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Dietary omega-3 but not omega-6 fatty acids down-regulate maternal dyslipidemia induced oxidative stress: A three generation study in rats

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ABSTRACT

Background: Maternal nutrition modulates fetal metabolic programming and development later. Maternal dyslipidemia effects on oxidative stress (OS) in offsprings and its modulation by dietary fatty acids over generations remains to be elucidated. The objective of present study was to assess the long-term (three generations) effect of omega-3 fatty acids on OS under dyslipidemia.

Methods: Weanling female Wistar rats were fed with control diet (7% lard), high fat diet (35% lard, HFL), high fat with fish oil (21% fish oil + 14% lard, HFF), high fat with canola oil (21% canola oil + 14% lard, HFC) and high fat with sunflower oil (21% sunflower oil + 14% lard, HFS). Following 60 days feeding, the female rats were mated with sexually matured males (fed normal chow diet) and continued with the above diet regimen during pregnancy and lactation. The pups after lactation were continued with their maternal diet for 60 days and subjected to mating and feeding trial as above for two generations. Serum lipid profiles, OS markers (lipid peroxidation, nitric oxide release and protein carbonyl) and antioxidant defence enzymes (catalase, SOD, glutathione peroxidase and glutathione transferase) were assessed in serum, liver and uterus of rats fed on experimental and control diets for three generations.

Results: Feeding HFL diet increased blood lipids, OS and lowered the antioxidant enzymes activity in serum, liver and uterus ($p < 0.05$). The reduction in the antioxidant enzymes in HFL group were higher in third followed by second generation compared to first generation ($p < 0.05$). Omega-3 fatty acids prevented the dyslipidemia induced loss of antioxidant enzyme activities in serum, liver and uterus.

Conclusions: Our data show for the first time that offsprings born to dyslipidemic mothers' exhibit diminished enzymatic antioxidant defence and its progressive reduction in future generation, and dietary omega-3 fatty acids restore the enzymatic antioxidant defence in offsprings and suppress the markers of OS.

1. Introduction

Increase in the intake of total fat and saturated fatty acids followed by decreased consumption of omega-3 fatty acids are reported across the population [1]. This coupled with low physical activity are linked to the onset of diabetes, hypertension and dyslipidemia [2]. Though dyslipidemia affect both men and women equally, the complications in women are significant during pregnancy and child birth. Metabolic adaptations during pregnancy, may lead to changes in the maternal lipid parameters thus affecting the lipid levels essential for fetal growth and development [3]. These changes in the lipids are reverted to pre-pregnancy levels after delivery under normal conditions. However, dyslipidemia during pregnancy may have long-lasting effects on mother as well as fetus. Offspring of such dyslipidemic mother may exhibit higher tendency to accumulate lipids later. Fetal exposure to consequences of maternal high fat intake and alterations in the intrauterine metabolic milieu may contribute to enhanced cardio metabolic risk later [4]. Management of metabolic complications during pregnancy through dietary interventions has gained importance [5]. This is particularly important to safely benefit and promote the fetal growth in the womb and later. Omega-3 fatty acids [alpha linolenic acid (LNA, 18:3 n−3), eicosapentaenoic acid (EPA, 20:5 n−3) and docosahexaenoic acid (DHA, 22:6 n−3)] are essential fatty acids with several health benefits [6,7]. They form structural component of cell membranes thus affecting its fluidity, development,

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid; HFL, high fat lard; HFF, high fat fish oil; HFC, high fat canola oil; HFS, high fat sunflower oil; OS, oxidative stress; ND, not detected.

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cognitive and visual functions [8]. Their dietary requirements during pregnancy exceed that of a nonpregnant state. However, its intake has decreased due to excessive use of oils rich in omega-6 fatty acids.

Pregnancy is coupled with elevated OS and may lead to pre-eclampsia [9,10]. Under normal condition, a delicate balance exists between the production of reactive oxygen species and the anti-oxidant defence; however, dietary modifications can significantly alter the balance. In animal models, it has been clearly established that OS during pregnancy is connected with the early development of endothelial dysfunction in offspring and may initiate in the uterus that could lead to increased risk of vascular disease later [10]. Increased free radicals and diminished antioxidant defence can lead to the modifications of structural components like lipids and proteins and eventually results in tissue dysfunction [11]. In animal models, many researchers have shown that high fat diet cause maternal obesity and increased metabolic perturbations in offsprings [12,13]. However, no studies have been reported on the high fat induced dyslipidemic effects on the antioxidant defence enzymes in offsprings over generations. Such studies are relevant as parental dietary practices influence the overall dietary pattern of children and carried to next generations. Hence, in the present study, the effect of omega-3 fatty acids in comparison with extensively consumed omega-6 fatty acids was undertaken in high fat fed rats over three generations to understand the degree of changes in the antioxidant defence enzymes over three generations.

2. Materials and methods

2.1. Materials

Ascorbic acid, adenosine diphosphate, (1-chloro-2,4, dinitrobenzene), cytochrome C, thiobarbituric acid, xanthine, xanthine-oxidase were obtained from Sigma Chemicals, St. Louis, MO, USA. Dinitrophenylhydrazine, EDTA, glutathione oxidized, glutathione reduced, malondehyde, NADPH, phosphoric acid, sulphanilamide, sulphosalicylic acid, sodium nitrate, t-butyl hydroperoxide, analytical grade solvents were obtained from SRL Chemicals, Mumbai, India. Fatty acid reference standards were purchased from Nu Chek Prep, Elysian, MN, USA. Refined sunflower oil, canola oil and lard were purchased from local market. Refined fish oil was procured from Janatha Fish Meal & Products, Udipi, India.

2.2. Diet and animal feeding

Animal protocols were approved by the Institutional Animals Care and Use Committee. Virgin female Wistar rats [OUTB-Wistar, IND-cft (2c)] weighing 50 ± 5 g were individually housed under 12 h light and dark cycle with water and standard chow diet available ad libitum. After acclimatization, the rats were randomly assigned to the respective diets with n = 4/group. Control diet had 7% of lard, HFL had 35% of lard, HFS (source of linoleic acid (LA) 18:2, n−6) had 2 parts of lard and 3 parts of sunflower oil, HFF (source of EPA + DHA) had 2 parts of lard and 3 parts of fish oil and HFC (source of LNA) had 2 parts of lard and 3 parts of canola oil [14]. After 60 days of feeding, the female rats were mated with sexually matured, normal chow fed males. Pregnancy was ensured and were continued on respective diets throughout gestation and lactation. After delivery, pups were counted; gender separated and assigned eight pups per mother (four males and four females). The feed intake and gain in the body weight were monitored and on completion of the lactation, the young females were kept on diets similar to their maternal group. Mothers were kept for overnight fasting and sacrificed for blood and tissue collection. The feeding trial, mating, pregnancy and lactation for second and third generation were similar to first generation as described above.

2.3. Measurement of serum lipids

Blood was allowed to clot, and spun at 4000 rpm to separate serum. Liver and uterus were harvested and washed with ice-cold saline and stored at −80 °C. The total lipid was extracted by Folch method [15]. Serum total cholesterol, LDL + VLDL cholesterol and triglycerides were measured using commercially available kit (Agappe diagnostics, Kerala, India) as per the kit instructions. The fatty acids methyl esters were prepared and estimated by GC [16]. Fatty acids in samples were identified by comparing the retention times of standards (Nu-Check prep., Elysian, MN). Free fatty acid (FFA) in the serum was measured by the method described by Brunk and Swanson [17].

2.4. Measurement of oxidative stress markers and antioxidant enzyme activity

Tissues were homogenized in KCl buffer and aliquots of supernatants were taken for the measurement of lipid peroxides level [18]. Serum lipid peroxides level was determined by measuring thiobarbituric acid (TBA) reactivity [19]. Nitric oxide level was measured using Griess reagent [20]. Protein carbonyls were measured by method as described by Mesquita et al. [21]. Tissues (100 mg) were homogenized in 1.0 mL phosphate buffer pH-7.0 and centrifuged at 600 g for 15 min. The supernatants and serum was used for the analysis of activity of antioxidant defence enzymes including catalase [22], SOD [23], glutathione peroxidase [24] and glutathione transferase [25] were measured spectrophometrically. The protein concentration was determined by method as described by Lowry et al. [26].

2.5. Comparisons of dietary groups and statistical analysis

Results are expressed as Mean ± SD. Using ANOVA; values in each dietary group between generations were compared. Data were analyzed using Graph Pad Prism and a p value <0.05 was considered statistically significant.

Table 1
Fatty acid composition of dietary fat.

Fatty acids (mg %)	Control	HFL	HFF	HFC	HFS
14:0	3.9	3.6	4.1	1.8	4.0
16:0	23.6	23.8	3.5	10.0	11.0
16:1	ND	ND	2.2	ND	ND
18:0	9.9	10.0	5.2	4.0	4.0
18:1 (n−9)	45.3	45.6	37.0	39.0	17.0
18:2 (n−6)	17.2	16.9	8.3	12.0	64.0
18:3 (n−3)	ND	ND	2.0	32.0	ND
20:4 (n−6)	ND	ND	17.9	ND	ND
20:5 (n−3)	ND	ND	10.5	ND	ND
22:6 (n−3)	ND	ND	9.2	ND	ND
S	37.4	37.4	12.8	15.8	19.0
M	45.3	45.6	37.0	39.0	17
P	17.2	16.9	47.9	44.0	64.0
S:M:P	1:1.2:0.45	1:1.2:0.37	1:2.9:3.7	1:2.5:2.8	1:0.9:3.4
N−6	17.2	16.9	26.2	12.0	64.0
n−3	0	0	21.7	32.0	0
n−6/n−3	NA	NA	1.2	0.4	NA

Values are Mean of triplicate samples. S: saturated fatty acids, M: monounsaturated fatty acids, P: polyunsaturated fatty acid, NA: not applicable and ND: not detected.

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