MATERNAL-FETAL METABOLISM DISORDERS INDUCED BY MATERNAL OBESITY IN AN ANIMAL MODEL

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Abstract

Context. Adipokines secreted by fat cells are vital to the control of energy metabolism, communicating the nutrient status with the tissues responsible for controlling both energy intake and expenditure and insulin sensitivity.

Objective. We aimed to prove in an experimental animal study that maternal obesity has long term adverse fetal metabolic consequences, which pass on even to the next generation of descendants.

Design. The effects of maternal obesity have been studied on animal model using 50 obese female Wistar rats, in which we induced obesity by high-calorie high-fat diet administered by gavage.

Subjects and Methods. Obese rat females were sacrificed at gestation term and we analyzed the secretion of adipokines from maternal venous blood: leptin and adiponectin, placental, pancreatic, liver and brain homogenates lipid peroxidation levels estimated by: MDA (malonyl-dialdehyde), total thiols and GSH – as antioxidant factors and routine biochemistry.

Results. Low levels of adiponectin and increased levels of leptin positively correlated with the value of placental and fetal tissue lipid peroxidation (from the liver, pancreas and brain) measured by elevated MDA and total thiols and low levels of GSH. The lipid peroxidation in the organs examined generated consistent results, showing high levels of peroxidation expressed through high values of MDA in the groups with Omega 6 supplements respectively no supplementation, and low levels of antioxidants expressed through glutathione and thiols.

Conclusions. Endocrine secretion of adipokines from the adipocytes and the recruited macrophages of obese mothers is positively correlated with placental and tissue lipid peroxidation level and routine biochemical parameters.

Key words: maternal obesity, metabolic disorders, lipid peroxidation, adipokine secretion.

INTRODUCTION

Obesity represents a pathological entity not yet fully elucidated, although increasingly more intensively studied. Maternal obesity during pregnancy associates metabolic risks, including the generation of a proinflammatory and lipid peroxidation state and hormonal imbalance. Besides them, it has long-term consequences for the offspring, the process of fetal metabolic programming appearing during early life with major physiopathological impact on later development. It has been considered a true pandemic of the 21st century, both its incidence and prevalence continuously rising. The incidence of obesity at the diagnosis of pregnancy has found an exponential increase in the latest 15 years. The prevalence of obesity in the first trimester of pregnancy has doubled between 1989 and 2007 and is still rising, while the prevalence of maternal obesity has been reported from 9-10% to 16-19% in different studies (1).

The real challenge in studying pregnancy obesity is that there are no criteria for defining obesity in pregnancy or metabolic syndrome in pregnant women. The classification of overweight and obese patients does not apply for pregnant women, the only criteria established until now being the weight gain during pregnancy. The Institute of Medicine has recommended in 2009 that the pregnancy weight gain for obese women to be 5 to 9.1 kilograms for singleton pregnancies, and 11 to 19 kilograms for twin or multiple pregnancies. These recommendations for overweight women are 6.8 to 11.3 kilograms during pregnancy (2). It is important to note, however, that body mass index - BMI can be misleading. That is why the American College of Obstetricians and Gynecologists has stated that there is no need to classify maternal obesity, as it

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brings the same maternal-fetal risks regardless of the weight excess (3).

Maternal obesity is one of the most common obstetrical risk factors, its early installation having a greater impact on the fetus by increasing the risk for cardiac malformations, neural tube malformations, omphalocele or long term metabolic alterations(4). The traditional idea that adipose tissue is an inert organ with energy storage function has been abandoned in 1987 when it has been discovered the metabolism of sex steroid hormones in the fat tissue and the production of adipsin, a protein secreted by adipocytes which is down-regulated in obese rodents. Adipocytes are also endocrine organs, with multiple metabolic roles in regulating whole-body physiology. Small adipocytes in lean individuals promote metabolic homeostasis, while the enlarged adipocytes of obese patients recruit macrophages and promote inflammation and the release of a range of factors that predispose towards insulin resistance. In 1994 leptin was discovered as the first described adipokine. Currently there are known a few hundred of adipocytokines which give fat tissue autocrine, paracrine and endocrine characteristics. Adipokines classification based on biological function divides them into four categories: factors with direct metabolic influence (adiponectin, leptin, retinol-binding-protein 4, adipsin, resistin, visfatin), pro-inflammatory and acute phase reactants (TNF α , interleukins 6, 8, 10, 18, monocyte chemoattractant protein1), extracellular matrix components (types I, III, IV, VI collagen, fibronectin, matrix metalloproteinases 1, 7, 9-11, 14, 15) and pro-mitogenic and pro-angiogenic factors (TGF β , IGF-1, FGF, VEGF) (5, 6). Altered adipokine levels have been observed in a variety of inflammatory conditions, although their pathogenic role has not been completely clarified.

Obesity is characterized by increased storage of fatty acids in an expanded adipose tissue mass and is closely associated with the development of insulin resistance in peripheral tissues like the skeletal muscle and the liver. Adipokines are vital to the control of energy metabolism, communicating the nutrient status of the organism with the tissues responsible for controlling both energy intake and expenditure as well as insulin sensitivity (7).

Hypertrophied adipocytes of obese women recruit macrophages in excess into the adipose tissue, which in turn secrete cytokines. The link between obesity and inflammation is represented in fact by the proinflammatory cytokines secreted by the fat tissue (8). Regular secretion of proinflammatory adipokines

and chemokines cause a subinflammatory chronic condition called metaflammation (9). Obesity specific metaflammation is the main metabolic risk factor for the endothelial dysfunction caused by the oxidative stress generated by proinflammatory adipokines (TNF, IL-6, IFN γ , MCP-1) and the lipotoxicity mediated by elevated free fatty acids - FFA levels (10, 11).

Our objective was to prove in an experimental animal study that maternal obesity has adverse fetal metabolic consequences, which pass on even to the next generation of descendants. The concept of fetal programming first described by Barker in 1990 was confirmed by our experiment concerning fetal imprinting of metabolic alterations generated by pregnancy obesity. We sought the possibility of a reprogramming phenomenon by subjecting the obese rodents to dietary interventions including food supplements or diet changes.

SUBJECTS AND METHODS

Design

The effects of maternal obesity have been studied on animal model using 50 female Wistar rats, weighting between 200g-250g (normal weight100-150g), in which we induced obesity by high-calorie high-fat diet administered by gavage (80% of food intake represented by fats, saturated fatty acids, respectively). After obesity was installed, the female rats became pregnant and were followed for the 3 weeks duration of their gestation. The obese pregnant female rats were divided into 5 groups (each one containing 10 females): Group 1- continued the high-calorie high-fat diet during pregnancy with supplementation of Omega 3 fatty acids (DHA - docosahexaenoic acid and EPA - eicosapentaenoic acid) 1 mL/kg, Group 2 – continued the high-calorie high-fat diet during pregnancy with supplementation of Omega 6 fatty acids, 1 mL/kg, Group 3 – continued the high-calorie high-fat diet during pregnancy with sea buckthorn fruits supplements 10g/ female, Group 4 – continued the high-calorie high-fat diet during pregnancy without supplements and Group 5 - received standard diet (normolipidic normocaloric) during pregnancy. The female descendants of the rodents were raised and at the age of adulthood; a part of them were fattened using the same protocol and also became pregnant, in order to study the effects of obesity on the next generation of pups.

Animal experiments have been conducted in agreement with the animal care procedures from the Guidelines from “Carol Davila” University of

Medicine and Pharmacy for the Care and Use of Laboratory Animals. Wistar female rats were assigned for the experiments from the University's biobase, all animals being born and raised under strict supervision of the specialized personnel from the University. Regarding the dietary supplements used for the rats, the docosahexaenoic and eicosapentaenoic acid and the sea buckthorn fruits were purchased from local pharmacy and weighed at the laboratory in order to adjust the dosages according to the required concentration and dose.

Methods

Obese rat females were sacrificed at gestation term and we analyzed the secretion of adipokines from maternal venous blood: leptin and adiponectin, placental lipid peroxidation levels estimated by: malonyl-dialdehyde (MDA), total thiols (proteins with cysteine) and oxidized glutathione and total (GSH) – as antioxidant factor. Tissue lipid peroxidation level was established by measuring the above-mentioned markers from pancreatic, liver and brain homogenates using spectrophotometric enzymatic measurement method with rat kits from Sigma Aldrich, Merck. For the dosage of the adipokine serum level the venous blood was collected on EDTA from the jugular vein, centrifuged and analyzed using an ELISA rat kit (Sigma Aldrich, Merck). The maternal organs were homogenated using a potter homogenator after reassuring that we collected samples from all organ parts and obtaining a 1:10 dilution using 0.9% sodium chloride, while the supernatant was subsequently analyzed using a spectrophotometric technique. Routine biochemistry was also collected and analyzed from maternal serum. In order to predict a negative outcome for the descendants we established associations between maternal diet and fetal metabolic status using the above mentioned biomarkers (12).

We searched the correlation between adipokines secretion and maternal placental and fetal tissue lipid peroxidation. Fetal status was evaluated initially

through placental homogenates analysis knowing that the placenta is the main maternal-fetal exchange organ and later by realizing a second part of the experiment using female pups of the obese mothers, which were subsequently followed throughout their gestation. That way we could evaluate the fetal consequences of maternal obesity both on short term and on long term, being able thus to follow two more generations of rat pups.

RESULTS

Low levels of adiponectin and increased levels of leptin as adipokines secreted by adipocytes of obese mothers correlated as expected with the value of placental and fetal tissue lipid peroxidation (from the liver and pancreas) measured by elevated MDA and low levels of GSH and total thiols as antioxidant capacity measurements. The rats that received Omega 3 and sea buckthorn decrease fruit supplements showed a statistical significant ($p < 0.05$, compared to the other groups) in the level of placental MDA, which positively correlated with the birth weight of pups, higher than in the other groups (see Tables 1, 2, 3). The antioxidants measured through total (both oxidized and reduced) glutathione and total thiols showed correspondingly high levels for these female Wistar rats, compared to the groups without supplementation, with Omega 6 supplementations or on standard diet. Also, for these two groups, the values of leptin and adiponectin were consistent with the positive effect of the supplements, leptin having lower values, while adiponectin increased ones (Fig. 1). The low levels of prooxidant molecules such as MDA correlated with increased antioxidant activity expressed through GSH and thiols, and favorable adipokine expression involving high adiponectin value and low leptin level in these rat groups which received auspicious supplementation. Considering these results obtained after dietary changes, our experiment confirmed the known fact that fat tissue is not inert, but a true

Table 1. Placental homogenates MDA, total thiols and total GSH levels correlated with pups birthweight

Group	MDA value (nmol/g placental tissue)	Total GSH value (μ mol/g placental tissue)	Total thiols value (μ mol/g placental tissue)	Offspring birthweight (g)
Group 1 – Omega 3	15.88 $\pm$ 15.73 ($p < 0.05$)	5.84 $\pm$ 0.93 ($p < 0.05$)	8.64 $\pm$ 1.16 ($p < 0.05$)	5
Group 2 – Omega 6	47.57 $\pm$ 16.08	3.33 $\pm$ 0.83	5.7 $\pm$ 0.93	2
Group 3 – Sea buckthorn	7.6 $\pm$ 15.4 ($p < 0.05$)	4.35 $\pm$ 0.72 ($p < 0.05$)	7.8 $\pm$ 0.15 ($p < 0.05$)	5
Group 4 – No supplements	45.2 $\pm$ 9.62	3.65 $\pm$ 0.61	6.94 $\pm$ 0.06	4
Group 5 – Standard diet	28.2 $\pm$ 0.48	5.41 $\pm$ 0.63	7.05 $\pm$ 0.01	5

Table 2. Liver homogenates MDA, total thiols and total GSH levels

Group	MDA (nmol/g liver tissue)	Total GSH (μmol/g liver tissue)	Total thiols (μmol/g liver tissue)
Group 1 - Omega 3	1.00±0.8 (p<0.05)	4.79±0.82 (p<0.05)	10.07±0.23 (p<0.05)
Group 2 - Omega 6	3.02±0.95	3.89±0.42	8.81±0.31
Group 3 - Sea buckthorn	1.74±0.47 (p<0.05)	4.95±0.2 (p<0.05)	10.21±0.57 (p<0.05)
Group 4 - No supplements	3.49±0.43	3.02±0.54	9.3±0.41
Group 5 - Standard diet	2.52±0.11	4.26±0.05	9.74±0.08

Table 3. Pancreatic homogenates MDA, total thiols and total GSH levels

Group	MDA (nmol/g pancreatic tissue)	Total GSH (μmol/g pancreatic tissue)	Total thiols (μmol/g pancreatic tissue)
Group 1 - Omega 3	2.4±1.78 (p<0.05)	1.76±0.35 (p<0.05)	3.91±0.48 (p<0.05)
Group 2 - Omega 6	6.68±1.24	0.55±0.5	1.64±0.47
Group 3 - Sea buckthorn	3.15±1.25 (p<0.05)	1.48±0.15 (p<0.05)	3.66±1.13 (p<0.05)
Group 4 - No supplements	7.07±1.51	1.3±0.03	1.61±0.95
Group 5 - Standard diet	5.31±0.27	1.2±0.04	1.66±0.45

Figure 1. Maternal serum adipokine levels. Group 1 - Omega 3; Group 2 - Omega 6; Group 3 - Sea buckthorn; Group 4 - No supplements; Group 5 - Standard diet.

endocrine organ that through the secretion of adipokines responds to energy and hormonal stimuli (13).

The lipid peroxidation measured in the organs examined (the placenta, liver, pancreas) generated consistent results, showing high levels of peroxidation

expressed through high values of MDA in the groups with Omega 6 supplements respectively no supplementation (>40nmol/g tissue), and low levels of antioxidants expressed through glutathione and thiols (see Tables 1, 2, 3). These results showed in Tables 1, 2 and 3 which were obtained from the initial five groups of rats have been similar for their female descendants which became pregnant and were followed through gestation as second generation of experiment rats, showing that these metabolic alterations not only pass on to the pups, but they also affect the next generation.

Concerning the routine biochemistry parameters, our results showed increased glucose, cholesterol, triglycerides, uric acid and liver enzymes level in obese rats with no dietary interventions, which were ameliorated by the administration of Omega 3 fatty acids and sea buckthorn fruits (see Table 4).

Fetal exposure to the chronic metainflammation status caused by maternal obesity is a mediator for

Table 4. Routine biochemistry results (maternal serum)

Group	Total Proteins (g/dL)	Glycemia (mg/dL)	Albumin (g/dL)	ALT (UI/mL)	AST (UI/mL)	Cholesterol (mg/dL)	GGT (mg/dL)	Tryglyceride (mg/dL)	HDL Chol (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)
Group 1 - Omega 3	5.79	84.71	3.32	61.11	30.81	46.9	0.74	168.59	29.2	1.57	38.31
Group 2 - Omega 6	4.74	80.42	2.84	62.97	37.08	68.11	2.32	267.23	21.3	2.37	43.24
Group 3 - Sea buckthorn	6.13	69.69	3.24	42.82	17.16	60.28	0.06	251.89	31.8	1.4	30.16
Group 4 - No supplements	5.54	106.89	3.11	77.68	81.77	69.83	3.2	480.48	21.1	4.34	50.12
Group 5 - Standard diet	5.43	87.02	3.21	61.58	32.22	66.13	2.14	263.8	24.7	1.82	43.92

programming insulin resistance and leads to long-term metabolic manifestations. Fetal programming is an inducible phenomenon that appears during critical periods of development that generates irreversible alterations through epigenetic changes with metabolic response even to the next generation (14, 15).

DISCUSSION

White adipose tissue used to be considered until recently an inert energy storage tissue. Recently however, it has been described its endocrine activity which contributes to metabolic homeostasis through multiple pathways. The components of adipose tissue include besides adipocytes also the stromal vascular fraction, which is mainly formed from preadipocytes, fibroblasts, endothelial cells and cells participating in immunologic response (16). In healthy individuals adipose tissue contains mainly preadipocytes and adipocytes and very few inflammatory leukocytes. In obesity, on the other hand, the composition, phenotype, and function of adipose tissue are shifted. The main physiological mechanism through which adipose tissue contributes to homeostasis disruption is through the secretion of a large variety of bioactive peptides, first described in 1980 (as adiponectin) and collectively referred to as adipokines (17). Their multiple actions include physiological roles like adipocyte differentiation, glucose and lipid metabolism control, satiety, immune response, cardiovascular and neuroendocrine regulation (18). Abnormal regulation of adipokine secretion causes multiple organs dysfunction and contributes to the development of obesity induced comorbidities such as peripheral insulin resistance and metabolic syndrome (19-22).

Persistent excess energy intake induces adipocyte hypertrophy in an attempt to meet the high storage needs, which generates complications like hypoxia as the blood supply becomes inadequate, causing adipocyte necrosis, chemokine secretion and compromised regulation of fatty acid flow (23). Hypertrophied adipocytes alter the balance of cytokines and adipokines to a low chronic proinflammatory state, which has been given the name of meta-inflammation, acting as a critical factor linking obesity to the pathogenesis of metabolic disease in both mother and offspring (24, 25). Adipose tissue of obese mothers is infiltrated with a wide range of immune cells including monocytes/macrophages, neutrophils, B lymphocytes, and T lymphocytes, which

secrete other inflammatory mediators contributing to dysfunctions like lipid peroxidation, insulin resistance and endothelial dysfunction in obesity (26).

Chronic fetal exposure to high glucose levels from maternal supply causes placental mitochondrial alteration of free fatty acids oxidation, with the accumulation of triglycerides into the placenta. A similar mechanism is present in other fetal organs, in different grades of intensity, like the liver, pancreas, kidney, heart or brain. Central obesity, defined as accumulation of fat into the maternal liver and placenta induces other metabolic dysfunctions including lipotoxicity, while fetal exposure to excess lipid levels causes overexpressed inflammatory response with high values of IL1, IL6, TNF α and CRP. That is why we can summarize that excessive fetal fuel sources exposure causes excessive growth, long term fat storage and high risk of pre and postnatal complications (27, 28).

Our study confirmed the high inflammatory and peroxidation state caused by altered adipocyte chemokine secretion present in case of maternal obesity, which is the source of organ and metabolic dysfunction. Although the experimental study did not include a large number of Wistar female rats, the results were conclusive, showing significant differences between the study groups. Obese pregnant female rats showed high levels of lipid peroxides both in serum and in tissue homogenates, positively correlated with high levels of leptin and low levels of adiponectin, dyslipidemia, moderate hepatic cytolysis, hypoproteinemia and abnormal glycemic control.

In conclusion, secretion of adipokines from the adipocytes and the recruited macrophages of obese mothers is positively correlated with placental and tissue lipid peroxidation level and routine biochemical parameters. We can suggest that fetal metabolic programming as inducible phenomenon is partly explained by the influence of abnormal maternal adipokine phenotype, causing a self-supporting pro-inflammatory cascade which promotes adipogenesis. This paper emphasizes the existence of a metabolic reprogramming phenomenon obtained by dietary interventions which diminish the negative consequences of maternal obesity.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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