JMBR: A Peer-review Journal of Biomedical Sciences
June 2012, Vol. 11 No.1 pp 62-70

GESTATIONAL AGE AND MANIFEST CARDIOVASCULAR RISK FACTORS IN NORMAL HUMAN PREGNANCY

OKWUSIDI JI[1]*, FOLAYAN A[1], OLATUNJI LA[1], FAWOLE AA[2] AND SOLADOYE AO[1].

ABSTRACT

Normal pregnancy (PREG) is a hyper-physiological state with unique challenges to maternal cardiovascular physiology including alterations in dynamics of lipid metabolism and potential disposition towards cardiovascular disease (CAD) risk with a gestational age (GA) bias. Thus manifestation of known CAD risk factors was evaluated in 75 human females, randomly assigned to 3 groups each of 25 subjects of different GA. Group1 served as Control (NO PREG), Groups 2 and 3 were PREG at GA of less than 20weeks (PREG<20wk) and of 20weeks or more (PREG ≥ 20wk) respectively. Following anthropometric and relevant obstetric history determination, anticoagulated blood samples were asceptically collected and clean plasma samples obtained for enzymatic determination of plasma concentrations of fasting blood sugar (FBS) and various lipid compartments: triglycerides (TRG), total cholesterol (CHOL) - (TPC), and high density lipoprotein CHOL (HDLc). Plasma low density lipoprotein CHOL (LDLc) concentration was estimated according to Okwusidi (1988)[1]. CHOL was compartmentalized in TPC and HDLc fractions. LDLc compartment was notably lesser. TRG/VLDLc fraction was the least of lipid compartments. TPC in PREG < 20wk was similar to Control value. All primary lipid variables in PREG ≥20wk were significantly ($p< 0.05$) elevated when compared to either the Control or PREG < 20wk. TPC and LDLc based AI were similar in both Control and PREG < 20wk. Only the TRG/VLDLc based AI of the group varied significantly in PREG < 20wk compared to Control ($p< 0.05$). All compartmental AI in PREG ≥20wk were significantly elevated compared to Control or PREG < 20wk. FBS concentration were similar in all study groups. The bulk of CAD risk potential was contributed by TPC/HDLc RAI and TRG/HDLc RAI respectively. The dreaded "bad" LDLc/HDLc RAI potential was essentially the same in Control and pregnant subjects (25%, 22%, 27%: Control, PREG < 20wk, PREG ≥20wk respectively). These results strongly suggest that normal pregnant state is not deleteriously or pro-pathologically disposed towards GA accentuated CAD risk.

INTRODUCTION

Normal pregnancy (PREG) is a hyper-physiologic state which presents a unique challenge to the maternal cardiovascular physiology. In addition to the changes brought about by mechanical factors such as the expanding size of the uterus, the development of uterine and placental circulations are themselves unique. Other cardiovascular and blood changes which take place are among the most extreme, occurring in normo-physiologic states. In addition, PREG associated endocrine changes are truly remarkable. In the first

KEY WORDS: *Gestational age, Cardiovascular risk factors, Normal human Pregnancy*

Departments of [1]Physiology and [2]Obstetrics and Gynaecology, College of Health Sciences, **University of Ilorin, Nigeria.**

***Corresponding Author Current Address:**
Department of Physiology, School of Basic Medical Sciences, Igbinedion University, **Okada, Edo State Nigeria**
E.mail: jiokwusidi@yahoo.co.uk
Tel: +234-803-567-2281

half of Pregnancy ($\leq$ 20 weeks) massive quantities of hormones such as human chorionic gonadotropin are secreted by the placenta. In the second half ($\geq$ 20 weeks) the pregnant female secretes daily milligram (and up to a gram) quantities of various other hormones. The coordinated adaptation by this human female body to these and other changes in PREG and again at parturition is a most impressive physiologic accomplishment. Due to the elevated functional state of virtually all the organ systems of the body, events of PREG allow for the study of several bodily processes and the dynamics of several other cellular interactions. It presents a good model to evaluate the parameters associated with these organ systems.

Cardiovascular disease (CAD) continues to be one of the leading global causes of death. The disease is insidious in nature and sometimes strike fatally without warning [1]. The etiological progression of CAD is not fully known, yet the burden of the disease in our society is enormous, and calls for serious contributions aimed at identifying the causes, ways and means of arresting the immense human death toll so involved. Current research efforts exploit natural incidences or lesions to investigate or re-evaluate known pathologic stances.

Because of the massive challenge, principally to the endocrine system during early PREG, there is an increase in body fat accumulation as a consequence of augumented maternal adipose. The breakdown of this accumulated fat is accelerated in the late PREG and plays a key role in fetal development. This hyperlipidemia on the other hand is supposedly a known CAD risk factor in both animal and human models [1-4] As a feature of PREG, hyperlipidemia has been well documented in women population of various races [5]. Although changing concentrations of hormones of PREG and alterations in lipoprotein concentration have been reported a while back now [6], the exact mechanism(s) of the hyperlipidemia of PREG remains to be fully delineated, and the associated morbidity or otherwise clearly defined.

This study was therefore construed to investigate the dynamics and lipid profile, a known cardiovascular risk factor in normal volunteers at different GA. Knowledge of such normo-lipidemic dynamics in normal PREG may yield insight into, as well as provide some index-of-profile assessment of ethiological progression of CAD morbidity and possibly contribute towards management of disease state and PREG related pathology.

SUBJECTS

Subjects and Selection criteria: Fifty healthy pregnant women of informed consent, attending the Ante-natal clinic at the maternity wing of the University of Ilorin Teaching Hospital of ages between 19-40 years participated in the study. Twenty-five (25) subjects each of the two study groups were of PREG at GA of less than 20 weeks (PREG < 20 wk) and GA of 20 weeks or more (PREG $\geq$20 wk) and were randomly selected. Twenty (25) apparently healthy non-pregnant (No PREG) subjects, matched for age, parity and body mass

index (BMI) mostly staff of the University of Ilorin served as the control.
None of the subjects from the Records and Assessment of the Ante-natal clinic had any cardiovascular, inflammatory or renal diseases. The subjects had neither an established cholestasis, diabetes mellitus or gestational diabetes nor any known obstetric complications such as pre-eclampsia, eclampsia or polyhydraminions.

They were not on (and were counseled against) any medications including oral and ingestible contraceptives in course of the study. Smokers and alcoholics or subjects who were not sure of their last menstrual period were also excluded from the study. All studies were conducted in the morning hours from 9:00AM after overnight fast. All the subjects gave their informed consent to participate in the study. The study was performed in accordance with international norms, declaration and conformed with the standard of the local University Research and Ethics Committee.

METHODS

Anthropometric measurement and Obstetric History: Anthropometric measurement as well as relevant obstetric history such as parity were determined. The subjects bodyweight (Bwt) and height were recorded without shoes. Bodymass index (BMI, kg/m^2) was calculated as summarized in Table 1.

Blood collection and preparation plasma samples: Following overnight fast, anticoagulated venous blood samples were aseptically collected through a clean venopuncture by clinically qualified staff of the Ante-natal clinic for the determination of lipids and glucose concentrations respectively. Collected blood samples were subsequently spun at 3000rpm for 10minutes to yield clean hemolysis free plasma. The plasma samples were separated and stored frozen in aliquots until analyzed usually in triplicates.

Determination of plasma lipids and glucose concentration: Concentrations of the various plasma lipids-triglyceride (TRG), total (TPC) – and high density lipoprotein (HDLc) – cholesterol and plasma glucose were all determined using Assay kits (Randox Laboratory Co, Antrim UK) following the instructions contained in the kits. The assays were monitored spectrophotometrically (Mann-heim Boehringer spectrophometer 4010) at appropriate wavelengths. The concentration of plasma low density lipoprotein cholesterol (LDLc) was estimated according to Okwusidi (1988)[1] as the difference between TPC and HDLc to obtain summed LDLc + VLDLc; with subsequent adjustment for determined TRG concentration assuming that TRG was carried mainly in the plasma VLDLc fraction [14]. These constituted atherogenic index (AI). Absolute AI (AAI) is the sum total of all AI irrespective of individual atherogenecity. Individual or specific

relative AI (RAI) is control corrected AAI. RAI was further expressed as percent of AAI to yield fractional atherogenecity (CAD risk potential) representing amount of CAD risk contributed by studied variable.

Data Handling and Statistical Analysis:
The study was a completely randomized experimental design to assess the manifest atherogenic indices (AI) and hence cardiovascular risk in control (No PREG), PREG < 20 wk and PREG ≥20 wk groups. AI are the various lipid compartments (including TRG) normalized for the HDLc compartment. Absolute AI (AAI) is the sum total of all AI irrespective of individual atherogenecity. Relative AI (RAI) is control corrected AAI (table 3). All values of variables are reported as mean± standard error of the mean (SEM). Data were analyzed by 2-way ANOVA with graphic post-hoc test of significance. A $p<0.05$ was considered significant [7,8,9]

RESULTS

The anthropometric and obstetric measurements of the study subjects are summarized in Table 1. The table validates the choice of the study subjects. There were no statistically significant differences in the study subjects as to linear age, height and parity. Similarly, no statistically significant differences were noted in the bodyweight and bodymass index (BMI) among the pregnant groups. However, obvious statistically significant differences were noticed in these parameters when compared with the control (No PREG) group ($p<0.05$).

Primary plasma lipids and fasting blood sugar (FBS) concentrations in the control and the pregnant groups are depicted in Table 2. As shown in the table, cholesterol was compartmentalized essentially in the total plasma cholesterol (TPC) and the HDLc fractions. The estimated LDLc concentration was notably lesser than these former compartments. The TRG/VLDLc was the least of the lipid compartments. The TPC in the PREG < 20wk was not statistically different in comparison to the control. In contrast, all the primary lipid variables in the PREG ≥ 20wk were significantly ($p<0.05$) elevated when compared to either the control or PREG < 20wk. No statistically significant variance was observed in the FBS concentrations of all the study groups (Table 2).

Table 3 summarizes the values of the atherogenic indices (AI) and the calculated absolute AI or the sum of all AI and specific relative effects of variables or CAD risk (RAI). In the control subjects, AI was highest for TPC compartment followed by the LDLc and TRG/VLDLc compartments respectively. Compared with the Control, early pregnacy (PREG < 20wk) did not significantly alter both the TPC and LDLc based AI. The only varied AI of the group was the significant ($p<0.05$) elevation of TRG/VLDLc based AI when compared to the control. All the compartmental AI (TPC, TRG/VLDLc and LDLc) in the PREG ≥ 20wk were significantly elevated in comparison to either the control or PREG < 20wk. PREG generally increased gross CAD

TABLE 1

Anthropometric Measurements and Obstetric History of the Study Subjects

Variable	Group[n]		
	No pregnancy (control)	**Pregnancy < 20wk**	**Pregnancy ≥ 20wk**
Age (yr)	26.7 ± 2.90[†]	29.1 ± 1.30[ns]	29.4 ± 1.20[ns]
Height (m)	1.60 ± 0.01	1.61 ± 0.02[ns]	1.63 ± 0.01[ns]
Weight (kg)	85.0 ± 3.30	66.2 ± 2.90*[#]	68.5 ± 3.60[*#]
BMI (kg/m^2)	22.7 ± 2.30	25.6 ± 1.60*	25.8 ± 1.60[*#]
Parity (n)	0-4	0-4[ns]	0-5[ns,#]

n, number of subjects per group = 25; [†] means ± SEM; ns, not significant versus No pregnancy (control); [#] not significant versus Pregnancy < 20wk; *p < 0.05 versus control. BMI, bodymass index.

TABLE 2

Plasma Lipids and Fasting Blood Sugar Concentrations (mMOL/L) in the Study Subjects

Variable	Group[n]		
	No pregnancy (control)	**Pregnancy < 20wk**	**Pregnancy ≥ 20wk**
TPC	3.80 ± 0.20[†]	3.90 ± 0.30[ns]	4.80 ± 0.20*,**
TRG	0.88 ± 0.03	1.60 ± 0.03[*]	2.20 ± 0.05*,**
LDLc	1.44 ± 0.05[e]	1.30 ± 0.04[ns]	1.90 ± 0.05*,**
HDLc	1.48 ± 0.05	1.00 ± 0.10*	0.68 ± 0.05*,**
FBS	4.40 ± 0.15	3.75 ± 0.04[ns]	4.31 ± 0.09[ns,#]

n, number of subjects per group = 25; [†] mean ± SEM; TPC, total plasma cholesterol; TRG, triglycerides carried mostly in very low density lipoprotein cholesterol (VLDLc) fraction; LDLc, low density lipoprotein cholesterol (estimated); HDLc, high density lipoprotein cholesterol; FBS, fasting blood sugar; ns, not significant versus No pregnancy (control); #, not significant versus Pregnancy < 20wk; *p< 0.05 versus control; **p< 0.05 versus Pregnancy < 20wk.

TABLE 3

Atherogenic Index of Cardiovascular Risk Factor in the Study Subjects

Variable	Group[n]		
	No pregnancy (control)	**Pregnancy < 20wk**	**Pregnancy ≥ 20wk**
TPC/HDLc	2.30 ± 0.20[†]	2.90 ± 0.30[ns]	4.50 ± 0.20*,**
TRG(VLDLc)/HDLc	0.60 ± 0.02	1.60 ± 0.01[*]	3.20 ± 0.04*,**
LDLc/HDLc	0.98 ± 0.04	1.30 ± 0.10[ns]	2.80 ± 0.20*,**
Absol AI (AAI)	3.88 ± 0.40	5.80 ± 0.60*	10.50 ± 1.00*,**
Rel AI (RAI)	0.0 ± 0.10	1.90 ± 0.20*	6.60 ± 0.70*,**

n, number of subjects per group = 25; [†]mean ± SEM; AI, atherogeic index: TPC/HDLc ratio; TRG(VLDLc)/HDLc ratio; LDLc/HDLc ratio; AAI, absolute AI is the sum of all AI; RAI, relative AI is control corrected AAI; ns, not significant versus control; *p< 0.05 versus No pregnancy (control); **p< 0.05 versus pregnancy<20wk.

TABLE 4

Atherogenic Index (RAI) As % of Absolute

Absolute Atherogenic Index AAI in the Study Subjects

Variable	Group[n]		
	No pregnancy (control)	**Pregnancy < 20wk**	**Pregnancy ≥ 20wk**
TPC/HDLc	59 ± 6[†]	50 ± 5*	43 ± 4*,**
TRG/HDLc	16 ± 2	28 ± 3[*]	31 ± 3*,[#]
LDLc/HDLc	25 ± 2	22 ± 2[ns]	27 ± 3[ns]
Absol AI (AAI)	0 ± 0.1	0 ± 0.1[ns]	0 ± 0.1[ns]
Rel AI (RAI)	0 ± 0.1	33 ± 3*	63 ± 6*,**

n, number of subjects per group = 25, [†]mean ± SEM, AI, atterogenic index: TPC/HDLc ratio; TRG(VLDLc)/HDLc ratio; LDLc/HDLc ratio; AAI, absolute AI is the sum of all AI; RAI, relative AI is control corrected AAI; AI are expressed as % of AAI for each variable in each group or regimen as depicted in table 3.*p< 0.05 versus No pregnancy (control) **p< 0.05 versus Pregnancy < 20wk; ns, not significant versus No pregnancy (control); [#] not significant versus Pregnancy < 20wk.

risk (AAI) of the study subjects. AAI in the PREG groups were significantly ($p<0.05$) elevated especially in late gestational age compared to control. The RAI in these study subjects were Control < PREG < 20wk < PREG ≥20wk (Table 3).

Finally, RAI was further expressed as percent of AAI for each individual variable in each group or regimen to yield the specific proportional or fractional relative atherogenecity (CAD risk potential). This resultant CAD risk potential represents the amount of CAD risk contributed by a particular studied variable towards the absolute or gross atherogenecity [2,3] and are displayed in table 4. As depicted in the table, the bulk CAD risk potential was contributed mostly by the TPC/HDLc RAI and TRG/HDLc RAI respectively. The potential contributed by the generally dreaded "bad" [10] LDLc/HDLc RAI was not significant in both the control and the pregnant subjects. Thus the CAD risk potential of 33% and 63% at < 20wk and ≥ 20wk gestation of the pregnant state appears less deleterious or pro-pathologic (Table 4).

DISCUSSION

The results of this study reveal a phasic manifestation of CAD risk factor in normal PREG: a less acute early pregnancy (< 20 wk) with TRG/VLDLc as the principal contributor to the overall atherogenecity; and a second LDLc – prone phase. CHOL mediates both phases. These results thus suggest that the state of PREG potentially predisposes towards atherogenic tendencies, increasing the blood level of culprit CAD risk factor especially in the later period of PREG (≥20wk).

These results are consistent with published data. Increases in total blood lipid have been described in pregnant human subjects. The increases were largely in neutral fat and cholesterol esters [5,11]. Blood cholesterol exhibited the most pronounced variance of any of the evaluated lipid especially in the later period (≥20wk). The plasma TRG/VLDLc concentration in this study was observed to increase with advancing PREG. This is consistent with the reported positive linear relationship between gestational age and serum TRG concentration in PREG [12]. On the other hand, significant decreases in the HDLc concentration were observed in the pregnant subjects in this study and apparently persisted throughout the PREG period. Reports on changes in HDLc concentration in PREG have been contradictory. The reports vary from decreases to increases or even non significant changes in serum HDLc level [5,6,13]. Most of the contrasting reports however originate principally from studies on pregnant subjects in Europe and America. Taken in concert, these evidences suggest that changes in blood HDLc level in pregnancy may be influenced by both socio-economic, genetic or ethnicity factors.

The overall CAD risk potency or atherogenecity of pregnant state was assessed in this study as AAI. The bulk of the atherogenic potential (or CAD risk) in this study was contributed essentially by TPC and TRG compartments. In conjunction with decreased HDLc concentration, increased levels of culprit plasma lipids as observed in this study are usually associated with increased cardiac

risk [14]. However these pregnancy elevated lipid compartments are themselves usually utilized for fetal development and as maternal metabolic substrate respectively [15]. Evidently,therefore in normal PREG as observed in this study the TPC and TRG based AI appear to be the factors with most significance, the latter especially from first trimester (< 20wk). The "bad" LDLc [10] based AI does not seem to exert a significant CAD potency in normal PREG. This is also evident in the non-significant CAD risk potential of 25%, 22% and 27% recorded in this study for Control, PREG < 20wk and PREG ≥20wk respectively (Table 4). Furthermore, even though the pregnant state manifested CAD risk potential of 33% in early pregnacy and 63% later (Table 4), these normal PREG did not progress onto frank CAD.

Mechanistically this may probably be due to various endogenous homeostatic controls. The hallmark of lipid (CHOL) homeostasis is receptor mediation [16] and the massive endocrine challenge of the pregnant state provides the impetus for its regulation. Hypercholesterolemia has been demonstrated to lower pituitary adrenocortocotropin output and hence exert a damping control of lipid metabolism by higher centers [1]. An uncoupling of such higher center control on hyperlipidemic metabolism [17] may find expression in atherocardiovascular disease associated with PREG for example. This may account for some of the unexplained PREG, related CAD morbidity.

In conclusion therefore, the results of this study indicate that normal PREG potentially predisposes towards the manifestation of GA accentuated CAD risk. At term, factors endogenous to a normal pregnant human female (perhaps higher center controls) preclude the progression of PREG induced CAD risks onto frank CAD.

REFERENCES

1. Okwusidi, JI. Effect of Psychosocial stress and Diazepam on aortic acylcoA: cholesterol acyltransferase and plasma adrenocorticotropin and corticosterone levels of cockerels fed an atherogenic diet. Howard university PhD-Dissertation Washington DC. 1987; pp 52-58.

2. Okwusidi, JI; Odewole, GA; Adebayo, JO; Akinyinka, AO; Oyesola, OA. Modulation of some coronary heart disease risk factors by caffeine and ambient (environmental) temperature in wister rats. J. Med. Biomed. Res. 2010; 9(1): 5-16

3. Okwusidi, JI; Osadare, GO; Olatunji, LA; Oyesola, TO; Oyesola, OA. Hypercalcemic diet impacts coronary heart disease susceptibility of oral contraceptive treated rat model. J. Med. Biomed. Res. 2010a; 9(2): 82-92.

4. Okwusidi, JI; Alabi, KI; Olatunji, LA; Oyesola, TO. Is hypercalcemic diet a possible antidote to oral contraceptive-induced hypertension? Nig. J. Physiol. Sci. 2010b; 25(2): 115-119.

5. Ajose OA, Fasuba OB, Thormas KD, Bolideoro JO. Serum lipids and lipoprotein cholesterol profiles in pregnant Nigerian Women J. Clin. Sci. 2003; 2:1-2.

6. Desoye G, Schweditssch MO, Peifter KP, Zechmer L, Kpstnar GM. Correlation of hormone with lipid and lipoprotein levels during normal pregnancy and post Partum. J. Clin. Endocrimol. Metabl. 1987; 64:704-12.

7. Finney, DJ. Experimental Design and its statistical Basis. The university of Chicago Press Chicago, 1955, 82-112.

8. Godfrey, K. Statistics in practice: Comparing the means of several groups. N. Eng. J. Med. 1985; 313: 1450-1456.

9. Daniel, WW. Biostatistics: A foundation for Analysis in the Health sciences. 3rd ed New York, John Willey and Son 1983; pp 319-320.

10. Lewis B. The LDL theory and the HDL hypothesis, in: G. Noseda, B. Lewis and R. Paolett (ed). Diet and Drugs in Atheroschlerosis. Reven Press, New York 1980; pp 1-8.

11. Brizzi P, Tonolo G, Esposito F, Puddu L. Lipoprotein metabolism during normal pregnancy and pueperium. Tropheo. Med. 2003; 33(3): 219-223.

12. Qureshi IA, Pasha N, Zheng HA, Huandy YB. Hyperlipideamia of normal pregnancy. Hsuch Tsa. Clin. 1998; 15(9): 529-35.

13. Taylor GO, Akinde EO. Serum lipid in pregnancy and socio-economic status. Brit J . Obstet. Gyne. 1975; 82:297-307.

14. Castelli WP, Doyle JT, Gordon T et al, HDL cholesterol and other lipids in coronany heart disease. Circulation. 1977; 55:767-772.

15. Herrera E. Lipid metabolism in pregnancy and its consequences in fetus and new born. Ann. Rev. Nutr. 2002; 19(1): 43-55

16. Brown MS and Goldstein, JL. A receptor-mediated pathway for cholesterol homeostasis, Science 1986, 232: 34-47.

17. Okwusidi J.I. Role of Neuroendocrine facilitators in lipid metabolism. Post Doctoral presentation (the Ganong Group). University of California San Francisco (Unpublished observation), 1988.