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Comparative studies on effect of Jadelle, Depoproveral and Implanon Contraceptives on APOA1 and APO B Concentration in Females Attending a Tertiary Hospital in Southern Nigeria

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Abstract

Background: Apo lipoproteins function as structural components of lipoprotein particles, cofactors for enzymes and ligands for cell-surface receptors. Apo A1 a major component of high density lipoprotein, while Apo B which is a primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL when in reference to both heart and vascular diseases indictment, helps in the transportation of fat molecules (lipids): cholesterol inclusive around the body. The study was carried out to compare Apo A1 and Apo levels in women on Implanon, Jadelle and Depo-Provera- the three most popular steroidal contraceptives used in south-south part of Nigeria.
Methodology: Ninety (90) female subjects in three groups of 30 each were recruited for Implanon, Jadelle and Depo-provera. Apo A1 and Apo B were determined using turbidimetric method on Baseline, 3rd, 6th, 9th and 12th month blood samples collected from these subjects.
Result: The result of the study showed that there was increased significant difference in Apo A concentration of females on Implanon, Jadelle and Depo-provera at3rd, 6th, 9th and 12th months compared with the baseline. Also, a significant increased difference ($P<0.05$) was observed at the 3rd, 6th, 9th and 12th month in ApoB when compared with the baseline. A monthly dependent increase was observed in the three contraceptives studied for ApoB, a pattern which is also observed in ApoA1 in jadelle and depo-provera only.
Conclusion: The study showed that there were significant increased changes in Apo A1 and ApoB concentrations in female subjects taking Implanon, Jadelle and Depo-provera at different months.
Keywords: Implanon, Jadelle, Depo-provera, Apo A1, Apo B.

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1. Introduction

High-dose estrogen formulations of oral contraceptives (OCs) were linked initially with an increased incidence of thromboembolic disease, which eventually led to an understanding of the profound effect estrogen has on the coagulation cascade. As a result, daily estrogen doses have been reduced nearly 80% – from 150 µg in the initial OC formulation to 20 µg/day in the lowest dose formulation. The risk of venous thromboembolism has dramatically declined with decreasing doses of estrogen [1], and it appears that the risk of acceleration of arterial disease is no longer present in healthy users of low-dose OCs [2]. However, oral progestogens are known to produce changes in lipoprotein metabolism that mirror those lipoprotein profiles that place an individual at high risk for

cardiovascular disease. Progestogens are also thought to be primarily responsible for the carbohydrate intolerance and insulin resistance noted with OC use; and as discussed later, high circulating insulin levels are thought by some to be atherogenic.

Subdermal implants are offered to women at family planning clinics in the tertiary/specialist hospitals, which are urban-based and staffed by gynecologists. Subdermal implants used in Nigeria include Norplant®, Jadelle®, and Implanon® are the current implants in use [3,4]. The mode of action is that these implants release low-dose of progesterone over an extended period of time, Norplant and Jadelle for five years and Implanon for three years. The levonorgestrel subdermal implant (Norplant®), introduced in 1985, and is the most commonly available long-acting progestin-only subdermal implant in Nigeria. During its first year of use, Norplant was shown to be highly effective and safe, and is considered an acceptable contraceptive method among Nigerian women of different ethnic groups [5]. The pooled Norplant continuation rate was shown to be 90.1% after 12 months, 84.9% after 24 months, and 77.1% after 36 months of use [5].

There are few studies in Nigeria concerning the use of hormonal contraceptive injections and subdermal implants, probably because these are not common choices. In addition, women fear the side effects of these hormonal methods of contraception, probably because of misinformation [6]. A study was conducted in Ibadan by Falase *et al* [6] which followed 810 patients who used depot-medroxy progesterone acetate (DMPA) as a contraceptive method over a period of 11 years. Amenorrhea, menorrhagia, and metrorrhagia were the major reasons for discontinuation of DMPA in only 11% of the patients. Progesterone only injectables (POIs) or progesterone injectables, e.g., DMPA and norethisteroneenanthate [7]. Act locally on cervical mucus and uterine endometrium preventing sperm transport and implantation of the fertilized ovum. Higher doses inhibit ovulation. DMPA benefits include decrease incidence of endometrial and ovarian cancers, ectopic pregnancies, iron deficiency anemia and PID. It is also useful in reducing the frequency of sickling and epileptic seizures in sickle cell anemia and epileptic patients.

In the mid 1940s, the lipoprotein major classes and subclasses were recognized and characterized. As awareness of the lipoproteins increased, apolipoprotein B (Apo B) was recognized to be present as one molecule per each LDL particle, and Apo A-I was shown to be present in about 70% of HDL particles [8,9] Apo B and Apo A-I are important components of non-HDL and HDL lipoprotein particles, respectively, and there is a wealth of evidence supporting their measurements to improve the prediction of CVD risk [10-13] . Studies by Rasouli *et al* [14] and Khadem-Ansari *et al* [15] indicated that the apoB/apoA-I ratio and apoB are independent risk factors for CAD assessment and prediction, and may be superior to any of the lipoprotein cholesterol or lipid ratios. Higher total cholesterol, LDL-C, apo B, triglycerides (TG), and lower HDL-C and apo A-I levels were found in patients with CAD [16].

The aim of this study was to determine the effects of Jadelle, depoproveral and implanon which are commonly used oral contraceptives on APO A1 and APO B concentrations of females in University of Port Harcourt Teaching hospital Nigeria

2. Materials and Methods

2.1 Study Area:

University of Port Harcourt Teaching hospital (UPTH).

2.2 Subject Selection:

Ethical clearance was obtained from the Ethics and Protocol Review Committee of the University of Port Harcourt Teaching hospital (UPTH). Written informed consent was obtained from each subjects. This was a study of adult females from the Rivers state attending a Reproductive Healthcare Clinic in University of Port Harcourt Teaching hospital. Subjects who were already on Oral Contraceptive depoprovera were recruited. Purposive random sampling technique was used. The inclusion criteria were as follows: females between the ages of 20 and 50 years, females on Oral Contraceptive depoprovera and females without predisposing factors or conditions to CV disease prior to contraceptive use.

2.3 Data and blood sample collection

A questionnaire was administered to obtain basic information on age, duration of drug use, contraceptive type, etc. Blood samples were collected by venepuncture from each subjects at baseline (0Months), 3months, 6months, 9months and 12 months respectively. After clotting, blood samples were centrifuged at 3,000 rpm for 10 minutes. Serum was aliquoted into Eppendorf tubes and stored at −20°C until use.

2.4 Biochemical Studies

The 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 peroxides [17]. Ten microlitre (10) of sample, Control, standard and distilled water was pipette into respective test tubes then 1000 of cholesterol working reagent was added. It was mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against the reagent blank at 520nm.The concentration of sample was calculated using the

absorbance of sample against absorbance of standard multiplied by concentration of standard.

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase [18].

Ten microlitre (10) of sample, Control, standard and distilled water was pipetted into respective test tubes then, 1000 of triglyceride reagent was added. It was mixed and incubated for 5 minutes at 370C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard.

Low density lipoproteins (LDL and VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic in the presence of Magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined. Five hundred microlitre (500ul) of sample, Control standard and distilled water was added into respective test tubes, 1000 of precipitant was added into all the tubes. It was mixed and allowed to stand for 10 minutes at room temperature. It was centrifuged for 2 minutes at 12,000 rpm. Then 10 of supernatant from Control, standard and distilled water was added into their respective test tubes and cholesterol concentration of supernatant was determined as shown above by the methods of Allain *et al*. (1974).

2.5 Statistical analysis:

Statistical analyses were performed using SPSS 21 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered significant. Data are presented as mean (standard deviation, SD). Differences in continuous data were compared using Student's *t*-test (two groups) and one-way analysis of variance (ANOVA; three or more groups) followed by the post-hoc test.

3. Result

APO A1(mg/dl) concentration in Depoprovera was 111.13±16.24, 111.03±15.96, 111.60±15.91, 113.47±16.09 and 116.43±19.27 while APO B (mg/dl) was 83.37±13.47, 84.07±14.34, 88.40±20.31, 90.00±14.59 and 93.00±15.28 at 0, 3rd, 6th , 9th and 12th month respectively. APO A1(mg/dl) concentration in implanon was 109.17±14.46, 99.47±13.21, 103.37±13.76, 109.10±14.58 and 118.13±20.91while APO B (mg/dl) was 81.10±9.56, 81.37±10.18, 84.23±11.52, 87.83±11.21 and 91.43±11.42 at 0, 3rd, 6th , 9th and 12th month respectively. In Jadelle, APO A1 (mg/dl) concentration was 100.60±15.10, 81.17±8.21, 84.60±9.11, 90.60±10.63 and 97.43±12.92 while APO B (mg/dl) was 82.10±12.09, 72.27±7.42, 73.93±6.93, 78.07±8.11 and 84.50±10.42 at 0, 3rd, 6th, 9th and 12th month respectively as shown in table 1 below.

Table 1: Effects of Depoproveral, Implanon and Jadelle on APO A1 and APO B at different Months

	Depoproveral		Implanon		Jadelle	
Month	**APO A1 (mg/dl)**	**APOB (mg/dl)**	**APO A1 (mg/dl)**	**APOB (mg/dl)**	**APO A1 (mg/dl)**	**APOB (mg/dl)**
Baseline	111.13±16.24	83.37±13.47	109.17±14.46	81.10±9.56	100.60±15.10	82.10±12.09
3rd month	111.03±15.96	84.07±14.34	99.47±13.21	81.37±10.18	81.17±8.21	72.27±7.42
6th month	111.60±15.91	88.40±20.31	103.37±13.76	84.23±11.52	84.60±9.11	73.93±6.93
9th month	113.47±16.09	90.00±14.59	109.10±14.58	87.83±11.21	90.60±10.63	78.07±8.11
12th month	116.43±19.27	93.00±15.28	118.13±20.91	91.43±11.42	97.43±12.92	84.50±10.42
F	0.560	1.981	6.112	5.021	15.441	9.585
P	0.692	0.101	0.000	0.001	0.000	0.000

APO A(mg/dl) concentration was 111.13±16.24, 111.03±15.96, 111.60±15.91, 113.47±16.09 and 116.43±19.27 while APO B(mg/dl) was 83.37±13.47, 84.07±14.34, 88.40±20.31, 90.00±14.59 and 93.00±15.28 at 0, 3rd, 6th , 9th and 12th month respectively in subjects taking depoprovera. APO A(mg/dl) concentration was 109.17±14.46, 99.47±13.21, 103.37±13.76, 109.10±14.58 and 118.13±20.91while APO B(mg/dl) was 81.10±9.56, 81.37±10.18, 84.23±11.52, 87.83±11.21 and 91.43±11.42 at 0, 3rd, 6th , 9th and 12th month respectively in subjects taking implanon. APO A (mg/dl) concentration was 100.60±15.10, 81.17±8.21, 84.60±9.11, 90.60±10.63 and 97.43±12.92 while APO B(mg/dl) was 82.10±12.09, 72.27±7.42, 73.93±6.93, 78.07±8.11 and 84.50±10.42 at 0, 3rd, 6th, 9th and 12th month respectively in subjects taking Jadelle.

Table 2: Comparative effect of Depoproveral, Implanon and Jadelle on APO A1 and APO B at different months

	Month	APO A (mg/dl)	APO B (mg/dl)
Depoprovera	Baseline	111.13±16.24	83.37±13.47
	3rd month	111.03±15.96	84.07±14.34
	6th month	111.60±15.91	88.40±20.31
	9th month	113.47±16.09	90.00±14.59
	12th month	116.43±19.27	93.00±15.28
Implanon	Baseline	109.17±14.46	81.10±9.56
	3rd month	99.47±13.21	81.37±10.18
	6th month	103.37±13.76	84.23±11.52
	9th month	109.10±14.58	87.83±11.21
	12th month	118.13±20.91	91.43±11.42
Jadelle	Baseline	100.60±15.10	82.10±12.09
	3rd month	81.17±8.21	72.27±7.42
	6th month	84.60±9.11	73.93±6.93
	9th month	90.60±10.63	78.07±8.11
	12th month	97.43±12.92	84.50±10.42
	F	17.683	7.114
	P	0.000	0.000

There was no significant difference in APO A (mg/dl) concentrations of 125.00±13.23, 109.22±15.40, 111.75±21.77, 124.33±13.86, 109.00±15.05, 112.75±21.50, 126.33±10.26, 109.91±15.59, 110.25±18.83, 127.33±13.20, 111.83±15.67, 112.50±19.26, 129.67±12.34, 115.57±19.58 and 111.50±21.56 of subjects taking Depoprovera at age groups (years) of 21-30 baseline, 31-40 baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively while there was significant in APO B(mg/dl) of 70.00±8.54, 85.52±13.64, 81.00±10.89, 69.67±13.42, 85.96±14.31, 84.00±11.43, 71.33±10.12, 90.70±21.75, 88.00±10.80, 73.67±9.87, 92.21±14.56, 89.50±11.56, 75.67±10.69, 95.35±15.24 and 92.50±11.67 at age groups(years) of 21-30 baseline, 31-40 baseline, 41-50 baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively.

There was significant difference in APO A (mg/dl) concentrations of 95.37±12.58, 103.37±15.46, 97.00±20.08, 80.25±7.69, 82.36±7.91, 76.00±12.28, 84.37±8.60, 85.32±9.57, 80.67±9.71, 90.37±7.85, 91.52±11.72, 85.33±11.71, 95.75±9.77, 98.68±13.95 and 94.00±17.05 in subjects taking implanon at age groups(years) of 21-30 baseline, 31-40 baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively while there was significant in APO B (mg/dl) of 79.50±19.55, 83.16±9.05, 82.33±2.51, 72.37±9.84, 71.94±6.89, 74.00±5.29, 73.62±9.97, 74.36±6.11, 72.00±2.00, 77.75±10.99, 78.79±7.36, 74.33±4.04, 84.37±14.73, 85.05±9.35 and 81.33±3.05 at age groups (years) of 21-30 baseline, 31-40baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively.

In subjects taking Jadelle, there was significant difference in APO A (mg/dl) concentrations of 125.67±8.14, 109.35±14.08, 95.75±3.30, 119.33±6.42, 97.47±12.60, 96.00±7.35, 127.00±1.73, 102.00±12.21, 93.50±5.75, 132.67±2.51, 107.96±13.14, 98.00±7.52, 137.00±6.24, 114.70±12.77 and 123.75±50.18 at age groups (years) of 21-30 baseline, 31-40 baseline, 41-50baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively while there was significant in APO B(mg/dl) of 73.67±4.72, 81.35±9.70, 85.25±10.04, 79.33±5.13, 80.47±10.32, 88.00±11.60, 80.33±6.65, 83.56±11.30, 91.00±15.38, 84.00±7.93, 86.70±10.98, 97.25±12.12, 82.67±6.42, 91.26±11.69 and 99.00±8.83 at age groups(years) of 21-30 baseline, 31-40baseline, 41-50 baseline, 21-30 3rd, 31-40 3rd, 41-50 3rd, 21-30 6th, 31-40 6th, 41-50 6th, 21-30 9th, 31-40 9th, 41-50 9th, 21-3012th, 31-40 12th and 41-50 12th respectively as shown in table 3 below.

Table 3: Effects of Depoproveral, Implanon and Jadelle on APO A1 and APO B in different age groups

	Depoprovera		Implanon		Jadelle	
Age Group	**APO A (mg/dl)**	**APO B (mg/dl)**	**APO A (mg/dl)**	**APO B (mg/dl)**	**APO A (mg/dl)**	**APO B (mg/dl)**
21-30 baseline	125.00±13.23	70.00±8.54	95.37±12.58	79.50±19.55	125.67±8.14	73.67±4.72
31-40baseline	109.22±15.40	85.52±13.64	103.37±15.46	83.16±9.05	109.35±14.08	81.35±9.70
41-50baseline	111.75±21.77	81.00±10.89	97.00±20.08	82.33±2.51	95.75±3.30	85.25±10.04
21-30 3rd	124.33±13.86	69.67±13.42	80.25±7.69	72.37±9.84	119.33±6.42	79.33±5.13
31-40 rd	109.00±15.05	85.96±14.31	82.36±7.91	71.94±6.89	97.47±12.60	80.47±10.32
41-503rd	112.75±21.50	84.00±11.43	76.00±12.28	74.00±5.29	96.00±7.35	88.00±11.60
21-306th	126.33±10.26	71.33±10.12	84.37±8.60	73.62±9.97	127.00±1.73	80.33±6.65
31-40 6th	109.91±15.59	90.70±21.75	85.32±9.57	74.36±6.11	102.00±12.21	83.56±11.30
41-50 6th	110.25±18.83	88.00±10.80	80.67±9.71	72.00±2.00	93.50±5.75	91.00±15.38
21-30 9th	127.33±13.20	73.67±9.87	90.37±7.85	77.75±10.99	132.67±2.51	84.00±7.93
31-40 9th	111.83±15.67	92.21±14.56	91.52±11.72	78.79±7.36	107.96±13.14	86.70±10.98
41-50 9th	112.50±19.26	89.50±11.56	85.33±11.71	74.33±4.04	98.00±7.52	97.25±12.12
21-3012th	129.67±12.34	75.67±10.69	95.75±9.77	84.37±14.73	137.00±6.24	82.67±6.42
31-40 12th	115.57±19.58	95.35±15.24	98.68±13.95	85.05±9.35	114.70±12.77	91.26±11.69
41-50 12th	111.50±21.56	92.50±11.67	94.00±17.05	81.33±3.05	123.75±50.18	99.00±8.83
F	1.016	1.903	4.678	2.742	5.244	2.457
P	0.442	0.031	0.000	0.001	0.000	0.004

Table 4: Comparative effect of Depoproveral, Implanon and Jadelle on APO A1 and APO B at different age groups

	Age Group (Years)	**APO A (mg/dl)**	**APO B (mg/dl)**
Depoprovera	21-30 baseline	125.00±13.23	70.00±8.54
	31-40baseline	109.22±15.40	85.52±13.64
	41-50baseline	111.75±21.77	81.00±10.89
	21-30 3rd	124.33±13.86	69.67±13.42
	31-40 rd	109.00±15.05	85.96±14.31
	41-503rd	112.75±21.50	84.00±11.43
	21-306th	126.33±10.26	71.33±10.12
	31-40 6th	109.91±15.59	90.70±21.75
	41-50 6th	110.25±18.83	88.00±10.80
	21-30 9th	127.33±13.20	73.67±9.87
	31-40 9th	111.83±15.67	92.21±14.56
	41-50 9th	112.50±19.26	89.50±11.56
	21-3012th	129.67±12.34	75.67±10.69
	31-40 12th	115.57±19.58	95.35±15.24
	41-50 12th	111.50±21.56	92.50±11.67
Implanon	21-30 baseline	95.37±12.58	79.50±19.55
	31-40baseline	103.37±15.46	83.16±9.05
	41-50baseline	97.00±20.08	82.33±2.51
	21-30 3rd	80.25±7.69	72.37±9.84
	31-40 rd	82.36±7.91	71.94±6.89
	41-503rd	76.00±12.28	74.00±5.29
	21-306th	84.37±8.60	73.62±9.97
	31-40 6th	85.32±9.57	74.36±6.11
	41-50 6th	80.67±9.71	72.00±2.00
	21-30 9th	90.37±7.85	77.75±10.99
	31-40 9th	91.52±11.72	78.79±7.36
	41-50 9th	85.33±11.71	74.33±4.04
	21-3012th	95.75±9.77	84.37±14.73
	31-40 12th	98.68±13.95	85.05±9.35
	41-50 12th	94.00±17.05	81.33±3.05
Jadelle	21-30 baseline	125.67±8.14	73.67±4.72
	31-40baseline	109.35±14.08	81.35±9.70
	41-50baseline	95.75±3.30	85.25±10.04
	21-30 3rd	119.33±6.42	79.33±5.13
	31-40 rd	97.47±12.60	80.47±10.32
	41-503rd	96.00±7.35	88.00±11.60
	21-306th	127.00±1.73	80.33±6.65
	31-40 6th	102.00±12.21	83.56±11.30
	41-50 6th	93.50±5.75	91.00±15.38
	21-30 9th	132.67±2.51	84.00±7.93
	31-40 9th	107.96±13.14	86.70±10.98
	41-50 9th	98.00±7.52	97.25±12.12
	21-3012th	137.00±6.24	82.67±6.42
	31-40 12th	114.70±12.77	91.26±11.69
	41-50 12th	123.75±50.18	99.00±8.83
	F	7.458	3.265
	P	0.000	0.000

4. Discussion

The result of the study showed significant difference in APO A1 and APO B in subjects taking implanon and Jadelle while the Depoprovera showed no significant increase in both APO A1 and APO B. This is suggestive that subjects taking implanon and Jadelle are prone to cardiovascular disorder**.** The result further showed significant difference in APO A1 and APO B among the three contraceptive users. The relationship between small, dense LDL and CHD risk has been described in a range of different situations [19-22] and HDL sub-fractions are among the new emerging CAD risk factors, and in particular HDL 2b has been shown to be linked to cardiovascular risk [23]. The measurement of apo B and total cholesterol or LDL-C does not discriminate between large LDL and small, dense LDL particle size and, in addition, the measurement of apo A-I also does not discriminate between larger cardio-protective HDL and smaller HDL particles.

There was significant difference in APO B of subjects using Depoprovera and APO A1 and APO B of subjects on implanon and jadelle at different age groups. This is suggestive that ages affect APO A1 and APO B in different contraceptive users. Apo B and apo A-I are important components of non-HDL and HDL lipoprotein particles, respectively, and there is a wealth of evidence supporting their measurements to improve the prediction of CVD risk [10-13].

ApoB and apoA-I are the two major apolipoproteins involved in lipid transport and in the processes causing atherosclerosis and its complications. ApoB is the major protein in Very Low Density (VLDL), Intermediate Density (IDL) and Low Density Lipoproteins (LDL), one protein per particle [24]. ApoA-I is the major protein in High Density Lipoprotein (HDL) particles. The apoB number indicates the total number of atherogenic particles, the higher the number the higher is the cardiovascular (CV) risk. ApoA-I reflects the anti-atherogenic potential in HDL particles, the higher the value the better protection of CV risk. The apoB/apoA-I ratio (apo-ratio) indicates the balance between atherogenic and anti-atherogenic particles, the higher the value, the higher is the CV risk.

5. Conclusion

The study has shown that Implanon and Jadelle caused changes in APO A1 and APO B concentrations.

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