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Effects of Maternal High-Fat Diets during Pregnancy and Gestation with Phytosterols Supplementation on Oxidative Status in Neonate, Sucking and Adult Offspring

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Abstract

To investigate the effects of maternal high fat and energy diets with or without phytosterols supplementation during pregnancy and gestation on the oxidative status in offspring, pregnant C57BL/6j mice were randomly assigned to three groups which are chow diet control group (NC), high fat and energy diet group (HFED) and the high fat and energy diet supplemented with phytosterols group (HFEP). Once weaned, the surviving offspring mice were given chow diet until 10 weeks old. Serum lipids were measured in 10 weeks offspring. Liver homogenates oxidative biomarkers analyzed according to the manufacture's protocol. The results indicated that the body weight, serum lipids were significantly increased in the HFED group compared with that in chow diet group. The oxidative stress in liver homogenate was much higher in maternal and neonatal mice liver in HFED group. The anti-oxidative abilities in neonatal and adult mice liver were damaged. The phytosterols supplementation during early life ameliorated the abnormal serum lipid metabolism but not the oxidative damage in offspring.

Keywords: Phytosterols; Maternal high fat and energy diet; Fetal development; Oxidant; Antioxidation; Lipid metabolism

Introduction

Fetal development *in utero* may be affected by the maternal diet, which was now considered as an origin of chronic non-communicable disease in adult life [1-3]. Bayol and his colleagues reported that rats exposed to maternal junk food diet at the fetal and sucking stage developed overweight at 10 weeks after birth [4]. Many other studies also revealed that rats from mothers fed with a high-fat diet during pregnancy and lactation showed features of metabolic syndrome such as obesity, sedentary behavior and vascular dysfunction in the offspring [1,5,6].

Consumption of high fat diets will disturb the balance among energy intake, expenditure and storage. It results in the increased adiposity and impaired regulation of multiple metabolic processes, including obesity and insulin resistance, and lipid oxidation [7,8]. It was reported that the initial events triggering the development of insulin resistance was the production of reactive oxygen species (ROS) in mice fed with a high fat diet [9], indicating that the production of ROS might be the initial key event triggering high fat diet-induced metabolic syndrome. In constant, maternal high fat feeding has been shown to associate with increased hepatic oxidative stress, reduced anti-oxidative capacity, and resulted in higher brain oxidative stress and reduced morris water maze performance in mice [10]. Whether a same trend of oxidative stress, changing affected by maternal high fat and energy diet HFED) in neonate, sucking and adult offspring was still unclear. Phytosterols are a group of steroids and esters that occur naturally in plants, which could be able to lower serum cholesterol concentrations by competing with dietary and biliary cholesterol for intestinal absorption and enhance cholesterol excretion [11,12]. Previously, we have reported that consumption of phytosterols from early in life may help alleviate the detrimental effects of maternal high fat diet on cholesterol metabolism in mice [13]. However, the effects of phytosterols on the oxidative stress trigged by maternal high-fat diet have not been elucidated. In this study, we investigated the effect of maternal high fat diet on off spring development and oxidative stress, and evaluated the effects of consumption of phytosterols during gestation and lactation.

Materials and Methods

Materials

The phytosterols were purchased from Bluesky Biological Engineering Co., Ltd (Xi'an, China) and the composition is shown in Table 1. Roche diagnostics kits from Baron medical equipment Co., Ltd (Beijing, China) was used to determine the serum lipid profile. Commercial kits were used to analyze the reactive oxygen species (ROS), superoxide dismutase (SOD) and malonaldehyde (MDA) in neonatal liver homogenates were obtained from the Jiancheng Institute of Biotechnology (Nanjing, China).

Phytosterols	No. of residues/100 residues
Brassicasterol	2.68
Campesterol	26.29
Stigmasterol	19.12
β-sitosterol	47.12
Total phytosterols	95.21

Table 1: The composition of phytosterols

Animals and diets

C57BL/6j mice weighing 18-22 g (aged 10 weeks) were received from the Animal service of Health Science Center, Peking University. All animals were adapted to the vivarium for 1 week before the treatment at the Laboratory Animal Center, Capital Medical University. There was automatic control of temperature, humidity and light cycle. Throughout the study, the temperature and humidity were 20-24°C and 40%-70% respectively, with a minimum 15 times per hour air changes. Light hours were 8:00-20:00. Animals were allowed free access to food and water *ad libitum*. After 1 week accommodation, two virgin female mice were caged overnight with one male in individual cage and the females were checked daily for vaginal plugs. Once confirmed pregnancy, female mice were randomly assigned to three groups (thirty mice per group) and treated as follows: chow diet (control group, NC, total energy 352 kcal/100 g), high fat and energy diet (HFED, 84% chow diet+16% lard fat+0.2% cholesterol, total energy 439.68 kcal/100 g), high fat and energy diet supplemented with phytosterols (HFEP, 82% chow diet+16% lard fat+0.2% cholesterol+1.5% phytosterols, total energy 439.68 kcal/100 g). These diets kept unchangeably during pregnancy and lactation and then the surviving offspring were given chow diet until 10 weeks old. Neonatal mice were randomly entering into three endpoints groups, which were newborn, weaning and aged 10 weeks. Mice were killed by cervical dislocation after recording body weight at 0 day, 21 days and 10 weeks old, respectively. Then liver was collected and weighed. Relative liver weight was calculated as the ratio between liver weight and body weight. All livers were immediately frozen in liquid nitrogen and stored at -80°C till further study.

All experimental procedures were carried out in accordance with the guidelines of the Principle of Laboratory Animal Care (NIH publication No.85-23, revised 1985) and the guidelines of the Capital Medical University Animal Research Committee.

Biochemical assay

Approximately 0.4 ml serum was separated by centrifugation at 3000 rpm for 10 min from whole blood of the mice offspring aged 10 weeks. The levels of serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were measured using an automatic biochemistry analyzer (Hitachi 7180, Tokyo, Japan).

Liver oxidative status measurement

Mice (dams and offspring) livers were washed, minced by ultrasonic cell smash and homogenized in saline solution 0.9 g/L, NaCl on ice. Liver reactive oxygen species (ROS) contents were evaluated in homogenate using 2',7'-dichlorofluorescin (DCFH) as a probe according to Bejma [14]. Livers (20 mg) were minced thoroughly in 80 µl PBS (0.1 mM, Ph 7.0) buffer and homogenized with a motor-driven Potter Elveljem glass homogenizer on ice. Liver homogenates were centrifuged (13000 r/min, 15 min) at 4°C. A portion of the homogenate (10 µl) was used to determine ROS production. The H_2O_2 standard curve (figure 1) was prepared according to the protocol provided by Cell Biolabs (San Diego, USA), with H_2O_2 concentration ranged from 0 to70 µM. The fluorescence was read with a Cary Eclipse Fluorescence Spectrophotometer at 485 nm excitation/530 nm emission.

The total antioxidative capability (T-AOC), SOD activity and MDA contents in liver homogenates were determined according to the manufacture's (NanJing Jiancheng Bioengineering Institute, China) protocols.

Statistical analyses

SPSS 18.0 for Windows software (SPSSInc., Chicago, IL, USA) was used for all statistical analyses. Data were analyzed by one-way ANOVA. The significance was considered when $p<0.05$.

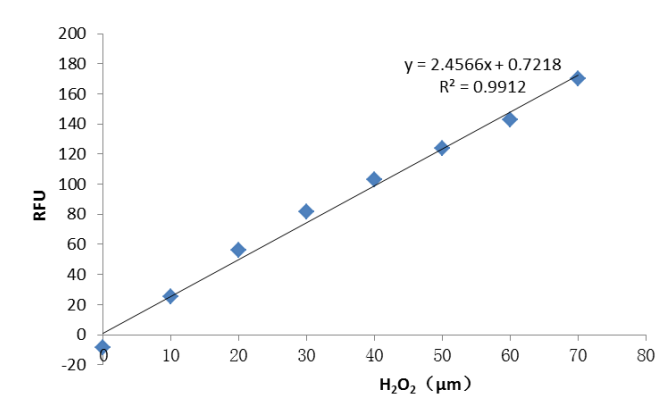


Figure 1: Hydrogen Peroxide Standard Curve

Results

Effects of HFED and HFEP on the development of male offspring

Compared with the chow diet group, the mean body weight of neonatal and sucking mice in the HFED group was increased significantly ($p<0.05$), while the body weights of adult offspring were not significant different among the three group. Compared with the HFED group, the bodyweight of neonatal mice in the HFEP group was significantly lower ($p<0.05$), but for sucking and adult mice, it was not significantly changed in HEFP group (Table 2). At the neonate stage, the relative liver weights of all tested male mice were not significantly changed, while the female of HFEP group showed slightly reduced the relative liver weight compared to the other groups. At the sucking stage (21 days old), the relative liver weights of the HFED group were significantly increased. While, at the adult stage (10 weeks old), the HFEP group showed the highest increase of relative liver weights (Table 3).

Serum cholesterol profile in adult offspring

The serum TC levels play an important role in the evaluation of early cardiovascular disease. The serum TC and HDL-C levels increased significantly in offspring from dams fed with HFED diet during gestation and lactation ($p<0.05$), and the phytosterols supplementation inhibited the increase ($p<0.05$). Serum LDL-C levels were not significantly changed on the maternal diet ($p<0.05$) (Table 4).

Oxidative status in dams and offspring (Figure 2)

At the neonatal stage, the activities of SOD, total anti-oxidative activity (T-AOC) and glutathione peroxidase (GSH-px) in the livers were significantly lower in the HFED group than the NC group (figures 2a, 2b and 2d). For the MDA and GSH levels, the treatment groups showed no difference (figures 2c and 2e). Interestingly, the ROS levels in liver homogenates of HFED-treated dams were significant increase, and a similar trend of increase was observed in their neonatal offspring (figure 2f). A correlation relationship between the maternal and neonatal liver homogenates was shown (figure 2g).

At the sucking stage, the activities of SOD, total anti-oxidative activity (T-AOC) and glutathione peroxidase (GSH-px) in the livers reversed the decrease, and showed trends of increase in the HFED group compared to the NC group (figures 2a, 2b and 2d). Meanwhile, the MDA level was decreased (figure 2c); and the GSH level was increased in the HFED group compared to the NC group (figure 2e).

For adult offspring (10 weeks old), the liver SOD activity was significant low in the HFED group than the NC group, and supplementation of phytosterols in the maternal diet could not affect its decrease. Other oxidative stress biomarkers were not affected by the maternal diet.

Group	0 d (n=30)		21 d (n=20)		10 w (n=30)	
	male	female	male	female	male	female
NC	1.33 ± 0.04	1.22 ± 0.06	9.13 ± 0.48	8.43 ± 0.99	25.53 ± 2.30	20.13 ± 1.25
HFED	1.44 ± 0.08**	1.31 ± 0.06**	11.16 ± 0.77**	10.58 ± 0.92**	26.30 ± 1.46	21.08 ± 0.49**
HFEP	1.38 ± 0.09*[a]	1.26 ± 0.08*[a]	11.06 ± 0.99**	10.67 ± 0.52**	26.09 ± 1.52	21.79 ± 1.45**

Table 2: Effects of maternal high fat and energy diet during pregnancy and lactation on body weight of mice offspring (g, mean ± SD)
NC: Chow diet, normal control group; HFED: High fat and energy diet; HFEP: High fat and energy diet supplemented with phytosterol; 0 d: the day which the mice offsprings were delivered; 21 d: the day which the mice offsprings were weaning; 10 w: 10 weeks old of the mice offsprings
**Indicate the significant difference compared with the control group (P<0.01)
*Indicate the significant difference compared with the control group (P<0.05)
[a]Indicate the significant difference compared with the HFED group(P<0.05)

Group	0 d (n=30)		21 d (n=20)		10 w (n=30)	
	male	female	male	Female	male	female
NC	4.64 ± 0.70	4.78 ± 0.64	4.49 ± 0.70	4.64 ± 0.62	4.69 ± 0.60	4.60 ± 0.50
HFED	4.46 ± 0.77	4.46 ± 0.58	5.36 ± 0.35**	5.20 ± 0.32**	4.48 ± 0.24	4.71 ± 0.37
HFEP	4.28 ± 0.66	4.38 ± 0.83*	4.47 ± 0.67[a]	4.43 ± 0.73[a]	4.72 ± 0.45[a]	5.10 ± 0.44**[a]

Table 3: Relative liver weight of mice offsprings treated with different diets during maternal pregnancy and lactation (g/100g.BW)
NC: chow diet, normal control group; HFED: High fat and energy diet; HFEP: high fat and energy diet supplemented with phytosterol; 0 d: the day which the mice offsprings were delivered; 21 d: the day which the mice offsprings were weaning; 10 w: 10 weeks old of the mice offsprings
**Indicate the significant difference compared with the control group (P<0.01)
*Indicate the significant difference compared with the control group (P<0.05)
[a]Indicate the significant difference compared with the HFED group (P<0.05)

Group	n	TC		HDL-C		LDL-C	
		male	female	male	female	male	female
NC	30	2.62 ± 0.36	2.02 ± 0.31	1.98 ± 0.28	1.44 ± 0.41	0.16 ± 0.04	0.23 ± 0.06
HFED	30/23	2.86 ± 0.23**	2.42 ± 0.33**	2.34 ± 0.26**	1.88 ± 0.23**	0.19 ± 0.04**	0.25 ± 0.04
HFEP	30	2.64 ± 0.39[a]	2.11 ± 0.24[a]	2.11 ± 0.31[a]	1.70 ± 0.18**[a]	0.17 ± 0.04[a]	0.22 ± 0.03

Table 4: Effects of maternal different diet during pregnancy and lactation on serum lipid of 10 weeks old mice offspring (mmol/L, mean ± SD)
NC: Chow diet, normal control group; HFED: High fat and energy diet; HFEP: High fat and energy diet supplemented with phytosterol.
**Indicate the significant difference compared with the control group (P<0.01)
*Indicate the significant difference compared with the control group (P<0.05)
[a]Indicate the significant difference compared with the HFED group (P<0.05)

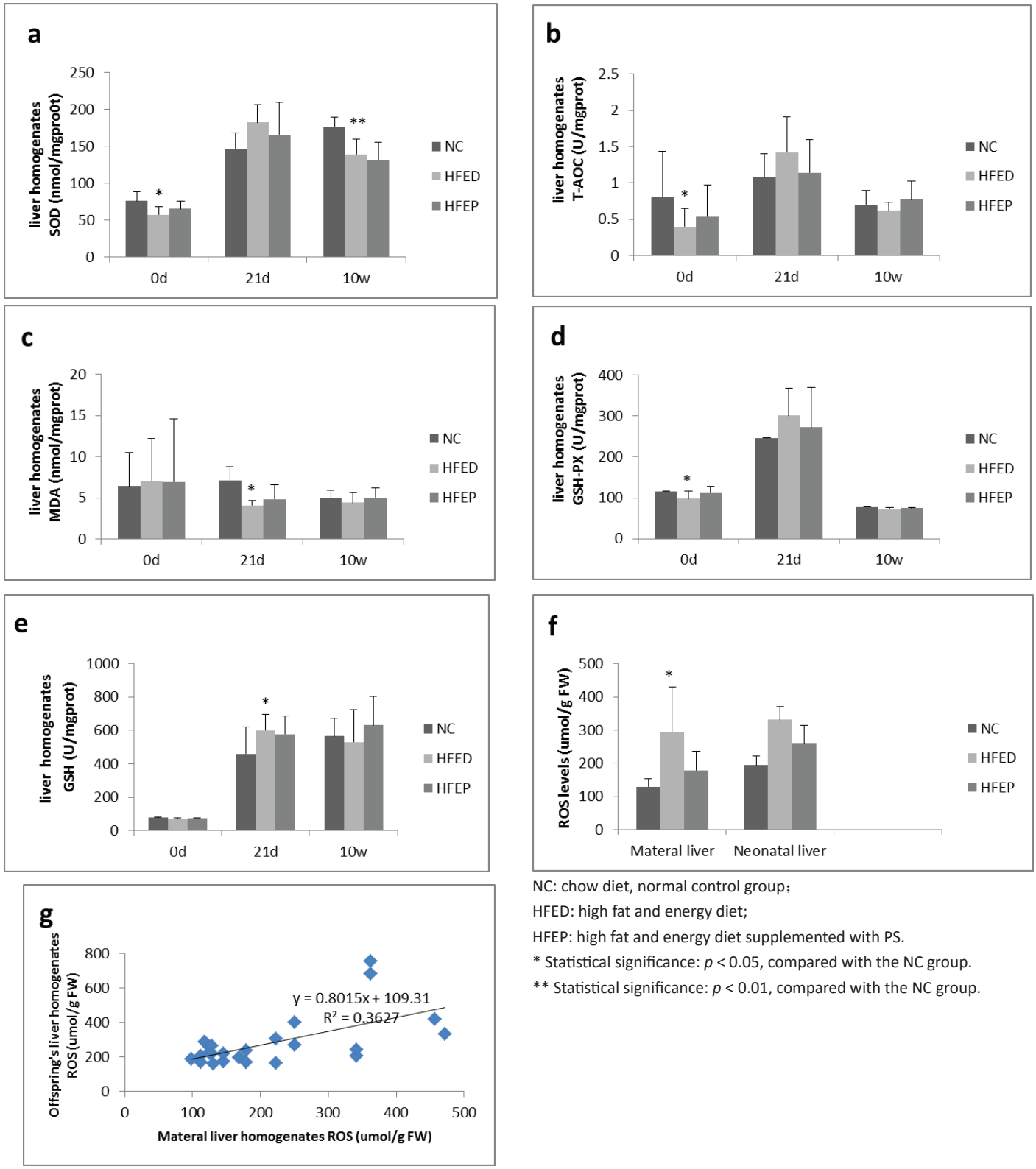
Discussion

Accumulating evidences indicate that the maternal diet rich in fat and energy increases the risk of insulin resistance, dyslipidemia, obesity and hypertension in the offsprings at their adult stage [15,16]. It has been suggested that oxidative stress can be a key for the fetus to develop disease at the adult stage [17,18]. In these studies, the effects of a maternal HFED diet on liver oxidative status in neonatal, sucking and adult offspring were evaluated and the effects of phytosterols supplementation in maternal diets were observed. The results indicated that the maternal HFED diet during gestation and lactation induced reduced anti-oxidative activity in the offspring. Meanwhile, we observed that phytosterols supplementation in maternal diet improved the development and serum cholesterol levels with no elevation of oxidative stress in offspring compared to the HFED group.

In the present study, our data is consistent with reports from others [3,5], that is that the serum TC and LDL-C levels in HFED group were significantly increased in the adult offspring. The relative liver weights in HFED group were significantly increased in sucking mice; however, in neonate and adult mice, this trend was not observed. For neonates, the nutrients are transported from maternal to fetus through placenta, which regulate the nutrients supply for fetus [19]. We found that the maternal body weight and food intake during gestation were not significantly changed by the HFED diet. In contrast, the serum TC, LDL-C, HDL-C, FFA, and insulin levels were significantly increased in dams [20]. In order to adaptive to the over nutrition status of dams, transcription of lipid synthesis genes might be inhibited [20]. This may explain the relative unaltered liver weight on the maternal diet.

During normal pregnancy, the circulating biomarkers of oxidative stress including lipid peroxides and MDA are significantly increased [21]. Oxidative stress is likely a causative agent in human pregnancy-related disorders, such as recurrent pregnancy loss, embryonic resorption, intra-uterine growth restriction (IUGR), pre-eclampsia and fetal death [22]. It is well known that chronic exposure to a high lipid diet in adult life causes oxidative stress and/or damage in the liver [23]. In our study, the maternal high lipid diet caused the increase of ROS level in the liver of dams indicating elevated oxidative stress. In another study, it also found that the HFED diet during gestation caused maternal oxidative [20]. Together, these results indicate that the maternal high lipid diet may play a causative role for pregnancy induced diseases.

We propose that maternal oxidative stress may translate to the fetus to affect the anti-oxidative ability in the offspring. Therefore, we analyzed the liver oxidative stress markers and anti-oxidative enzymes activity in the offspring. Our results showed that a reduced anti-oxidative activity in livers of both neonates and adult offspring, however, increased anti-oxidative activity was induced in the liver of sucking mice. Consistent with our observations, it has been reported that maternal consumption of



NC: chow diet, normal control group;

HFED: high fat and energy diet;

HFEP: high fat and energy diet supplemented with PS.

* Statistical significance: $p < 0.05$, compared with the NC group.

** Statistical significance: $p < 0.01$, compared with the NC group.

Figure 2: Effects of a maternal high-fat diet during pregnancy and gestation with or without phytosterols supplementation on oxidative status in neonate, sucking and adult mice. (a)&(d) In neonatal mice, the active of SOD and GSH-Px in liver were significantly lower in the HFED group than that in chow diet group. **(b)** The total anti-oxidative ability (T-AOC) was significantly decreased in the HFED group. **(c)** MDA content of sucking mice liver was significant low in the HFED group than that in chow diet group. **(e)** Total GSH levels in sucking mice liver homogenates were significantly increased in the HFED group comparing to that in chow diet group. **(f)** The HFED induced the ROS levels in liver homogenates of dams significant increase and then in their neonatal offspring. **(g)** Correlation between materal liver homogenates ROS and neonatal liver homogenates ROS concentrations. Pearson Correlation Coefficients: r=0. 602, p=0.002.

a HFED diet during pregnancy decreased the SOD activity and the mRNA expression in fetal liver of rats and increased the levels of 8-hydroxy-deoxyguanisine (8-OH-DG) and 4-hyroxy-2-nonenal (HNE) in the fetal liver in non-human primates [24,25]. These results indicate that the anti-oxidative activity in livers of fetus or neonates was reduced.

In adult offspring delivered by dams fed with HFED diet during gestation and lactation, genes responsible for the antioxidant defense capacity in the liver were 191 inhibited despite feeding a chow diet after weaning [26]. In our study, we observed the decreased SOD activity in the HFED group. However, the activity of GSH-px in the HFED group was not significantly lower than the NC group. Our key discovery is that we observed an increased anti-oxidative activity in the liver of sucking mice delivered by dams fed with the HFED during gestation and lactation. After birth, the pups were fed by dams that fed with the HFED diet; the milk from these dams might have a high content of energy [27]. It has been reported that high fat and high energy diet increases the oxidative stress in mice [9]. Thus in responses to the high energy content diet, the anti-oxidative activity in liver were induced. The mechanism for this response waits for future investigation.

It is well-known that consumption of foods supplemented with phytosterols may 202 help reduce LDL-C levels in animal models or humans [28]. Recently, researchers found that phytosterols alleviated the oxidative stress in hyperglycemia and colon cancer animal models and hypercholesterolemia patients [29-31]. We propose that phytosterol supplementation may alleviate the oxidative damage induced by the HFED diet in dams and offspring. The results indicate that the anti-oxidative activity if effective in dams but not so good in offspring, indicating that supplementation of phytosterols in the maternal diet may not be a good way to prevent the detrimental effects of the HFED diet consumed by dams to the offspring.

Conclusion

In summary, our current investigation suggests that the oxidative stresses in neonatal, sucking and adult offspring from dams fed with the HFED diet during pregnancy and gestation were different. Phytosterols supplementation can alleviate the oxidative damage in dams but not in offspring.

Conflict of Interest

The authors declare that there is no conflict of interests.

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