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Effects of Selected Antioxidant Vitamins on Lipid Profile in Early Pregnancy

[1]O.I. Iribhogbe, [1]A. Aigbiremolen, [2]U. Akpamu, [1]J.E. Emordi and [2]E.O. Nwoke
[1]Department of Pharmacology and Therapeutics,
[2]Department of Physiology, College of Medicine Ambrose Alli University, Ekpoma, Edo State Nigeria

Corresponding Author: Dr. O.I. Iribhogbe, Department of Pharmacology and Therapeutics, College of Medicine, Ambrose Alli University Ekpoma, P.M.B. 14, Edo State Nigeria Tel: +2348065794437, +2348065794437

ABSTRACT

The purpose of this study is to evaluate the effects of vitamin A, C and E supplementation on lipid profile in early pregnancy. A total of 85 adult female wistar albino rats weighting 225-300 g were used and randomly grouped into 5 groups (2 control groups of five rats each, 3 test groups with 5 sub- groups of 5 rats each). After, pregnancy was confirmed, the control groups were administered distilled water and tween 80 respectively, while test groups A, C and E received vitamin A, C and E supplements, respectively. At the end of the 11th day of the experiment, blood samples were collected and TC (total cholesterol), TG (Triglyceride), LDL-C (low density hpoprotein cholesterol and HDL-C (high density lipoprotein cholesterol) were assayed using standard procedure. The test group had significantly increased TC, LDL- C and HDL- C but decreased TG levels following vitamin A administration when compared with control ($p<0.05$). The group supplemented with vitamin C also had significantly higher TC, TG and LDL- C and unchanged HDL-C compared with control ($p<0.05$). The group supplemented with vitamin E had a non significant TC levels, a significantly increased TG and HDL- C and a significantly reduced LDL- C levels ($p<0.05$). Based on results observed, we suggest that, in pregnancy, dietary supplementation with Vitamin A and E may be cardioprotective. This is because vitamin A and E significantly increase HDL- C, although this is accompanied by increase of other serum lipid component. Vitamin C, however was not beneficial.

Key words: Pregnancy, hyperlipidemia, antioxidant vitamins, cardiovascular diseases, lipid peroxidation, pre-eclempsia

INTRODUCTION

Lipid peroxidation is a reaction whereby molecular oxygen is incorporated into Poly-Unsaturated Fatty Acids (PUFA) to yield lipid peroxides which generate free radicals; a mechanism of cell membrane destruction and damage and a key factor in the pathophysiology of pre-eclampsia (Ozan *et al*., 2002). The unsaturated lipid component and thiol containing proteins of the cell membranes are susceptible to free radical attack formed in both physiological and pathological conditions in mammalian tissues (Krishna Mohan and Venkataramana, 2007; Plaa and Witschi, 1976). But normally, defense mechanisms of the body play an important role in the form of antioxidants, making every effort to minimize the damage, as an adaptation to stressful situations. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals, or oppose their actions (Sies, 1991). Hyperlipidemia in pregnancy in humans was first

described over 150 years ago (Bequerel and Rodier, 1844) and has been studied extensively since then (Oliver and Boyd, 1955; Potter and Nestel, 1979; Knopp *et al.*, 1992). A positive association of this hyperlipidemia with later development of cardiovascular disease has been suggested (Van Stiphont *et al.*, 1987) and a supra-physiologic increase has been proposed as a marker for development of non-pregnancy-associated hyperlipidemia in later life (Montes *et al.*, 1984). Hyperlipidemia occurs virtually in all pregnant women and concentrations tend to normalize in the year after pregnancy (Erkkola *et al.*, 1986; Kallio *et al.*, 1992). Studies show association with higher than normal serum lipid levels during pregnancy (Johnson *et al.*, 1975; Reyes, 1992), however, epidemiological studies of the relationship between pregnancy and later risk of cardiovascular disease have shown mixed results (Ness *et al.*, 1993).

This study was carried out based on the fact that hyperlipidemia in pregnancy is associated with increased risk of cardiovascular disease. Rats were chosen for the study because like humans, they develop hyperlipidemia during pregnancy (Fillios *et al.*, 1958; Bosch and Camejo, 1967; Knopp *et al.*, 1975) and also cholestasis (Kern *et al.*, 1978). Thus, they serve as a suitable model for studying the outcome of antioxidant vitamins (A, C and E) supplement on lipid profile during early pregnancy, which is the major objective of this study.

MATERIALS AND METHODS

Animals: Eighty-five adult female Wister albino rats (225-300 g) used in this study were obtained in November, 2009 from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma and were housed in groups in a stainless steel cage with plastic bottom grid and a wire screen top in Physiology Lab 1 of the Department of Physiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. They were assigned into four groups; a control group made up of two sub- groups with 5 rats each (a control and a vehicle group; tween -80 was the vehicle used for the dispersal of lipid soluble vitamin A and E) and three test groups (A, C and E) made up of five sub- groups of 5 rats each (Table 1). They were fed *ad libitum* with tap water and pellated feeds purchased from

Table 1: Treatment administered to different rat groups (n = 5 rats per group)

Group	Treatment
Control	0. Normal feed +Distilled water 1 mL
	0^1. Normal feed + Tween 80 Vehicle 1 mL
Vitamin A	1 Normal feed + Vehicle + Dist H_2O + Vit A 0.6 mg kg^{-1}
	2 Normal feed + Vehicle + Dist H_2O+ Vit A 0.7 mg kg^{-1}
	3 Normal feed + Vehicle + Dist H_2O + Vit A 0.8 mg kg^{-1}
	4 Normal feed + Vehicle + Dist H_2O + Vit A 0.9 mg kg^{-1}
	5 Normal feed + Vehicle + Dist H_2O + Vit A 1.0 mg kg^{-1}
Vitamin C	1 Normal feed + distilled water + Vit C 200 mg kg^{-1}
	2 Normal feed + distilled water + Vit C 250 mg kg^{-1}
	3 Normal feed + distilled water + Vit C 300 mg kg^{-1}
	4 Normal feed + distilled water + Vit C 350 mg kg^{-1}
	5 Normal feed + distilled water + Vit C 400 mg kg^{-1}
Vitamin E	1 Normal feed + Vehicle + Dist H_2O + Vit E 16.4 mg
	2 Normal feed + Vehicle + Dist H_2O + Vit E 18.4 mg
	3 Normal feed + Vehicle +Dist H_2O + Vit E 19.4 mg kg^{-1}
	4 Normal feed + Vehicle + Dist H_2O + Vit E 20.4 mg kg^{-1}
	5 Normal feed + Vehicle + Dist H_2O + Vit E 22.4 mg kg^{-1}

Bendel feeds and flour meal Ewu, Nigeria Limited and allowed to acclimatize for 2 weeks. After which 2 male Wister albino rats were introduced into each group for mating. The animals were allowed to mate for 6 days after which the male animals were removed from the cage. Pregnancy was confirmed by palpation method as described by Agematsu *et al.* (1983) and vaginal smear microscopy method typified by the occurrence of scanty epithelial cells and leucocytes (Long and Evans, 1922; Daly and Kramer, 1998).

From the 7th day, administration of the different vitamins began (Table 1) using orogastric tubes and syringes to minimize occurrence of loss of the test substance (Ejebe *et al.*, 2009). This lasted for a period of 11 days. The administrations were conducted between the hours of 08:00 am and 10:00 am daily.

Antioxidant vitamins preparation: Vitamin A, C and E (commercial) were purchased from Clarion Medical Pharmaceuticals Nigeria Limited. Tween 80 vehicle (commercial) was purchased from Sigma Pharmaceuticals Limited. Two hundred microgram of the powdered form of vitamin C was dissolved in 10 mL of distilled water and the appropriate dose per kg were prepared for administration. Vitamin A (25,000 IU equivalent to 6 mg retinol and vitamin E, 100 mg) was dissolved in 0.2 mL of tween 80 and water in a ratio of 0.2:0.2:9.6. The test groups received vitamin A, C and E, respectively (Table l).

Sample collection and analysis of the lipid profile: Twenty-four hours after the last administration of the vitamins, the animals were sacrificed after inhalation of chloroform. Cardiac and jugular vein puncture were used to collect blood samples into sterilized test tubes containing EDTA as anticoagulant (Raederstorff *et al.*, 2002).

Serum from blood samples were obtained by centrifugation at 2500 rpm and serum Total Cholesterol (TC) and triglyceride (TG) concentrations were determined as described by Erickson *et al.* (1990), while low-density lipoprotein cholesterol (LDL- C) and high density lipoprotein cholesterol (HDL-C) were determined according to the method of Nichols *et al.* (1986).

Data analysis: The mean±standard deviation was determined and the one-way ANOVA statistical test was performed using SPSS version 17 soft ware. The significance level was set at $p<0.05$.

RESULTS

Vitamin A increased TC, LDL-C and HDL-C, it however decreased TG levels in a dose dependent fashion as shown in Fig. 1. The increase in LDL-C and HDL-C were gradual and steady as dose increased, while that of TC was interrupted in the 3rd treatment. As shown in Fig. 2, serum TC was more markedly elevated in the vitamin C treated group when compared to the vitamin A and E treated group (Fig. 1, 3). After the 5th treatment, peak TC was 5.71 mg dL^{-1} for vitamin A, 6.44 mg dL^{-1} for vitamin C and 4.91 mg dL^{-1} for vitamin E, respectively (Table 2-4). Statistical analyses revealed that vitamin A and E cause significant reduction in serum TG levels ($F = 6.542$, $p<0.001$; $F = 2.622$, $p<0.05$), respectively, while serum TC, HDL-C and LDL- C levels were significantly increased in the vitamin A and E treated groups ($p<0.05$) as shown in Table 2 and 4.

However, vitamin C administration did not alter serum HDL-C levels even with increasing dosage. This was justified by the level of significance [$F (5, 24) = 0.397$, $p>0.05$] as shown in Table 3. However, TC, TG and LDL-C levels were significantly increased (Table 3 and Fig. 2 for details). The effect of vitamin E on TC levels is however, not significant ($F (6, 28) = 1.503$, $p>0.05$).

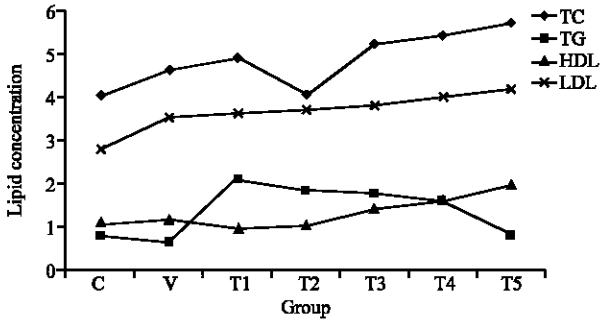

Fig. 1: Pattern of distribution of serum lipid profile after vitamin A supplementation in pregnancy; C: Control group; V: Vehicle/tween 80; T: Treatment; TC: Total cholesterol, TG: Triglyceride, HDL- C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol

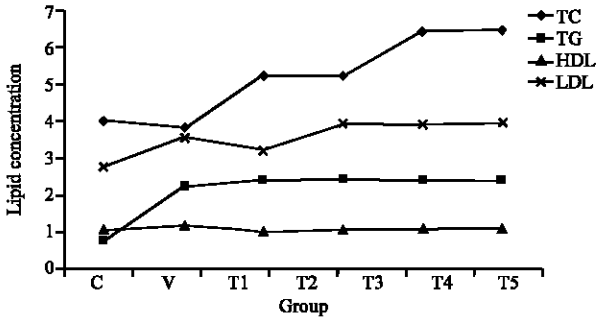

Fig. 2: Pattern of distribution of serum lipid profile after vitamin C supplementation in early pregnancy; C: Control group; V: Vehicle/tween 80; T: Treatment; TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol

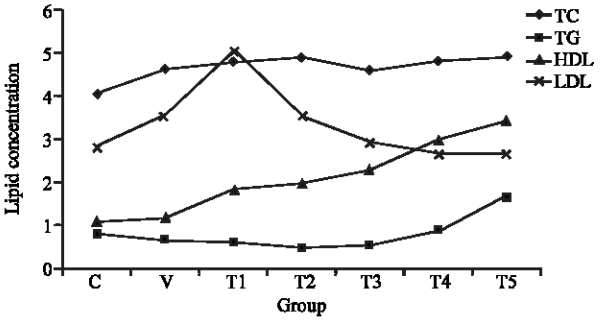

Fig. 3: Pattern of distribution of serum lipid profile after vitamin E supplementation in early pregnancy; C: Control group; V: Vehicle/tween 80; T: Treatment; TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol

DISCUSSION

Normal pregnancy is accompanied by a high metabolic demand and elevated requirements for tissue oxygen, which results in increased oxidative stress and antioxidant defenses (Knapena *et al.*, 1999). Arikan *et al.* (2001) reported significant increase in the level of thiobarbituric acid during

Table 2: Serum lipid profile in various treatment groups

	Serum lipids (mg dL^{-1})			
Group	TC	TG	HDL	LDL
C	61±0.43	0.80±0.31	1.08±0.32	2.78±0.49
V	4.61±0.98	0.66±0.20	1.18±0.21	3.53±0.48*
Vitamin A				
T1	4.87±1.42	2.10±0.73*	0.96±0.51	3.61±0.60*
T2	4.02±0.47	1.81±0.68*	1.04±0.33	3.71±0.54*
T3	5.21±0.57*	1.77±0.67*	1.42±0.44	3.19±0.68*
T4	5.40±0.77*	1.69±0.42*	1.61±0.56	3.99±0.50*
T5	5.71±0.56*	0.83±0.30	1.96±0.85*	4.18±0.40*

C: Control group; V: Vehicle/tween 80; T: Treatment; TC: Total cholesterol, TG: Triglyceride, HDL-C : High-density lipoprotein cholesterol; LDL- C: Low density lipoprotein cholesterol; *Significant difference with control C ($p<0.05$)

Table 3: Serum lipid profile in various treatment groups

	Serum lipids (mg dL^{-1})			
Group	TC	TG	HDL	LDL
C	4.02±0.43	0.80±0.31	1.08±0.32	2.78±0.49
Vitamin C				
T1	3.81±0.40	2.24±0.46*	1.20±0.22	3.57±0.38*
T2	5.22±0.80*	2.41±0.84*	1.02±0.08	3.19±0.45
T3	5.22±0.40*	2.44±0.31*	1.05±0.05	3.95±0.32*
T4	6.40±0.40*	2.39±0.24*	1.09±0.32	3.92±0.34*
T5	6.44±0.31*	2.36±0.77*	1.10±0.18	3.97±0.61*

C: Control group; T: Treatment; TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol; LDL- C: Low density lipoprotein cholesterol; *Significant difference with control C ($p<0.05$).

Table 4: Serum lipid profile in various treatment groups

	Serum lipids (mg dL^{-1})			
Group	TC	TG	HDL	LDL
C	4.61±0.43	0.80±0.31	1.08±0.32	2.78±0.49
V	4.61±0.98	0.66±0.20	1.18±0.21	3.53±0.48
Vitamin E				
T1	4.79±0.50*	0.58±0.17	1.82±0.30*	5.04*±0.27
T2	4.88±0.3*	0.47±0.20	1.98±0.75*	3.53±0.65
T3	4.59±0.33	0.52±0.17	2.26±0.74*	2.92±0.65
T4	4.80±0.5*	0.89±0.22	2.96±0.32*	2.65±0.55
T5	4.91±0.4*	1.66±0.60*	3.41±0.57*	2.66±1.11

C: Control group; V: Vehicle/tween 80; T: Treatment; TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol; LDL- C: Low density lipoprotein cholesterol; *Significant difference with control C ($p<0.05$)

normal pregnancy. The human placenta produces lipid peroxides that are secreted mainly to the maternal side of the placenta (Walsh and Wang, 1993a) and makers of increased lipid peroxidation are observed during normal pregnancy (Morri *et al*., 1998).

Blood vitamin A concentration decline gradually in pregnancy because of hemo-dilution and evidence exists that inadequate dietary vitamin A intake can also lower blood concentrations (Wallingford and Underwood, 1986). From the result of this study, vitamin A supplementation in

early pregnancy increased HDL- C levels in a dose dependent fashion. Furthermore, TC and LDL-C levels were also increased while TG levels were decreased.

From this study, vitamin C supplementation appears not to have any beneficial effect in early pregnancy. HDL-C levels were not affected by vitamin C supplementation, while TC, TG and LDL-C levels were increased. This finding is not supported by the study of Olayaki *et al.* (2008) who reported that vitamin C intake during pregnancy reduce lipid peroxidation and Eteng *et al.* (2006) who revealed that vitamin C produced a hypocholesterolaemic effect in albino non pregnant rats. The effect observed in this study may be due to reduced activity of some antioxidant enzymes in pregnancy. Vitamin C supplementation may be important in pregnancy, in that its deficiency has been associated with facilitated placental infection (Casanueva and Viteri, 2003), complications of pregnancy like gestational hypertension, intrauterine growth retardation and gestational diabetes (Rumbold and Crowther, 2005).

Vitamin E concentration is known to increase during gestation, probably because of the hyperlipidemic state associated with pregnancy (Wickens *et al.*, 1981). Results from our study suggest that, vitamin E supplementation in early pregnancy reduce LDL- C and increase HDL- C profile in a dose dependent fashion. Furthermore, while TC levels remained unchanged, TG increase was not favoured as dose increases. Thus, vitamin E at moderate doses improves lipid profile and may be beneficial in hyperlipdemic states in early pregnancy. Although, this study suggest a promising effect of vitamin E supplementation which is corroborated with studies performed in cell culture and animal models, the results of its supplementation in humans in randomized prospective clinical trials were disappointing (Miller *et al.*, 2005; Bjelakovic *et al.*, 2007).

Epidemiologic studies suggest an association between antioxidant intake, especially vitamin E and reduced rates of morbidity and mortality from coronary artery disease (CAD) (Jialal and Devaraj, 2005; Meydani, 2004; Traber, 2007). Several studies have indicated that the antioxidative defense system is modified during pregnancy. Wisdom *et al.* (1991) showed that the activity of an important family of antioxidative enzymes, the superoxide dismutase (SOD), is reduced in the blood of pregnant women. In addition, Walsh and Wang (1993b) reported a deficiency in another antioxidative enzyme glutathione peroxidase (GPx) during pregnancy. Glutathione peroxidase and superoxide dismutase activities have been found to be reduced during the second trimester of pregnancy in humans (Zachara *et al.*, 1993; Qanungo and Mukherjea, 2000). Most other studies report low levels of specific antioxidants in pregnancy (Morri *et al.*, 1998; Uchenna and Fidelis, 2005). Multivitamins and mineral supplementations have been shown to have beneficial effect on lipid profile (Li *et al.*, 2010). However, evidence from our study reveals that vitamin E supplementation in pregnancy may be beneficial as a cardioprotective agent due to its ability to elevate serum HDL-C and reduce serum LDL-C levels.

CONCLUSION

Vitamin E supplementation apart from having cardioprotective and hypolipidemic potential may help reduce the risk of pregnancy complications involving oxidative stress, such as pre-eclampsia. The lipo-protective benefit of vitamin A and C when used as monosupplements in pregnancy is minimal. The disappointing result of vitamin C may be as a result of modification of antioxidant defense system and enzymes during pregnancy.

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