# Apolipoproteins Distribution in the three Trimesters of Pregnancy in Women attending Antenatal Clinic in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

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## ABSTRACT

**Background**: During human pregnancy, apolipoproteins play crucial roles in influencing metabolic adaptations essential for fetal growth and development, significantly impacting maternal health. **Objectives**: To determine apolipoproteins levels (Apo A-I, Apo A-II, Apo B100) in each trimester of pregnancy in women attending Nnamdi Azikiwe University Teaching Hospital. **Materials and Methods**: This cross-sectional study involved a total of 232 participants recruited using simple random sampling. The participants comprised 58 apparently healthy pregnant women in their first, second and third trimesters of pregnancy each and 58 apparently healthy non-pregnant women as the control group. Apolipoprotein A-I and Apolipoprotein B100 levels were determined using immunoturbidimetric methods. Apo A-II levels were determined using Enzyme-linked Immunosorbent assay (ELISA). **Results**: The findings showed that Apo A-I levels were significantly higher in the first and second trimesters compared with the control group (*P*-value = 0.054 and <0.001) respectively. The mean levels of Apo A-II were significantly lower in the first, second, and third trimesters compared with the control group (*P*-value = <0.001). A significantly lower mean level of Apo A-II was observed in the first trimester compared with the second trimester (*P*-value = 0.016). The mean levels of Apo B100 were significantly higher in the first, second, and third trimesters compared with the control group (*P*-value = 0.002, 0.001 and <0.001) respectively. **Conclusion**: This study showed significantly higher serum levels of Apo A-I and Apo B100 with significant reduction in Apo A-II levels in pregnant women, suggesting the need for monitoring during pregnancy.

**Keywords**: Apolipoproteins; Gestation; Pregnancy; Trimesters.

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## INTRODUCTION

Pregnancy, also known as gestation is the period from conception to child birth during which fetal development occurs [1]. Fetal development and growth depend on appropriate in utero homeostasis, which is regulated by maternal nutrients metabolism and placental transport into the fetal circulation [2]. Pregnancy is divided into three trimesters with the average duration of 40 weeks from the start of the Last Menstrual Period (LMP) or about 38 weeks when measured from fertilization [3].

Human pregnancy is characterized by striking changes in maternal metabolism associated with an increase in cell proliferation due to uterine enlargement, increased blood volume, placental development and fetal growth [4]. These changes may involve alterations in maternal lipid metabolism associated with hormonal changes [5]. Decreased tissue sensitivity to insulin induced by hormonal alterations has been reported in pregnancy [6]. The insulin resistance that develops is characterized by an increased basal insulin concentration and decreased tissue sensitivity to insulin [7].In the maternal lymphatic and blood circulations, cholesterol is transported in the form of lipoproteins such as high-density lipoprotein (HDL), low-density lipoprotein (LDL) or very low-density lipoprotein (VLDL). During early developmental stages, when the fetus is incapable of its own cholesterol synthesis, placental transfer of maternal cholesterol is critical. Besides its beneficial roles, an excess of cholesterol can be a predisposing factor to adverse health outcomes [8]. Several studies consistently show an association between elevated LDL-cholesterol and high prevalence of cardiovascular disease in adults, and, on the other hand, between high HDL-cholesterol and reduced prevalence of cardiovascular diseases [2, 9].

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in developed nations and has recently emerged as one of the most prominent health challenges facing the developing countries [10]. According to the recent reports from the World Health Organization, CVD is the number one cause of death globally and over 75% of these deaths occur in low and middle income countries [11]. There has been a significant steady rise in the prevalence of CVD among Nigerian women within the last decade [12]. The risk of developing CVD is directly proportional to the number of circulating atherogenic particles which Apo B correctly correlates [13]. Most recent studies have shown that conventional lipid indices are not adequate in its assessment and the risk of CVD is primarily determined by the balance between pro-atherogenic particles (ApoB) and anti-atherogenic particles [13].

Apolipoproteins are important structural and functional proteins in lipoprotein particles. Apolipoprotein A-I (Apo A-I), the major structural protein of High Density Lipoprotein (HDL) is synthesized predominantly in the liver and the small intestine [14]. Apo A-I is involved in the regulation of reverse cholesterol transport by being a co-factor for Lecithin cholesterol acyl transferase (LCAT), while they also exert anti-inflammatory, anti-apoptotic, anti-aggregatory and anti-oxidant actions [15-17]. Apo A-I is a key mediator of plasma cholesterol transport and cellular cholesterol homeostasis through its interaction with different receptors and transporters like ABCAI (ATP Binding Cassette AI), ABCGI (ATP Binding Cassette GI) and Scavenger Receptor BI with structural changes in HDL [18]. Thus, this contributes towards its novel anti-atherogenic function in the prevention of coronary artery disease (CAD) [19]. In addition to its anti-atherogenic function, Apo A-I has antidiabetic properties by increasing insulin sensitivity [20]. Previous studies have shown that addition of Apo A-I to myocytes in culture also stimulates glucose uptake in an insulin-dependent and insulin-independent manner [21, 22]. Apolipoprotein A-II (Apo A-II) is the second most abundant protein component of HDL, accounting for approximately 20% of total HDL protein [23]. Apo A-II is reported to be more hydrophobic than apolipoprotein A-I (Apo A-I), and is closely associated with modulation of HDL metabolism and alteration of HDL conformation with its interaction with Apo A-I and other apolipoproteins by influencing hepatic lipase-mediated lipolysis [24].Apolipoprotein B is a large amphipathic glycoprotein with two isoforms; Apo B100, which is synthesized in the hepatocytes and Apo B48, synthesized in the small intestine [25]. It is now evident that an

increased serum Apo B100 concentration is an important coronary heart disease (CHD) risk factor because it is a component of all atherogenic or potentially atherogenic particles including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and lipoprotein (a) [Lp(a)] and each particle contains one molecule of Apo B; thus, Apo B levels indicate the atherogenic particle concentration independent of the particle cholesterol content, which is variable [26]. The Apo B/Apo A-I ratio is a strong predictor of cardiovascular risk which reflects the cholesterol balance of the potentially atherogenic and antiatherogenic lipoprotein particles. Its high value would indicate an increased risk of cholesterol deposition, endothelial dysfunction and hence, higher risk of atherosclerosis [27]. Metabolic changes occur in pregnancy due to maternal metabolism accompanied with hormonal changes [28]. Previous studies have reported that adverse pregnancy outcomes can affect short and long term maternal and fetal health [7].Substantial knowledge on apolipoproteins levels in the different trimesters of pregnancy are limited. This study provides useful insights on the roles of apolipoproteins during human pregnancy in influencing metabolic adaptations essential for fetal growth and development.

## MATERIALS AND METHODS

### Study Area

This research was conducted at Nnamdi Azikiwe University Teaching Hospital Antenatal Clinic, Nnewi, Anambra state, Nigeria.

### Study design

This cross sectional study was conducted on pregnant women who enrolled in the antenatal program at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra state, Nigeria. Its goal was to evaluate the levels of apolipoproteins (Apo A-I, Apo A-II and Apo B100) in each trimester of pregnancy. A total number of 232 participants, including 174 test participants (pregnant women) and 58 individuals (apparently healthy non-pregnant women) as the control group were recruited for the study using a simple random sampling procedure. The test participants comprised 58 pregnant women in their first trimester (8-12 weeks gestation), 58 pregnant women in their second trimester (20-24 weeks gestation), and 58 pregnant women in their third trimester (30-36 weeks gestation), all of who appeared to be in good health.

### Sample size

The sample size was calculated using the software package, G*power (version 3.0.10). Power analysis for one-way ANOVA, was calculated using the software to determine a sufficient sample size of 232 using the alpha of 0.05, a power of 0.90 and a medium effect size (d=0.25) (Faul*et al*., 2013). Based on these, the calculated sample size of 232 has 90% power to detect a difference of 0.25 (medium effect size) at significance level of 0.05.

### Inclusion Criteria

Apparently healthy pregnant women within the age range of 18-35 years and age matched non-pregnant women (control group) were recruited for this study.

### Exclusion Criteria

Obese participants and participants with known history of diabetes, renal and cardiovascular diseases were excluded from this study.

### Informed consent

Prior to the study, informed consent was sought and obtained from the participants

### Sample collection

Three (3) milliliters of fasting blood sample was collected from each participant by venepuncture technique and dispensed into plain container. The blood samples were centrifuged at 3000 rpm for 5 minutes after clotting to obtain the serum for apolipoproteins assay.

### Biochemical analysis

### Determination of Apolipoprotein A-I levels

Serum apolipoprotein A-I levels were assayed using immunoturbidimetric method.

### Procedure

All reagents, standards and samples were brought to

room temperature before use. Two hundred and twenty five microliters (225µl) of R1 and two microliters (2µl) of the sample and standard were pipetted into the appropriate wells in the microtiter plate. The mixture was incubated at 37$^0$C for 5 minutes and the absorbance A1 was read. Seventy five microliters (75µl) of R2 was added to each well and the mixture was incubated at 37$^0$C. The absorbance A2 was read after 5 minutes.

Abs = (A2 – A1) sample or calibrator

**Determination of Apolipoprotein A-II levels**

Serum apolipoprotein A-II levels were assayed using Enzyme Linked Immunosorbent Assay (ELISA) technique.

**Procedure**

All reagents, standards and samples were brought to room temperature before use and serial dilutions of the standard were made. Fifty microliters (50µl) of standards or sample were pipetted into the appropriate wells in the microtiter plate and 100µl of HRP-conjugate reagent wasadded to each well. The plate was closed with closure plate membrane and incubated at 37$^0$C for 60 minutes. The closure plate membrane was uncovered and the liquid discarded. The washing buffer was added and the contents of the wells drained and dried repeatedly for five times. Fifty microlitres (50µl) of substrate A and B were added to each well and incubated at 37$^0$Cfor 15 minutes. Enzyme reaction was stopped by adding 50µl of stop solution to each well, changing the blue color to yellow color. The optical density was read at 450nm within 15 minutes using microtitre plate reader.

**Determination of Apolipoprotein B100 levels**

Serum apolipoprotein B100 levels were assayed using immunoturbidimetric method.

**Procedure**

All reagents, standards and samples were brought to room temperature before use. Two hundred and twenty five microliters (225µl) of R1 and two microliters (2µl) of the sample and standard were pipetted into the appropriate wells in the microtiter plate. The mixture was incubated at 37$^0$C for 5 minutes and the absorbance A1 was read. Seventy five microliters (75µl) of R2 was added to each well and the mixture was incubated at 37$^0$C. The absorbance A2 was read after 5 minutes.

Abs = (A2 – A1) sample or calibrator

**Statistical Analysis**

The statistical package for social sciences version 23.0 was used to analyze the data. Specific statistical tools utilized were Analysis of Variance (ANOVA) and post hoc tests. Results were considered significant at $p<0.05$.

## RESULTS

**Table 1** shows the mean level of Apo A-I in the test participants (pregnant women in the first, second and third trimesters of gestation) and the control Group. There was a significant difference in the mean levels of Apo A-I in the first trimester, second trimester, third trimester of pregnancy and in the control group (f-value = 6.435; *P*-value = <0.001). The mean Apo A-I levels of the pregnant women in the first and second trimesters were significantly higher compared with the control group (*P*-value = 0.054 and <0.001) respectively. However, there were no significant differences in the mean levels of Apo A-I of the pregnant women in the first trimester compared with those in the second and third trimesters of gestation (*P*-value = 0.517; *P*-value = 1.000) respectively. Similarly, no significant differences were observed in the mean levels of Apo A-I in the second trimester compared with third trimester (*P*-value = 0.406) and in the third trimester compared with the control group (*P*-value = 0.074). See table 1.

Table 2 shows the mean levels of Apo A-II and Apo B100 in pregnant women in the first, second and third trimesters of gestation and in the control group. There was a significant difference in the mean levels of Apo A-II in the first trimester, second trimester, third trimester of gestation and in the control group (f-value =18.350; *P*-value < 0.001).

The mean Apo A-II levels of the pregnant women in the first, second, and third trimesters were significantly lower compared with the control group (*P*-value < 0.001; < 0.001 and < 0.001) respectively. A significantly lower mean level of Apo A-II was observed in the first trimester compared with the second trimester of gestation

**Table 1**: Apo A-I levels in the test and control groups (Mean±SD).

| Groups | Apo A-I (mg/dl) |
|---|---|
| **A** – Pregnant women in first trimester of gestation (n=58) | 173.60±14.95 |
| **B** – Pregnant women in second trimester of gestation (n=58) | 179.19±14.68 |
| **C** – Pregnant women in third trimester of gestation (n=58) | 173.24±17.60 |
| **D** – Apparently healthy non-pregnant women (n=58) | 165.07±21.70 |
| F-test | 6.435 |
| p-value | <0.001* |
| A vs B | 0.517 |
| A vs C | 1.000 |
| A vs D | 0.054* |
| B vs C | 0.406 |
| B vs D | <0.001* |
| C vs D | 0.074 |
| p<0.05 = *significant | |

Apo A-I = Apolipoprotein A-I

**Table 2**: Apo AII and Apo B100 levels in the test and the control groups (Mean±SD).

| Groups | Apo A-II (mg/dl) | Apo B100 (mg/dl) |
|---|---|---|
| **A** – Pregnant women in first trimester of gestation (n=58) | 463.85 ± 77.11 | 128.50±44.97 |
| **B** – Pregnant women in second trimester of gestation (n=58) | 529.95 ± 115.16 | 130.64±31.98 |
| **C** – Pregnant women in third trimester of gestation (n=58) | 516.85 ± 129.47 | 137.47±32.99 |
| **D** – Apparently healthy non-pregnant women (n=58) | 622.66 ± 138.54 | 101.47±49.25 |
| F-test | 18.350 | 8.871 |
| p-value | <0.001* | <0.001* |
| A vs B | 0.016* | 1.000 |
| A vs C | 0.095 | 1.000 |
| A vs D | <0.001* | 0.002* |
| B vs C | 1.000 | 1.000 |
| B vs D | <0.001* | 0.001* |
| C vs D | <0.001* | <0.001* |
| p<0.05 = *significant | | |

*Apo A-II = Apolipoprotein A-II*
*Apo B100 = Apolipoprotein B100*

(*P*-value = 0.016). However, no significant differences were observed in the mean levels of Apo A-II in the first and second trimesters compared with the third trimester (*P*-value = 0.0095; *P*-value = 1.000) respectively. See table 2.

There was a significant difference in the mean levels of Apo B100 in the first trimester, second trimester, third trimester of gestation and in the control group (f-value = 8.871; *P*-value < 0.001). There were significantly higher mean levels of Apo B100 in the first, second, and third trimesters compared with the control group (*P*-value = 0.002, 0.001 and < 0.001) respectively. However, no significant differences were observed in the mean levels of Apo B100 in the first trimester compared with the second and third trimesters ((*P*-value = 1.000, and 1.000) respectively. Similarly, there was no significant difference in the mean levels of Apo B100 in the second trimester compared with third trimester (*P*-value = 1.000). See table 2.

## DISCUSSION

During pregnancy, significant changes in maternal metabolism occur to support fetal demands and these changes begin after conception [29]. It is accompanied by extra demand of energy with a well-integrated metabolic shift to ensure adequate and continuous supply of nutrients to a constantly feeding fetus from an intermittently fasting and feeding mother [4]. The decreased tissue sensitivity to insulin induced by these hormones cause changes in maternal metabolism which may be predisposing to short and long term adverse maternal and fetal health outcomes. These metabolic changes involve alterations in maternal lipid metabolism, which are associated with a cluster of cardiovascular risk factors. Evidences suggest an increased risk of cardiovascular diseases (CVDs) in metabolic syndrome, as CVDs is one of the major causes of morbidity and mortality in women worldwide [30]. This study evaluated levels of apolipoproteins in pregnant women in each trimester of gestation.

The findings of this study indicated a significant difference in the mean levels of Apo A-I in the first trimester, second trimester, third trimester of gestation and in the control group. A significantly higher mean level of Apo A-I was observed in the first and second trimesters compared with the control group. The mechanisms underlying the higher levels of Apo A-I during pregnancy may involve combination of hormonal, metabolic and physiological adaptations to support the demands of gestation. Apo A-I is involved in the regulation of reverse cholesterol transport while they also exert anti-inflammatory, anti-apoptotic, anti-thrombotic and anti-oxidant roles [17, 31]. As such, Apo A-I has crossed its boundary of its potential of protecting cardiovascular system and lowering cardiovascular disease risk due to its anti-atherogenic effects [14].Pregnancy is associated with significant

hormonal changes, including an increase in estrogen levels. Estrogen has been shown to influence lipid metabolism by promoting the production of HDL particles and Apo A-I. Beazer and Freeman (32) reported that increase in plasma HDL-C concentration is usually concomitant with pregnancy-associated estradiol production that has a multitude of direct and indirect effects on lipid and lipoprotein metabolism. HDL-associated estradiol may directly increase Apo A-I functions or increased estradiol may stimulate the production of Apo A-I, either of which could counteract the adverse maternal metabolic adaptations to pregnancy.

The higher mean level of Apo A-I could also be attributed to its anti-inflammatory role. Each stage of pregnancy is characterized by a unique inflammatory environment [33]. This suggests the role of Apo A-I in controlling inflammation as numerous cells of the placenta synthesize and secrete pro-inflammatory cytokines throughout pregnancy [34]. These maternal inflammatory states usually play a key role in immune activation during pregnancy [35]. This finding is in agreement with the report of Woo *et al*. [36] who reported a 25% increase in Apo A-I concentration with minimal change in HDL-C concentrations during pregnancy in Gambian women. Ezeugwunne *et al*. [37] reported a significantly higher level of Apo A-I in symptomatic HIV subjects on therapy when compared to before therapy. This suggested an immune recovery, impaired by HIV infection and hence, the cardioprotective role of Apo A-I. The mechanisms in pregnancy may be similar as the significantly higher Apo A-I levels could be due to its cardioprotective role. Samangooei *et al*. [38] showed the role of Apo A-I in inflammatory process performed in the first-degree family members of relapsing-remitting multiple sclerosis patients which revealed a significant decrease, which was considered as one of the contributing factors in the pathogenesis. Wu *et al*. [22] reported that Apo A-I has the capacity to reduce pregnancy-induced insulin resistance in rats by increasing glucose uptake in adipose tissue and skeletal muscle and thus, reducing systemic inflammation.

The findings of this study revealed a significant difference in the mean levels of Apo A-II in the first trimester, second trimester, third trimester of gestation and in the control group. A significantly lower mean level of Apo A-II was observed in the first, second, and third trimesters compared with the control group. Also, a significantly lower mean level of Apo A-II was observed in the first trimester compared with the second trimester of gestation. Apo A-II is the second most abundant protein component of HDL particles. While Apo A-II levels are generally lower in HDL compared to Apo A-I, it still plays a significant role in lipid metabolism, being directly or indirectly involved in vascular diseases [23]. The lower levels of Apo A-II during pregnancy suggest a shift in HDL function as HDL-Apo A-II mediates cholesterol efflux. The actions of pregnancy associated hormones and their varying concentrations during pregnancy can lead to alterations in lipoprotein metabolism as gestation progresses. Parrettini *et al*. [5] reported a positive correlation between changes in the lipid and lipoprotein concentrations and the changes in the concentrations of the pregnancy hormones estradiol, progesterone and human placental lactogen (HPL) during gestation. These changes are not unexpected as estrogen increases activity of cholesteryl ester transfer protein and decreases hepatic lipase activity, leading to a redistribution of lipids and an increase in the larger particles [39]. This finding is in agreement with the report of Woo *et al*. [36] who showed that Apo A-II reduced greater than 50% during gestation in Gambian women. Ramanjaneya *et al*. [40] reported a decrease in Apo A-II levels in pregnant women with gestational diabetes and that Apo A-II may also play a role in inflammation, as it negatively correlated with C-reactive protein (CRP).

Pregnancy often triggers a mild inflammatory response in the body. Elevated inflammatory markers such as C-reactive protein (CRP) and interleukins can influence Apo A-II levels [41]. Inflammation may reduce the synthesis of Apo A-II and other apolipoproteins involved in High Density Lipoprotein (HDL) metabolism. Feingold and Grunfeld [42] stated that inflammation can lead to structural changes in HDL and marked decrease in HDL associated proteins (Apo A-II) and transfer proteins involved in HDL metabolism and function. Kauss *et al*. [43] showed that smoking is associated with reduced levels of Apo A-II thereby confirming the impact of inflammation due to smoking. Ezeugwunne *et al*. [37] reported a significantly lower level of Apo A-II in symptomatic HIV subjects on 3,

6, 9 and 12 months therapy when compared with value before therapy. However, the levels of Apo A-II observed with lengthened therapy suggested an immune recovery. The significantly lower mean level of Apo A-II observed in the first trimester compared with the second trimester of gestation could be attributed to fetal growth and development. As the pregnancy progresses from the first trimester to the second trimester, the demands for nutrients and lipids for fetal growth and development increase and this could lead to changes in lipoprotein composition, including Apo A-II. Ramanjaneya *et al*. [40] reported that Apo A-II correlated to gestational age at delivery in pregnant women with, as well as without gestational diabetes and raised the hypothesis that Apo A-II may be useful as a biomarker of premature delivery.

The findings of this study indicated a significant difference in the mean levels of Apo B100 in the first trimester, second trimester, third trimester of gestation and in the control group. A significantly higher mean level of Apo B100 was observed in the first, second, and third trimesters compared with the control group. This could be attributed to hormonal changes that significantly influence lipid metabolism including the increased production and decreased clearance of Apo B100 containing lipoproteins.

The higher mean level of Apo B100 could be due to fetal demands since triglyceride-rich lipoproteins are likely the main source of energy and Low Density Lipoprotein (LDL) carry cholesterol, which is a critical component of cell membranes, necessary for the formation of the fetal nervous system including the brain and spinal cord.

This finding is in agreement with the report of Woo *et al*. [36] who reported a significant increase in Apo B100 levels throughout gestation in Gambian pregnant women. Similarly, Melchoir *et al*. [3] reported a parallel increase in Apo B100 concentrations in pregnant women with no significant differences in plasma triglycerides or triglyceride-rich lipoprotein (TRL-C) concentrations. This suggested that the elevation in total cholesterol during pregnancy was due to increased number of LDL particles as opposed to increased cholesterol content of the LDL fraction. Conversely,Wolter *et al*. [44] reported that maternal Apo B100 serum levels are decreased in women whose pregnancies are complicated by Intrauterine Growth Restriction.

An elevated Apo B100 level represents a more atherogenic lipid profile and is known to be related to several chronic diseases including metabolic syndrome, insulin resistance and cardiovascular disease among non-pregnant population [45]. Zhang *et al*. [46] stated that Apo B100 clearance is decreased and the levels of plasma Apo B100 are increased in patients with Type 2 Diabetes Mellitus. Martson *et al*. [47] showed the association of apolipoprotein B-containing lipoproteins and the risk of myocardial infarction, independent from lipid content (cholesterol or TG). This suggested that Apo B may be the primary driver of atherosclerosis and that lowering the overall concentration of all Apo B-containing lipoproteins should be the focus of therapeutic strategies. Although, this study revealed a non-significant increase in the mean levels of Apo B100 as gestation progresses. Napso *et al*. [48] stated that during the first two trimesters, lipid metabolism is primarily anabolic. There is an increase in lipid synthesis and fat storage in preparation for the exponential increases in fetal energy needs in late pregnancy. Additionally, the increased synthesis of progesterone, cortisol, leptin and prolactin contributes to increased fat storage [48]. Lipid metabolism in the third trimester is in a 'net catabolic phase' associated with a decrease in insulin sensitivity. The decrease in insulin sensitivity is associated with enhanced lipolysis of stored triglycerides in adipocytes. In addition, insulin resistance results in a decrease in lipoprotein lipase in adipocytes leading to a decrease in the uptake of fatty acids from plasma triglyceride-rich lipoproteins [49]. Inflammatory states are associated with increased Apo B100 levels, and consequently, predisposition to cardiovascular diseases. Ihim *et al*. [50] reported an increased serum Apo B100 levels among *Mycobacterium tuberculosis* infected patients compared to non-infected healthy subjects and suggested a predisposition to cardiovascular disease.

Most recent studies have shown that conventional lipid indices are not adequate in its assessment, as lipoprotein-related risk of cardiovascular disease is

primarily determined by the balance between atherogenic and antiatherogenic particles. The finding of this study strengthens our knowledge about the metabolic alterations during pregnancy and emphasizes the significance of trimester-specific differences in apolipoprotein levels. Although, it is limited by the fact that the participants were not followed up to term.

## CONCLUSION

This study has shown that women in the first and second trimesters of pregnancy had significantly higher Apo A-I levels than non-pregnant women. The study also indicated significantly lower mean Apo A-II levels in the first, second, and third trimesters of pregnancy compared with non-pregnant women as well as a significantly lower mean Apo A-II level in the first trimester compared with the second trimester of pregnancy. The mean levels of Apo B100 were significantly higher in the first, second, and third trimesters of pregnancy compared with non-pregnant women. Routine monitoring for all women at risk of dyslipidemia is recommended throughout all trimesters of pregnancy.

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**Author Contributions**

CSO and POM conceptualized, designed and contributed to the implementation of the project. NAM, CEO, ECO and ACI contributed to the literature review, data collection and analysis. ESO, GUE, KEN, CJN, EEN and CFN participated in the revision of the manuscript. All authors were involved in the writing and revision of the manuscript. The authors read, approved the final manuscript and agree to be accountable for all aspects of the work.

**Data Availability**

The data used to support the findings of this study are available from the site publicly.

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**Conflict of Interest**

None declared

**Ethical Approval**

The study was approved by the Institutional Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi (Reference number: NAUTH/CS/66/VOL.14/VER 3/31/2021/029).

## REFERENCES

1. Obrowski S, Obrowski M, Starski K. Normal Pregnancy: A Clinical Review. AJPN. 2016; 1 (1): 15-18.
2. Melhem H, Kallol S, Huang X, Luthi M, Ontsouka CE, Keogh A, et al. Placental secretion of apolipoprotein A-I and E: the anti-atherogenic impact of the placenta. Sci. Rep.2019; 9: 6225.
3. Melchior JT, Swertfeger DK, Morris J, Street SE, Warshak CR, Welge JA, et al. Pregnancy is accompanied by larger high density lipoprotein particles and compositionally distinct subspecies. J Lipid Res. 2021; 62: 1-5.
4. Armistead B, Johnson E, VanderKamp R, Kula-Eversole E, Kadam L, Drewlo S, et al. Placental regulation of energy homeostasis during human pregnancy. Endocrinol. 2020; 161 (7): 1-13.
5. Parrettini S, Caroli A, Torlone E. Nutrition and Metabolic adaptations in physiological and complicated pregnancy: Focus on obesity and gestational diabetes. Front. Endocrinol. 2020; 11: 611929.
6. Kampmann U, Knorr S, Fuglsang J, Ovesen P. Determinants of Maternal Insulin Resistance during Pregnancy: An Updated Overview. J. Diabetes Res. 2019; 19:10-12.
7. Song C, Lyu Y, Li C, Liu P, Li J, Ma RC, et al. Long term risk of diabetes in women at varying durations after gestational diabetes; a systematic review and meta analysis with more than 2 million women. Obes Rev. 2018; 19: 421-429.
8. Fuenzalida B, Sobrevia B, Cantin C, Carvaja L, Salsoso JG, Susana CD, et al. Maternal

supraphysiological hypercholesterolemia associates with endothelial dysfunction of the placental microvasculature. Sci. Rep. 2018; 8 (7690): 1-3.
9. Florea G, Tudorache IF, Fuior EV,Ionita R, Dumitrescu M, Fenyo IM, et al. Apolipoprotein A-II, a player in multiple processes and diseases. Biomedicines. 2022; 10 (7): 1578-1579.
10. Keteepe-Arachi T, Sharma S. Cardiovascular disease in women: Understanding symptoms and risk factors. Eur. Cardiol. 2017; 12: 10-13.
11. WHO. Cardiovascular Key Facts. 2020; Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases- (cvds).Accessed on 25 January, 2023.
12. Adedapo AD. Rising trend of cardiovascular diseases among South-Western Nigerian female patients. Nig J Cardiol. 2017; 14: 71-74.
13. Ayoade OG, Essien SI, Sonuga O. Apolipoprotein B / A1 ratio as a potential marker of cardiovascular risk in women. Sahel Med. J. 2021; 24 (3): 99-103.
14. Cochran BJ, Ong KL, Manandhar B, Rye KA. Apo A-I: A protein with multiple therapeutic functions. Curr. Atheroscler. Rep. 2021;23 (3): 11.
15. PensonPE, Long DL, Howard G, Toth PP, Muntner P, Howard VJ, et al. Associations between very lowconcentrations of low density lipoproteincholesterol, high sensitivity C-reactive protein, and health outcomes in the Reasons for Geographical andRacial Differences in Stroke (REGARDS) study. Eur. Heart J. 2018; 39: 3641–3653.
16. Sathiyakumar V, Kapoor K, Jones SR, Banach M, Martin SS,Toth PP. Novel Therapeutic Targets forManaging Dyslipidemia. Trends Pharmacol. Sci. 2018; 39: 733–747.
17. Bhale AS, Venkataraman K. Leveraging knowledge of HDLs major protein Apo A-I: Structure, function, mutations and potential therapeutics. Biomed. Pharmacother. 2022; 154: 113634.
18. VanderVorst EPC. High Density Lipoproteins and Apolipoprotein A1. Subcell. Biochem. 2020; 94: 399-420.
19. Rahim S, Abdullah HA, Ali Y, Khan UI, Ullah W, Shahzad MA, et al. Serum Apo A-I and its role as a biomarker of coronary artery disease. Cureus. 2016;8 (12): 941.
20. King TW, Cochran BJ, Rye KA. Apo A-I and Diabetes. Arterioscler.Thromb. Vasc. Biol. 2013; 43 (8):1362-1368.
21. Tang S, Tabet F, Cochran BJ, Cuesta Torres LF, Wu BJ, Barter PJ, et al. Apolipoprotein A-I enhances insulin-dependent and insulin independent glucose uptake by skeletal muscle. Sci. Rep. 2019; 9: 1350.
22. Wu BJ, Sun Y, Ong KL, Li Y, Tang S, Barter PJ, et al. Apolipoprotein A-I protects against pregnancy-induced insulin resistance in rats. Arterioscler. Thromb. Vasc. Biol. 2019; 39: 1160-1171.
23. Hisamatsu T. Apolipoprotein A2 Isoforms: New insight into the risk of myocardial infarction. J. Atheroscler. Thomb. 2021; 28 (5): 469-470.
24. Yang M, Liu Y, Dai J, Li L, Ding X, Xu Z, et al. Apolipoprotein A-II induces acute phase response associated AA amyloidosis in mice through conformational changes of plasma lipoprotein structure. Sci. Rep. 2018; 8 (1): 5620.
25. Jialal I, Duell PB. Diagnosis of Familial Hypercholesterolemia. Am. J. Clin. Pathol, 2016; 145 (4): 437-439.
26. Behbodikhah J, Ahmed S, Elyasi A, Kasselman LJ, DeLeon J, Glass AD, et al. Apolipoprotein B and Cardiovascular Disease: Biomarker and Potential Therapeutic Target. Metabolites. 2021; 11: 690-700.
27. Walldius G. The Apo B/Apo A-I is a strong predictor of cardiovascular risk. Lipids Health Dis. 2012; 39545: 95-148.
28. Wang C, Liu C, Zhang Z. Transthyretin and normal human pregnancy: mini review. Crit. Rev. Eukaryot. Gene Expr. 2016; 26: 273-277.
29. Poon LC, McInytre HD, Hyett JA, DaFonseca EB, Hod M. The first-trimester of pregnancy-A window of opportunity for prediction and prevention of pregnancy complications and future life. Diabetes Res. Clin. Pract. 2018; 145: 20-30.
30. Dietrich E, Jomard A, Osto E. Crosstalk between high-density lipoproteins and

endothelial cells in health and disease: Insights into sex-dependent modulation. Front. Cardiovasc. Med. 2022; 9: 1-19.

31. Georgila K, Vyrla D, Drakos E. Apolipoprotein A-I (Apo A-I), Immunity, Inflammation and Cancer. Cancers. 2019; 11 (8): 1097.
32. Beazer JD, Freeman DJ. Estradiol and HDL function in women- A partnership for life. J. Clin. Endocrinol. Metab. 2022; 107 (5): 2192-2194.
33. Ravi M, Bernabe B, Michopoulos V. Sress-related mental health disorders and inflammation in pregnancy: The current landscape and the need for further investigation. Front. Psychiatry. 2022; 13: 1-11.
34. Woollett LA, Catov JM, Jones HN. Roles of maternal HDL during pregnancy. Biochim. Biophys. Acta Mol. Cell Biol. Lipids. 2022; 1867 (3): 159106.
35. Han VX, Patel S, Jones HF, Nielsen TC, Mohammed SS, Hofer MJ, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. Transl. Psychiatry. 2021; 11 (1): 71-72.
36. Woo JG, Melchoir JT, Swertfeger DK, Remaley AT, Sise EA, Sosseh F, et al. Lipoprotein subfraction patterns throughout gestation in the Gambia: changes in subfraction composition and their relationships with infant birth weights. Lipids Health Dis. 2023; 22 (19): 1-12.
37. Ezeugwunne IP, Ogbodo EC, Analike RA, Ifeanyichukwu M, Ogah HGO, Amah AK, et al. Evaluation of apolipoprotein and lipid profiles in HIV symptomatic subjects before and after 12 months antiretroviral therapy in NAUTH Nnewi, South Eastern Nigeria. J. Med. Lab. Sci. 2019; 29 (1): 52-60.
38. Samangooei M, Farjam M, Etemadifar M, Taheri A, Meshkibaf MH, Movahedi B, et al. (2022). Evaluation of S100A12 and Apo A-I plasma level potency in untreated new relapsing-remitting multiple sclerosis patients and their family members. Sci. Rep. 2022; 12 (1): 2160.
39. Roland MCP, Godang K, Aukrust P, Henriksen T, Lekva T. Low CETP activity and unique composition of large VLDL and small HDL in women giving birth to small-for-gestational age infants. Sci. Rep. 2021;11: 6213.
40. Ramanjaneya M, Butler AE, Bashir M, Bettahi I, Moin ASM, Ahmed L, et al. Apo A2 correlates to gestational age with decreased apolipoproteins A2, C1, C3 and E in gestational diabetes. BMJ Open Diabetes Res. Care. 2021;9 (1):1925.
41. Koohdani F, Sadrzadeh-Yeganeh H, Djalali M, Eshraghian M, Zamani E, Sotoudeh G, et al.Diabetes Metab. J. 2016; 40 (3): 222-229.
42. Feingold KR., Grunfeld C.The effect of inflammation and infection on lipids and lipoproteins. In: Feingold KR, Anawalt B, Blackman MR, et al., editors.Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2022. PMID: 26561701.
43. Kauss AR, Antunes M, Bourdonnaye G, Pouly S, Hankins M, Heremans A, et al. Smoking and apolipoprotein levels: A meta-analysis of published data. Toxicol. Rep. 2022; 9: 1150-1171.
44. Wolter M, Okai CA, Smith DS, Rub M, Rath W, Pecks U, et al. Maternal Apolipoprotein B100 serum levels are diminished in pregnancies with Intrauterine Growth Restriction and differentiate from controls. J. Proteomics. 2018; 12 (6): 1-5.
45. Liu Q, Wu L, Wang L, Chen K, Wu Y, Xia J, et al. Associations between maternal mid-pregnancy apolipoprotein A-I, apolipoprotein B, apolipoprotein B/ apoliporotein A-I ratio and preterm birth. Clin. Chim. Acta. 2022; 536: 12-17.
46. Zhang P, Gao J, Pu C, Zhang Y. Apolipoprotein status in type 2 diabetes mellitus and its complications (Review). Mol. Med. Rep. 2017; 16(6): 9279-9286.
47. Marston NA, Giugliano RP, Melloni GEM, Park JG, Morrill V, Blazing MA, et al. Association of apolipoprotein B-containing lipoproteins and risk of myocardial infarction in individuals with and without atherosclerosis. J. Am. Coll. Cardiol. 2022; 7 (3): 250-256.
48. Napso T, Yong HEJ, Lopez-Tello J, Sferruzzi-Perri AN. The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. Front. Physiol. 2018;

9: 1091-1092.
49. Zeng Z, Liu F, Li S. Metabolic adaptations in pregnancy: A review. Ann.Nutr. Metab. 2017; 70: 59-65.
50. Ihim AC, Meludu SC, Onyenekwe CC, Anyabolu AE, Akujiobi CN. Serum apolipoprotein B increased among tuberculosis patients compared to healthy subjects. Univ. Med. 2021; 40 (1): 45-51.