Maternal Use of Cod Oil Fish, Nigella Sativa Oil and Sea Buckthorn Fruit During Pregnancy and Lactation Period Have Protective Effects on Offspring from High Fat Diet Dams

A comparative study

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The aim of this study was to compare the effects of three different supplements: Cod oil fish (EPA and DHA 0.1 g/kg), Nigella Sativa oil (0.1 g/kg), and Sea Buckthorn fruit 1g/kg on pups at weaning from females with high-fat diet model of Wistar rats. Female Wistar rats (age 10 weeks) were mated and kept on high caloric/ high fat diet (30%) and allocated in three groups according to the supplement associated to the fat diet. After delivery, the dams from these groups continued the treatment but with standard diet until weaning. Two more female groups were kept in parallel on the same high fat diet during gestation, but during breastfeeding one group switched on standard chow while the other continued the high fat diet. At weaning, the offspring and their dams were sacrificed and the pups' liver and pancreas were harvested. Histopathological exam was performed and tissue oxidative stress and blood biological parameters were measured. At weaning, the pups from the high fat diet dams without supplements had minor hepatopathy and minor pancreatopathy, increased tissue oxidative stress and altered metabolic profile, whereas each administered supplement proved to preserve the normal tissue aspect and also to improve biological blood and tissue oxidative stress parameters. In conclusion, maternal use of Cod oil fish, Nigella sativa oil and Sea buckthorn fruit during pregnancy and lactation period have similar protective effects on offspring from obese high fat diet dams.

Keywords: high fat diet, pregnancy, lactation, offspring, Cod oil fish, Nigella sativa oil and Sea buckthorn

One of the biggest errors done during pregnancy is having an inappropriate hyper-caloric alimentation.

Pregnancy obesity includes a wide range of pathologies with implications on both fetal and maternal metabolism. Dyslipidemia and oxidative stress during pregnancy are more severe in obese mothers and have impact on the fetus development. The newborn offspring from obese mothers are either with low weight or macrosomic or have high risk for neural tubular defect and other congenital defects [1]. On long term, the offspring of obese mothers are prone to become overweight in childhood and in early adulthood. Also, it was demonstrated that hyperlipidemic environment during intrauterine life increases the susceptibility of these children to early atherosclerosis [2].

High caloric/high fat diet during pregnancy and lactation represents an important source of additional energy which is transferred to the offspring, increasing the risk for obesity in the pups. Also, the mother's diet affect the composition of the maternal milk and this can influence the development of the offspring [3].

In order to prevent obesity complications, the best time to do a good management is to target gestational and lactation period, to reverse the adverse outcome of high caloric/high lipid diet during pregnancy and this can be done by using different supplements.

On the one hand, studies focused on perinatal Omega 3 fatty acids deficiency or on supplementation with Omega 3 fatty acids formula gave conflicting results regarding the fat mass in the offspring during the first year and later during childhood [4-6]. On the other hand, according to the European Directive, supplementation of infant formula with long chain PUFA is recommended because of beneficial effects on long term in lowering blood pressure during later childhood [7].

Obesity is associated with prediabetes and there are studies which demonstrated that the Sea Buckthorn berries [8] have a good potential to diminish metabolic syndrome complications [9].

The main constituents of Sea Buckthorn berries are carbohydrates, essential fatty acids, amino acids, antioxidants (i.e. vitamins C and E, β-carotene, and lycopene), phytosterols, and flavonoids, in addition to chemical elements (i.e. iron, calcium, etc.) [10]. Sea buckthorn supplements are considered safe in children and adults [11, 12] but there is a lack of studies according the effects of Sea buckthorn berry fruit during pregnancy in obese young women.

Nigella sativa oil has a long history of folklore usage in different diseases and it is rich in timoquinone, unsaturated fatty acids, vitamins and minerals. It is a multipotent supplement having antioxidant, anti-inflammatory, immunomodulatory, antihypertensive, antidiabetic, lipid-lowering, hipofagic effects being beneficial in obesity [13].

The aim of this experimental study was to compare the effects of dam intake of three different supplements: Omega 3 fatty acids (EPA and DHA), Nigella Sativa oil and Sea Buckthorn fruit, on their weaning pups and observe if the therapy alleviate the adverse effects of the high-fat diet during pregnancy obesity.

Experimental part

Materials and methods

Thirty six female Wistar rats (50-60 g) purchased from Animal Facility of Carol Davila University of Medicine and

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Pharmacy, Bucharest, Romania, were raised on standard chow until maturity age (10 weeks, 180-190 g). Male rats were introduced in the cages for mating and the females were then divided into three big groups and for six weeks, throughout gestation and lactation as following: group CON (control group, *n=6*), continued with the standard chow while the second, H group *(n=6*) was fed with high caloric/ high fat diet (30%). The third big group *(n=24*) was subdivided into other four groups (*n=6*), all receiving high/ caloric high fat diet during gestation and standard chow during lactation. The differentiation between these four groups was made by the supplement that was associated with the above described diet, as follows: one group received Omega 3 fatty acids as a mixture of EPA and DHA, 0.1mg/kg/day *(O group*), the second group took Nigella sativa oil 0.1mg/kg/day (*N group*), the third group eat 1g/kg/day Sea buckthorn frozen fruit (*F group*), and the forth group did not receive any supplement (*S group*) (table 1).

Each corresponding group of the offspring was formed of six to eight pups.

The lactation period was of 3 weeks and at weaning the pups were sacrificed by cervical dislocation and their liver and pancreatic tissues were harvested and immediately placed in an ice bath and divided for tissue oxidative stress measurement and for histopathological assessment. Blood samples from carotid arteries were taken from pups and also from their dams. The blood was centrifugated at 4000 g for 10 min and on the serum usual biological parameters like glucose, total cholesterol, triglycerides, uric acid, albumin, HDL-c, urea, total proteins were done by using Hospitex Diagnostics kits, Romania).

The experimental procedures were carried out under Convention 86/609/E.E.C. from November 24, 1986, for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. Tissue samples were homogenized either in KCl 0.15 M, 10% dilution for glutathione (GSH) assay and 25% dilution, for lipid peroxides, or in EDTA 0.02 M, *p*H 4.7 for total thiol. A Potter–Elvehjem instrument was used as a tissue homogenizer. The measurements were done on the supernatant of the centrifugated homogenate (10000 g for 10 min at 4^0C in a Heraeus Multifuge 3 S-R centrifuge). The protein content was determined by using the biuret reagent (Sigma Chemical Co., St. Louis, MO, USA), at 550 nm.

Determination of total glutathione and total glutathione on liver and pancreatic tissue

Liver total GSH and total thiols content were measured following the Beutler E *et al* method by using the colour reactant 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma Chemical Co.) The absorbance was read at 412 nm and calculation was done using the molar extinction coefficient for glutathione [14].

Estimation of liver and pancreatic lipid peroxidation

Esterbauer H and Cheeseman KH method was used for lipid peroxidation assessment in liver and pancreatic homogenates[10]. The pink colored complex betweem MDA and thiobarbituric Acid (TBA, Sigma T-5500) was measured at the wavelength 532 nm[15].

Histopathological studies

Fresh samples of liver and pancreas were fixed in 10% formalin for at least 24 h. After paraffin-embedding, the samples were cut into 5μm-thick sections using a rotary microtome and stained with Hematoxylin–Eosin. The histopathological slides were examined and images were acquired using a Carl Zeiss Jena photomicroscope.

Results and discussions

Histopathological exam (fig.1 and 2)

O group: The general architecture of the liver was preserved, the hepatocytes exhibiting a normal arrangement, with a moderate cytoplasmic load of glycogen and the Kupffer cells being present in a moderate number with no signs of activation. No detectable microscopic lesions were identified, which indicates normal liver histology.

N group: The typical liver architecture, with small loads of glycogen in hepatocytes, a moderate number of Kupffer cells and hyperemia in the centrilobular veins indicate a normal liver histology.

F group: Normal liver architecture with multifocal presence of extramedullary hematopoiesis islands and, isolated in the sinusoid capillaries, discrete leukocytosis represent a normal liver histology.

H group: The sections displayed hepatocytes with moderate loads of glycogen, with increased volume and active Kupffer cells in a moderate amount. Areas with focal hyperemia in the sinusoidal capillaries and discreet leukocytosis are present. Multifocal islands of extramedullary hematopoiesis are present. The general liver architecture was preserved so the hepatopathy characteristics fall into the category of minor degree.

S group: The general liver architecture is intact, with the presence of hepatocytes with low glycogenic load, inactive Kupffer cells, discrete leukocytosis in the sinusoidal capillaries and islands of extramedullary hematopoiesis. The hepatopathy characteristics fall into the category of minor degree.

CON group: The general liver architecture is preserved (normal liver aspect).

O group: The pancreatic general architecture is preserved. These features indicate normal pancreatic tissue histology.

N group: Pancreatic general architecture preserved.

F group: The pancreatic tissue has a normal histological aspect.

H group: Perilobular and periacinar a discreet edema can be observed. The Langerhans islets cells present focal cytoplasmic vacuolation and around the islets, multifocal mononuclear inflammatory infiltrate can be seen, along with

Table 1

BIG GROUPS AND SMALL GROUPS DIVISION, ACCORDING TO THE DIET AND THE SUPPLEMENT GIVEN

First group CON	**Second group H**	**Third group**			
		O	**N**	**F**	**S**
Gestation: standard chow Lactation: standard chow	Gestation: high caloric/high fat diet Lactation: high caloric/high fat diet	Gestation: high caloric/high fat diet+Omega 3 fatty acids oil Lactation: standard chow+Omega 3 fatty acids oil	Gestation: high caloric/high fat diet+ Nigella sativa oil Lactation: standard chow+ Nigella sativa oil	Gestation: high caloric/high fat diet+Sea buckthorn fruit Lactation: standard chow+Sea buckthorn fruit	Gestation: high caloric/high fat diet Lactation: standard chow

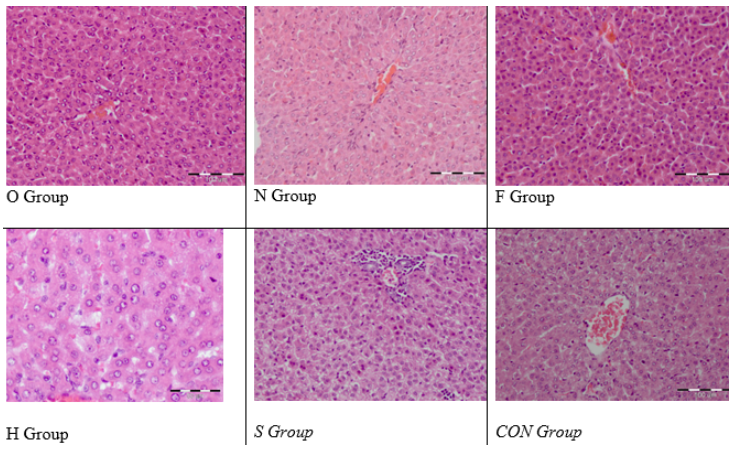

Fig 1.The histopathological aspect of the pups liver, Hematoxilin-Eosin dye, ob 40 x for:O group (with Omega 3 fatty acid oil as supplement), N group (with Nigella sativa oil supplement), F group (with Sea buckthorn fruit), H group (fat diet during gestation and lactation), S group (fat diet during gestation, standard diet during lactation), CON group (only standard diet)

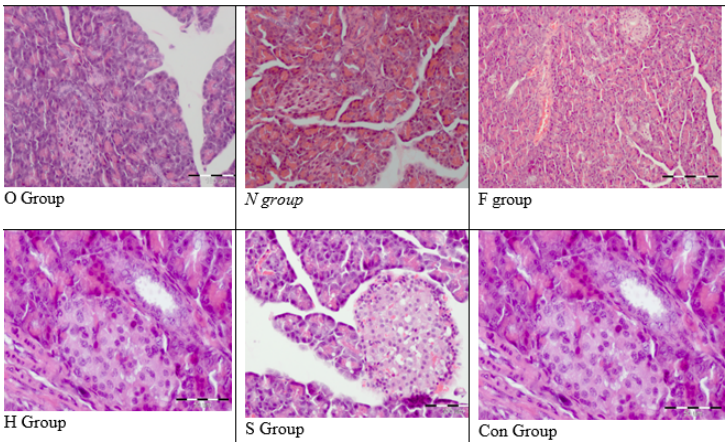

Fig 2. The histopathological aspect of the pups pancreas, Hematoxilin-Eosin dye, ob 40 x: O group (with Omega 3 fatty acid oil as supplement), N group (with Nigella sativa oil supplement), F group (with Sea buckthorn fruit), H group (fat diet during gestation and lactation), S group (fat diet during gestation, standard diet during lactation), CON group (only standard diet)

capillary hyperemia. Peripancreaticthere is an inflammatory cell infiltration in the adipose tissue.

S group: Discreet periacinar and perilobular edema, andperipancreaticinfiltrated by inflammatory cells from adipose tissue can be observed.

CON group: The pancreatic tissue has a normal histological aspect.

Obesity in pregnancy has effects on child health and the implications are even on long term, the new born having an increased risk for developing obesity, diabetes and cardiovascular diseases later in life [16]. The hypothesis of the *thrifty phenotype* by David Baker and Nicholas Hales underlines that the phenotypic changes are induced by nutritional imbalance of intrauterine life [17]. These changes provide an immediate survival advantage, but the individual has an increased risk of developing cardiometabolic diseases, later during lifetime. Altered hormone levels like hyperleptinemia associated with high caloric/high fat diet in pregnancy may impact the offspring development. And more, relatively recent studies highlight that different nutrients from the breastfeed milk diet in neonates can affect gene expression by epigenetic modification and influence the child development [18].

Franco *et* al. demonstrated that milk composition is influenced by high fat diet during lactation period, resulting in obesity and hyperglycaemia in male offspring at weaning [19].

In our study, the offspring at weaning age from the studied groups had different metabolic profiles. Impaired biological plasma levels were demonstrated in H group, as high triglyceridemia, uricemia and low HDL-c. In O group, the supplement rich in Omega 3 fatty acids decreased the increased level of plasma triglycerides ($p<0.04$) and increased the decreased level of HDL cholesterol ($p<0.05$), compared with the H group. In N group, compared to H group, the supplement Nigella sativa oil improved the activity of AST ($p<0.05$), demonstrating that this supplement has protective effect on the pups liver. In F group, the supplement Sea buckthorn fruit significantly decreased the level of plasma glucose ($p<0.05$) in comparison to the H group (table 2).

Table 2

SERUM BIOLOGICAL PARAMETERS OF OFFSPRING

Parameter	Group				
	O	N	F	H	S
Total proteins [g/dL]	5.97±0.61	5.22±0.72	5.97±0.69	5.85±0.43	5.72±0.48
Albumin [g/dL]	3.26±0.25*	3.15±0.58	3.12±0.77	3.10±0.53	3.01±0.41*
ALT [UI/L]	53.27±8.6	53.27±8.6	53.28±4.6	58.82±4.2	55.04±5.9
AST [UI/L]	30.26±5.1	24.09±1.9	20.70±3.2*	45.88±2.8*	33.71±6.3
Uric acid [mg/dL]	0.56±0.32*	1.20±0.27	0.75±0.52	2.29±0.34*	1.89±0.23
Glycaemia [mg/dL]	91.03±9.2	81.22±7.4	78.35±9.3	94.77±12.1	73.99±7.9
Cholesterol [mg/dL]	91.95±11.1	93.55±12.3	80.23±12.7	84.63±13.5	69.02±8.3
Triglycerides [mg/dL]	36.59±9.1*	58.48±8.9	47.72±9.8	71.83±10.2*	53.85±6.2
Urea [mg/dL]	36.96±5.2	47.85±6.1	34.15±4.7	45.05±3.8	30.56±4.6
HDL-c [mg/dL]	41.1±5.6*,#	35.8±4.7	28.8±3.9	27.5±3.8*	25±1.9#

t-test, p* values were obtained by the comparison of the H group with the other groups;($p<0.05$) for AST between H and F group; ;($p<0.05$) for uric acid between H and O group; $p<0.04$ for triglycerides between H and O; $p<0.05$ for HDL between H and O.
p# values were obtained by the comparison of the S group with the other groups;($p<0.05$) for albumin between S and O group; $p<0.05$ for HDL between S and O

Maternal high caloric/fat diet during breastfeeding is associated with offspring obesity. Following the pathogenic events, the offspring obesity is associated with hyperleptinaemia, which stimulates adrenal medullary and thyroid function. The high levels of cathecolamines increase gluconeogenesis [19] and these may explain the high glycemia observed in the pups from high fat diet dams.

We analysed also the liver and pancreas oxidative stress in pups because dysfunctions in sugar and lipid metabolism can trigger the oxidative stress. In our study, in the H group, the liver peroxidation had the highest value and both remedies Nigella sativa oil and Omega 3 fatty acids used in the dams diet reduced their pups liver MDA level (table 3). The Sea Buckthorn fruit may be of great value in pancreatic protection by increasing the tissue glutathione level (table 3). We consider that breast milk enriched in antioxidants, like that from dams taking supplements, has beneficial effects on pups because it was demonstrated that antioxidants in the diet could reduce tissue oxidative stress. In vitro studies demonstrated that polyphenols and flavonoids extracted from plants have hepatoprotective effects, reducing liver oxidative stress [20]. Polyphenols extracted from Sea Buckthorn are natural antioxidants having the capacity to chelate transition metals ions and scavange radicals [21].

Experimental studies on female Wistar rats with streptozotocinic diabetes mellitus, demonstrated that different compounds from breast milk can protect the pups pancreas. One study showed that the intake of flaxseed oil rich in Omega 3 fatty acids, reduced the damage caused by maternal hyperglycaemia, and maintained the β-cell pancreatic mass in female offspring promoting normal pancreas [22]. Another study demonstrated that nutritional supplementation of gestational mothers with the natural antioxidant thymoquinone, during pregnancy and lactation periods had protective effect in pups [23].

In our study, the high caloric/high fat diet during pregnancy and during breastfeeding was associated with a high lipid peroxidation in the offspring's liver and a lower pancreatic antioxidant defense estimated as total glutathione. The switch of the diet from a high caloric/high fat diet during pregnancy to a standard chow during lactation is not enough to correct the abnormal metabolic consequences of the unhealthy dam diet during gestation. As a strong argument is the histopathological result showing minor hepatopathy and peripancreatic inflammation in the offspring of both groups H and S. Opposite, the liver and the pancreas of pups at weaning age from dams with Supplements associated to the fat diet during gestation and standard diet during breastfeeding were normal. It is important to observe that all the supplements reversed the tissue alterations (fig.1 and 2).

In pups, from group H, the liver histopathological aspect characterized by inflammation and the increased liver oxidative stress were in accordance with the plasma parameters as high triglyceridemia, low HDLc, high AST and high uric acid. In S group, the albuminemia had the lowest level between the studied group and Omega 3 fatty acids increased the decreased levels, improving the liver synthesis capacity (table 2).

In first natural source of fatty acids in neonates is human milk. The polymorphism of desaturases and diet influence the quality and quantity of the fatty acids [24]. It was demonstrated that in neonates, dietary arachidonic acid plays a minor role on growth and development relative to the impact of dietary docosahexaenoic acid [25] and that the dietary trans fatty acid intake during pregnancy and lactation influence maternal and infant adiposity [26, 27]. On the other hand, in the prospective Pregnancy, Infection and Nutrition Study (n = 358) done on human subjects in the first 4 months post-partum, no correlation was observed between infant development and the long chain polyunsaturated acid content of breast milk [28]. The improvement of the metabolic picture in the pups from dams who associated one of the supplement during gestation and lactation is due to the antioxidant and anti-inflammatory effects of the supplement components. The assay of the milk samples from 60 breastfeeding women at one month postpartum showed that polyunsaturated fatty acids do not lower the milk antioxidant capacity [29]. Franco et al. [19] demonstrated that even consumption of a normocaloric diet during pregnancy and lactation may cause deleterious effects in the offspring if the quality of the diet is not appropriate. Also, during lifetime, diet is important. The IDEFICS study, published recently, done on 1400 children, 2-9 years old, showed the relation between whole blood fatty acids and inflammation [30].

In moderate doses, Omega 3 fatty acids (EPA and DHA) are safe for pregnant and postpartum women and have minor side effects [31]. Nowadays there is a growing interest in supplementing pregnant and breastfeeding women with Long chain polyunsaturated fatty acids and daily intake of at least 200 mg DHA is recommended during gestation and breastfeeding [32].

In other words, the fetus and neonate should receive long chain fatty acids which are very important for visual and cognitive development. Moreover, the consumption of oils rich in n-3 LC-PUFA during pregnancy reduces the risk for early premature birth [33]. Experimental studies

Table 3

OXIDATIVE STRESS IN LIVER AND PANCREAS IN OFFSPRING

Parameter	Group				
	O	N	F	H	S
Liver MDA [nmol/g tissue]	3.26±0.76*#	3.12±0.54*#	3.44±0.35	7.85±2.33*	6.15±1.12#
Liver total glutathione [µmol/ g tissue]	5.1±0.92	4.4±0.87	5.3±0.99	4.7±0.43	5±0.37
Liver total thiols [µmol/ g tissue]	7.2±0.98	7.05±0.85	6.98±0.78	6.3±0.93	6.5±0.72
Pancreatic MDA [nmol/g tissue]	2.28±0.22	2.42±0.24	2.69±0.27	3.05±0.30	2.3±0.23
Pancreatic total glutathione [µmol/g tissue]	3.05±0.51	2.9±0.49	3.35^±0.34	2.41^±0.32	2.63±0.38
Pancreatic total thiols [µmol/g tissue]	4.87±0.63	4.5±0.58	4.68±0.71	4.71±0.84	5.04±0.96

t-test for liver: p* values were obtained by the comparison of the H group with the other groups; p<0.04 for MDA between H and O, p<0.04 for MDA between H and N; p# were obtained by the comparison of the S group with the other groups

t-test for pancreas: p^ values were obtained by the comparison of the H group with the other groups

Table 4
SERUM BIOLOGICAL PARAMETERS OF DAMS

Parameter	Group				
	O	N	F	H	S
Total proteins [g/dL]	6.80±1.21	6.24±0.72	6.51±0.87	6.33±0.56	6.34±1.23
Albumin [g/dL]	3.08±.0.56	3.21±0.47	3.00±0.78	3.01±0.97	3.39±0.66
ALT [UI/L]	54.56±7.86	80.25±10.59	83.01±9.87	88.77±11.34	63.79±8.89
AST [UI/L]	40.07±6.23	39.84±3.45	50.34±8.45	93.96±12.76	69.06±8.98
Uric acid [mg/dL]	1.19±0.34	0.97±0.12	0.84±0.22	2.13±0.44	1.30±0.21
Glycaemia [mg/dL]	103.12±13.76	74.65±6.88	45.31±5.66	111.56±14.75	93.96±11.78
Cholesterol [mg/dL]	47.14±6.12	59.75±7.21	58.27±5.76	56.86±6.34	51.02±5.67
Triglycerides [mg/dL]	77.14±8.32	114.53±12.64	115.46±12.99	154.02±16.76	101.90±12.9
Urea [mg/dL]	41.30±6.43	48.73±5.22	43.07±6.44	53.54±7.47	20.52±3.2
HDL-c [mg/dL]	23.0±3.14	31.9±3.5	25.7±2.8	27.5±3.41	29.2±3.56

t-test, p values were obtained by the comparison of the H group with the other groups ($p<0.05$) for AST between H and N group;
t-test, p values were obtained by the comparison of the H group with the other groups $p<0.05$ for glycaemia between H and F, $p<0.05$ for triglycerides between H and O.

demonstrated that thymoquinone during pregnancy and lactation period is recommended in gestational diabetes mellitus [34, 23]. There is lack of data on Sea Buckthorn effect on gestation and lactation period.

This study is focused on the effects of three different maternal supplements on pups at weaning from obese Wistar females with high-fat diet during pregnancy. But we observed also that the dams' blood tests from high fat diet group without supplements were in an abnormal range. In mother rats, the metabolic picture was similar to one described by Auberval et al. and the high fat diet model of Wistar rats is considered a good tool for the biological validation of drugs and supplements[35].

In our study, in each dam group, the supplement improved at least one parameter. The Omega 3 fatty acids lowered the increased triglyceridemia, the Sea Buckthorn fruit decreased the increased glycaemia and Nigella Sativa improved the AST level (table 4). All these supplements, rich in mono- and polyunsaturated fatty acids, associated to the dam's diet influence their blood lipid profile. There is a strong relation between fatty acids composition, modified LDL, lipid peroxidation and cardiovascular diseases [36], so the treatment is very important in the dams also.

The study opens new lines for pregnancy obesity management. The type of supplement during breast-feeding and lactation should address the picture of the dysmetabolism in mother and the expected results in the offspring. As an example, if the metabolic disturbance in pregnancy obesity is obvious dyslipidemia, the polyunsaturated fatty acids oils like Omega 3 fatty acids may be the first choice as Supplements, if it is insulin resistance or dysglycemia, the berry fruit (like Sea Buckthorn) or timoquinone from Nigella Sativa should be used. The beneficial effects of the supplements have two targets: the mother and the offspring.

Conclusions

Maternal use of Nigella sativa oil or Omega 3 fatty acids during pregnancy and lactation have protective effects on offspring from high fat diet dams. These two oils are good remedies, reversing the pups liver injuries and reducing the increased liver oxidative stress in offspring at weaning. Maternal use of Sea Buckthorn fruit supplements during gestation and lactation period increased the pancreatic glutathione, improved the plasma level glucose and maintained a normal histological aspect of the liver and the pancreas in offspring from high fat diet dams.

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