
VLDL	0.73	< 0.05 *
TC/ HDL	0.87	< 0.05 *

\* = Statistically Significant, P= values of significance with difference of each group at 95% confidence level, TC: Total cholesterol, HDL: High-density lipoprotein, TG: triglyceride, LDL: Low-density lipoprotein VLDL: Very low-density lipoprotein

**Table 4: Test of difference (Mean ± SD) between lipid fractions (mmol/l) in pregnant women in their first trimester and control subjects (25-34years)**

Parameter	Test (n=25)	Control (n=28)	P-value
TC	4.43 ± 0.19	4.04 ± 0.13	<0.05 *
HDL	1.05 ± 0.08	0.92 ± 0.06	<0.05 *
TG	1.04 ± 0.06	0.86 ± 0.11	<0.05 *
LDL	2.90 ± 0.17	2.71 ± 0.20	<0.05 *
VLDL	0.48 ± 0.04	0.39 ± 0.05	<0.05 *
TC/HDL	4.23 ± 0.29	4.42 ± 0.30	<0.05 *

\* = Statistically Significant, n = number of subjects, P= values of significance with difference of each group at 95% confidence level, TC: Total cholesterol, HDL: High-density lipoprotein, TG: triglyceride, LDL: Low-density lipoprotein VLDL: Very low-density lipoprotein

**Table 3: Test of difference (Mean ± SD) between lipid fractions (mmol/l) in the two age brackets of pregnant women in their first trimester**

Parameter	Test (25- 34 years) (n=25)	Test (35- 45years) (n=35)	P-value
TC	4.43 ± 0.19	4.47 ± 0.22	>0.05
HDL	1.05 ± 0.08	1.08 ± 0.06	>0.05
TG	1.04 ± 0.06	1.05 ± 0.08	>0.05
LDL	2.90 ± 0.17	2.91 ± 0.24	>0.05
VLDL	0.48 ± 0.04	0.47 ± 0.03	>0.05
TC/HDL	4.23 ± 0.29	4.14 ± 0.33	>0.05

\* = Statistically Significant, n= number of subjects, P= values of significance with difference of each group at 95% confidence level, TC: Total cholesterol, HDL: High-density lipoprotein, TG: triglyceride, LDL: Low-density lipoprotein VLDL: Very low-density lipoprotein

**Table 5: Test of difference between (Mean ± SD) lipid fractions (mmol/l) in pregnant women in their first trimester and control subjects (35-45years)**

Parameter	Test subjects (n=35)	Control (n=32)	P-value
TC	4.47 ± 0.22	3.96 ± 0.20	<0.05 *
HDL	1.08 ± 0.06	0.90 ± 0.05	<0.05 *
TG	1.05 ± 0.08	0.87 ± 0.11	<0.05 *
LDL	2.91 ± 0.24	2.66 ± 0.19	<0.05 *
VLDL	0.47 ± 0.03	0.40 ± 0.05	<0.05 *
TC/HDL	4.14 ± 0.33	4.44 ± 0.32	<0.05 *

\* = Statistically Significant, n= Number of subjects, P= values of significance with difference of each group at 95% confidence level, TC: Total cholesterol, HDL: High-density lipoprotein, TG: triglyceride, LDL: Low-density lipoprotein VLDL: Very low-density lipoprotein

is in contrast with the finding of Butte,<sup>[22]</sup> who reported an initial decrease in the TC and LDL concentrations in the first trimester, followed by an increase in the second and third trimesters. The TC results were also in agreement with the finding of Adegoke *et al.*,<sup>[24]</sup> who reported continuous significant increases in TC with advancing gestational age and Takahashi *et al.*<sup>[25]</sup> that reported significantly increased levels of TC, TG, LDL and VLDL in all trimesters.

The mean increase in TC from the first to the third trimester (0.67 mmol/L) was higher than that of TG (0.36 mmol/L). This was in contrast with the work of Herrera *et al.*,<sup>[26]</sup> who reported significant increases in the maternal plasma TC and TG levels from the first to the third trimester of pregnancy, with the change in TG being greater than that for TC.

The cardiac risk factor (TC/HDL) was calculated for the different trimesters as a predictor of atherosclerosis in pregnant women. A continuous decrease was observed which corresponds with the findings of De *et al.*,<sup>[2]</sup> who reported a decrease in TC/HDL during pregnancy. In addition to the relevance of the TC/HDL as a predictor of atherosclerosis, the significance of altered TC/HDL ratio indicates additional risks in pregnancy-induced hypertension (PIH).<sup>[2]</sup>

Winkler *et al.*<sup>[8]</sup> reported an association between elevated plasma TG concentration, small dense LDL and decreased HDL. This, however, does not hold true in our study which revealed an increased concentration of all the lipid fractions. Elevated TG levels already present in the first trimester due to hyperestrogenemia may be responsible for the increase in LDL seen in the early stages of pregnancy.<sup>[8]</sup>

The classification of the parturients in their first trimester, into two age groups (25-34) and (35-45) years revealed statistically significant increases ( $P < 0.05$ ), compared to their age-matched controls. Comparison of the two groups, however, showed nonsignificant difference ( $P > 0.05$ ) in the two age groups, possibly indicating no age-dependent effect on the lipid metabolism in first trimester. The study questionnaires revealed a remarkable increase in the antenatal visits of parturients of the lower socioeconomic status, possibly as a result of the highly subsidized cost of infant and maternal care in the Government Teaching Hospitals. Also the parturients were shown to be involved in increased physical activities, as a result of their less-sedentary lifestyle. Weight gain in pregnancy was also moderate, probably due to the social status.

The changes in HDL, LDL and VLDL metabolism presented in this study are compatible with the well

established reduction of hepatic TG lipase activity during pregnancy. These changes are related to the etiology of hyperlipidemia and cholestasis of pregnancy. There was very insignificant drop-out rate. This was probably because, the lipid profile tests which are otherwise expensive were carried out and the results issued to the patients free of charge as an incentive for the study. The decrease in TC/HDL ratio in the pregnant women can be attributed to Nigerian diet and the less sedentary professions of such women such as hawking, farming and trading all of which predispose them to increased activity and possible protection from abnormal lipid profile.

### Study limitations

The limitations encountered were initial refusal of some of the women to be enrolled in the study. This was, however, overcome by the confidentiality of the recruitment, which made them give their consent. The follow-up rate from the first to the third trimester proved to be a bit strenuous and necessitated frequent visits to the clinics during each trimester, until the women have been seen. The sample size was also limited, as this depended on the women that were not ruled out by the exclusion criteria and who were willing to participate.

## CONCLUSIONS

In conclusion, data from this study does not support the idea that normal pregnancy may still put women at risk for vascular damage as previously thought. The corresponding increase in HDL alongside other lipid fractions significantly reduces the cardiac risk factor (TC/HDL); a sign that pregnant women are in fact protected from the risk of atherosclerosis more than the nonpregnant women, as the gestational age advances.

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