Original Article

Effect of high salt intake on plasma lipid profile in pregnant wistar rats

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Abstract: Many physiological and behavioral changes take place during pregnancy to ensure the growth and development of a healthy fetus. This study investigates the effects of high maternal salt intake during pregnancy on lipid parameters in Wistar rats. Twenty female Wistar albino rats (200-250 g) were used for the study. The rats were time-mated and day 1 of pregnancy was determined by the presence of spermatozoa after a vaginal lavage. Animals were then randomly divided into two groups: a standard control diet and high-salt diet (8% NaCl) of 10 rats each. On the 19th day, the animals were fasted overnight and sacrificed under anaesthesia. Blood samples were collected via cardiac puncture for determination of lipid parameters triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), and very low density lipoprotein-cholesterol (VLDL-c) using enzymatic colorimetric method. Atherogenic indices, triglyceride/HDL-C (TG/HDL-C) and total cholesterol/HDL-C (TC/HDL-C) ratios were calculated. SPSS 21.0 package was used for data analysis and level of significance was analyzed using student t-test. Significance was set at P<0.05. Result showed significant (P<0.05) increases in plasma level of TG, TC, LDL-C, VLDL-C, TG/HDL-C and TC/HDL-C ratios in high salt fed pregnant rats compared to control. No significant (P>0.05) change was observed in HDL-C level in high salt fed pregnant rats when compared with control. High salt intake during pregnancy has detrimental effect on maternal lipid profile which can threaten both maternal and the fetal life.

Keywords: Pregnancy, lipid profile, atherogenic indices, salt (NaCl)

Introduction

Pregnancy is a period from fertilization to development of one or more offspring, known as a fetus or embryo, in a woman’s uterus [1]. It is a state of multiple and critical changes in the morphology and physiology of women, which play a fundamental role in meeting the mother’s basal needs and the requirements of the developing fetus [2]. The body undergoes physiological changes in the cardiovascular, metabolic, renal, respiratory and gastrointestinal systems [3]. Additionally, there is an increase in triglyceride (TG) levels mediated by inactivation of hepatic lipase secondary to the action of high-density lipoprotein (HDL) and to the elevation of very low-density lipoprotein (VLDL). Together, these changes are due to the increase in estrogen levels during pregnancy [4, 5]. The energy provided by lipid is used in cellular proliferation of uterus, blood volume expansion of mother, implantation of fetus in uterus, uteroplacental, and fetal development [6].

During pregnancy, many conditions such as overheating hemorrhage, diarrhea, and hyperemesis may result in sodium deficiency and a change in salt appetite, so pregnant women experience sodium deficiency and tend to prefer salty food [7, 8]. Salt is sodium chloride with 39.34% sodium and 60.66% chloride on a molecular-weight basis. Sodium is found naturally in many foods especially processed foods where large amounts are used mainly for the purposes of conserving and accentuating food taste [9, 10]. Sodium is the most abundant electrolyte found in extracellular fluid, where it determines the osmolality and volume of this compartment, and directly contributes to arterial pressure maintenance [11]. It also trans-
mits nerve impulses, assists muscle relaxation and contraction, and transports nutrients to cells [12, 13]. There has been a fairly large body of research on the impacts of salt exposure in pregnancy [14, 15]. A comment about the salt content of the high-salt diet is worthwhile. Eight percent (8%) NaCl is a very high salt content in human parameters, and this level may influence rat appetite [14]. It is reported that high dietary salt intake increased risk of gestational hypertension and preeclampsia [16, 17]. So far, no work has been done on health impacts of high dietary salt intake on lipid profile during pregnancy. Therefore, this study aimed at investigating the effects of maternal high salt intake (8% NaCl) on plasma lipid parameters in pregnant Wistar rats.

Materials and methods

Experimental animals

Twenty (20) female Wistar albino rats weighing 200-250 g were received from the Animal House of Physiology Department, Ladoke Akin tola University of Technology, Ogbomoso, Oyo State, Nigeria. They were housed in well ventilated plastic cages and acclimatized for a week under controlled conditions of temperature (23±5°C), humidity (30-55%) and light (12 h light/dark cycles). They were fed with standard laboratory animal feed and water ad libitum prior to the experiments. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH). The experimental protocol was approved by the Ethical committee of the Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Formulation of high salt feed

Eight percent of common salt (8% NaCl) was added to grower’s mash feed (i.e. 8 g of salt was thoroughly mixed with 92 g of feed) which were then formed to pellets.

Experimental treatments

The phases of the estrous cycle of the rats were determined by vaginal smear cytology [18]. At proestrus, the rats were mated (one couple per cage) and mating was verified by the presence of sperm in the vaginal smear, this being considered the first day of pregnancy. The confirmed pregnant animals were then divided into two groups of 10 rats each (n=10) as follows: Group I: Control (Pregnant rats fed with standard diet and water); Group II: High salt (Pregnant rats fed with 8% NaCl diet and water). Treatments lasted for 19 days in accordance with gestation period of Wistar rats.

Blood sampling and biochemical analysis

At the end of the 19th day, the rats were fasted overnight for 12 hours and sacrificed under anaesthesia using sodium pentobarbitone. Blood samples was collected from each rats via cardiac puncture into heparin tubes centrifuged at 3500 rpm for 15 min, and the clear supernatant plasma were separated and stored at -20°C for biochemical analyses.

The levels of plasma triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were evaluated according to the methods [19-22] respectively, using enzymatic colorimetric determination. Very low density lipoprotein-cholesterol (VLDL-C) was estimated according to the formula [23]:

$$\text{VLDL-c (mg/dL)} = \frac{\text{Triglycerides concentration}}{5}$$

Atherogenic indices were calculated as the ratio of triglycerides to HDL-cholesterol (TG/HDL-C), and ratio of total cholesterol to HDL-cholesterol (TC/HDL-c).

Statistical analysis

Statistical analysis was performed with SPSS 21.0. Data were expressed as means ± standard error of the mean (Mean ± SEM). Student t-test was used for significance differences between mean values. The results of the mean differences were considered statistically significant at P-values were less than 0.05 (P<0.05).

Results

Effect of high salt intake on Triglyceride (TG), total cholesterol (TC), High density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C) and Very low density lipoprotein-cholesterol (VLDL-C) (Figure 1)

There were significant increases in TG, TC, LDL-C and VLDL-C levels of high salt fed preg-
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nant rats compared with control. However, no significant difference in HDL-C level.

Effect of high salt on the ratio of Triglycerides to HDL-cholesterol (TG/HDL-C), and ratio of Total cholesterol to HDL-cholesterol (TC/HDL-C) (Figure 2)

There were significant increases in TG/HDL-C and TC/HDL-C in high salt fed pregnant rats compared with control.

Discussion

The current study provided information on the influence of high salt intake during pregnancy on lipid parameters in Wistar rats. Pregnancy is one of the most important periods in human life with hormonal, immunologic, vascular, metabolic and psychological changes in order to nurture and accommodate the developing fetus [24, 25]. According to Wollett, as gestational age progresses, women show an increase in lipid levels [26]. Therefore, excessive dietary salt ingestion may significantly alter the normal biochemical values of lipid parameters including levels of triglycerides (TG) and total cholesterol.

In the present study, there was significant (P<0.05) increase in triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) in high salt fed pregnant rats compared with control which similar with other pre-

vious findings. The increased levels of TG, TC and LDL-c and VLDL-c may be likely due to the fact that lipid metabolism changes during pregnancy due to an increase in hepatic lipase (HL) activity, a decrease in lipoprotein lipase (LPL) activity, delayed uptake of the remnant chylomicrons and hormonal changes as they are modulators of lipids metabolism [27-29] which increases during pregnancy. High levels of maternal TC and TG are associated with cardiovascular risks [30, 31], preeclampsia [32], and pregnancy-induced hypertension (PIH) [33].

On the other hand, supraphysiological changes in blood cholesterol during pregnancy also seem to impact significantly on maternal, fetal, and offspring outcomes. For instance, high LDL-cholesterol during pregnancy has been associated with preterm delivery [34, 35] as well as retarded intrauterine growth [36]. Finally, severe hypertriglyceridemia in pregnancy is a very high risk condition for acute pancreatitis [37].

A controversial pattern of high density lipoprotein cholesterol (HDL-c) changes has been reported by others previous studies during pregnancy. The present study observed a non significant change (P>0.05) in HDL-c level when control and high salt fed pregnant rats were compared. In consonance with the present study, Kar & Sinha did not find any significant alteration of HDL-c in normotensive pregnant mothers compared with non pregnant females [38]. Loke et al. showed increases in HDL-c level during the second trimester but a decrease during the third trimester [39]. Pusukuru et
al. observed a decrease in HDL-c as pregnancy progressed from second trimester to third trimester [40]. The varying pattern of HDL-c changes reported by several studies could be due to variation in the nature (normal, complicated, multiparous and primiparous pregnant women). Multiparous women tend to have a relative decrease in HDL-c levels in comparison to their primiparous counterparts [41]. Studies have linked the progressive raise in HDL-c in the pregnant mother as a protective measure to offset elevations in atherogenic LDL-c and TG levels in normal pregnancies [42].

In a recent study, Khosrowbeygi et al. found the atherogenic indices including the LDL-C/ HDL-c, TG/HDL-c, and TC/HDL-c ratios were higher in gestational diabetes mellitus compared with normal pregnancy and especially, the TG/HDL-c ratio showed a significantly positive correlation with insulin resistance, which might be used as a simple surrogate marker for assessing insulin resistance in pregnancy [43]. In this study, the TG/HDL-c and TC/HDL-c ratios increased significantly (P<0.05) in the high salt fed group when compared with control, which indicated progressive insulin resistance during normal pregnancy. Maternal insulin resistance is reported to be mediated by increase in the levels of estrogen, progesterone, human placental lactogen, human placental growth hormone, cortisol and inflammatory cytokines [44, 45]. Elevated level of insulin resistance has been shown to be associated with development of dyslipidemia [46].

Conclusion

The present study showed that high salt (8% NaCl) intake during pregnancy caused significant increase in TG, TC, LDL-c, VLDL-c TG/HDL-c, TC/HDL-c levels and non-significant difference in HDL-c level which indicate dyslipidemia, a risk factor for cardiovascular disease and can threaten the life of both maternal and the fetus. Therefore, high salt intake during gestation should be restricted to overcome critical problem of heart diseases, pre-eclampsia, preterm delivery with all the adverse pregnancy outcomes for mother and fetus.

Disclosure of conflict of interest

None.

References

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