The role of adipose tissue as endocrine organ in the metabolic programming of fetuses derived from obese mothers

Abstract
We aim to present the endocrine and paracrine role of the cytokines secreted by hypertrophied maternal fat cells on fetal and later on neonatal metabolic profile. The study used an animal model in order to analyze the influence of maternal adipokines secretion of fetal metabolism. We selected 30 female Wistar rats weighing between 200-250 g (normal weight 100-150g) to whom we induced obesity by high-fat, high-calorie food intake (80% of diet saturated fatty acids) and tracked the correlation of maternal adipokines secretion with placentual and fetal lipid peroxidation level. The low adiponectin and increased leptin values as adipokines secreted by adipocytes of obese mothers were correlated with the level of placental and fetal tissue lipid peroxidation (from the liver, pancreas, brain), measured by elevated malondialdehyde and total thiols and the decreased levels of glutathione. It has been known for decades that fat tissue is not inert, but a true endocrine organ, which responds by secreting adipokines to different energy and hormonal stimuli. Endocrine secretion of adipokines from the adipocytes of obese mothers is positively correlated with placentual and fetal lipid peroxidation levels. Fetalm metabolic programming as an inducible phenomenon is explained partly by the influence of excess secretion of maternal adipokines. Keywords: adipose tissue, adipokines, maternal obesity, fetal metabolic programming

Introduction
Obesity is a pathological entity not fully elucidated yet, although increasingly more intensively studied. It was declared a pandemic of the XXIth century, given the incidence and prevalence continues to raise, both in the developed and in the developing(1). Thus, multiple epidemiological studies reported an exponential increase in the incidence of maternal obesity at the onset of pregnancy in the last 15 years, while the prevalence of maternal obesity is described with rates from 9-10% to 16-19% in different studies(2,3). The prevalence of maternal obesity in the first quarter of pregnancy has doubled between 1989 and 2007(4). Globally, one in three women is obese, more than half of pregnant women are overweight or obese, and 6-9% of these present morbid obesity, falling into the category of maximum obstetrical risk(5).

Institute of Medicine proposed in 2009 actually waiving the classification of obesity, considering the risks induced by it significantly equal for any value of body mass index (BMI) higher than 30 kg/m2 and recommend an optimal weight gain during pregnancy for obese women between 5 and 9.1 kg for single pregnancies, respectively between 11 and 19 kg for multiple pregnancies(6). Early onset of obesity in pregnancy or previous existence has consequences with a major impact on the fetus: increased incidence of cardiovascular and neural tube malformations, increased incidence of omphalocele and not least the long-term fetal metabolic alterations(7).

White adipose tissue is no longer considered for decades an inert organ with an exclusive energy reserve role. This myth was abandoned in 1987, with the discovery that adipose tissue is a major location for the metabolism of sex steroid hormones(8) and the identification of the first molecules produced by adipocytes - adipin, an endocrine factor down-regulated in obese rodents(9). Confirmation that adipose tissue is an essentially active organ with intense metabolic activity and endocrine, inflammatory and immunologic function appeared in 1994, when leptin was the first identified adipokine secreted by it. Nowadays several hundred of adipocytokines are known to be bioactive peptides secreted by adipose tissue, which gives its character of autocrine and paracrine organ by their local actions and endocrine by their distance actions(10). These were classified according to their biological role into 4 categories: factors with direct metabolic influence (leptin, adiponectin, retinol-binding protein, adipin, resistin), proinflammatory factors and acute phase reagents (tumor necrosis factor (TNF) alpha, interleukin (IL)-1β, IL4, IL 6, IL 8, IL 10, IL 18), extracellular matrix components (α2-macroglobulin, collagen I, III, IV, VI, fibronectin, matrix-metallo-proteinase 1, 7, 9, 10, 11, 14, 15) and pro-mithogenic and pro-angiogenic (transforming growth factor-β, insulin-growth factor-1
In addition to adipocytes, it includes mixed connective tissue, nerve, vascular stromal and immune cells (macrophages, T cells), which operates as a whole, with involvement in the regulation of physiological and pathological processes. Macrophages and lymphocytes also secrete inflammatory or anti-inflammatory peptides according to the signals received, which helps create an environment characterized by chronic inflammation at a low level defined as specific obesity meta-inflammation\(^{(12)}\). The neuroendocrine function described by constant communication with central nervous system, the inflammatory and immune function by secreting molecules involved in immune response and the regulation of energy metabolism by modulating insulin resistance through pro-inflammatory molecules generated chronically, gives fatty tissue unique characters with capital role in the maintenance of homeostasis\(^{(13)}\). The importance of the endocrine function of adipose tissue is underlined by the adverse metabolic consequences of both its excess and deficit\(^{(14)}\).

The study aims to present the endocrine and paracrine role of cytokines secreted by hypertrophied maternal fat cells on fetal and later on neonatal metabolic profile. Confirmation of fetal metabolic programming process by inducing an adverse metabolism as a consequence of endocrine molecules secreted in excess by hypertrophied adipocytes of obese mothers is studied based on an animal model in which obesity was induced by diet. It is also studied the possibility of a metabolic reprogramming phenomenon by subjecting the laboratory animals to nutritional interventional therapies.

**Methods**

The effects of maternal obesity have been studied in an animal model using 50 female Wistar rats weighing 200g to 250g (normal weight 100-150g), to whom we induced obesity by high-fat, high-calorie food intake administered by gavage (80% of dietary fat accounting or saturated fatty acids) and tracked the correlation of the secretion of maternal adipokines with the placental and fetal lipid peroxidation. Some females were bred with high calorie diet and subsequently get pregnant, while in the second group obesity was induced during pregnancy. We checked for the existence of a possible metabolic reprogramming process by subjecting female obese interventional therapies, such as dietary changes or supplements anti-inflammatory.

They were divided into 5 groups, after becoming pregnant by type of nutritional interventions that have undergone: Group 1 received high-calorie/high-fat diet during pregnancy and supplementation of polyunsaturated omega 3 fatty acids docosahexaenoic acid and eicosapentaenoic acid 1 mL/kg, Group 2 received high-calorie/high-fat diet during pregnancy and supplementation of polyunsaturated Omega 6 fatty acids 1 ml/kg, Group 3 received high-calorie/high-fat diet during pregnancy and supplements of sea buckthorn fruits 10g/female, Group 4 received high-calorie/high-fat diet without supplements during pregnancy and Group 5 received standard diet (normal calorie, normal fat) during gestation.

Females were sacrificed at gestation term and analyzed: the secretion of maternal adipokines from venous blood (leptin, adiponectin), the placental lipid peroxidation (estimated by malonyl-dialdehyde (MDA) values, thiols total - proteins with cysteine, total and oxidized glutathione (GSH) as an antioxidant factor) and the markers of lipid peroxidation aforementioned from the pancreatic, liver and fetal brain and placenta tissue homogenates.

In order to predict a negative outcome for descendants we have established associations between maternal diet and fetal metabolic status using the mentioned biomarkers. Adipokines secretion was correlated with the maternal placental fetal and tissue lipid peroxidation.

**Results**

Using an animal model we showed that maternal obesity induces a fetal meta-inflammation process, which has the result of speeding adipogenesis in descendants. This process is due to the secretion of adipocytokines from adipose tissue with pro-inflammatory, chemotactic and proatherogenic role. Adipokines secreted in excess create a favorable environment for joining inflammatory cells and support the creation of a vicious circle which is self-sustaining the process.

We dosed in the blood of pregnant females the most important two adipokines with complementary roles: leptin and adiponectin. Their values were then correlated with markers of lipid peroxidation from placental and fetal tissue homogenates (liver, pancreas and brain) and values of usual biohumoral markers in maternal serum (urea, creatinine,
glucose, lipid profile, alanine transaminase, aspartate transaminase, gamma-glutamyl transpeptidase, albumin, total proteins). According to data published in the literature, maternal leptin value was significantly increased in the high fat diet group without supplements and decreased in the groups that were given Omega 3 fatty acids and sea buckthorn fruits. Surprisingly, the administration of omega 6 fatty acids did not determine the decrease of leptin levels, which remained similar to the group without supplements. The group with standard diet during the gestation showed intermediate leptin levels between the extremes mentioned above. Regarding adiponectin, its values were complementary to those of leptin levels (increased in females with omega 3 and sea buckthorn fruits, low in those without dietary supplements and intermediate the group with normolipidic normocaloric diet), finding the same favorable effect of supplementation with polyunsaturated omega 3 acids and sea buckthorn berries (Figure 1 and 2).

Studying the lipid peroxidation by the values of MDA and total GSH (reduced and oxidized) on placental tissue homogenates, we observed increased levels induced by maternal obesity in the group without nutritional interventions, while supplementation with omega 3 acids and sea buckthorn fruit significantly reduced the peroxidation rate (p < 0.05) (Figures 3 and 4). The values of total placental thiols, which consist largely of proteins containing cysteine residues, were higher in females given omega 3 compared to other groups, including the standard diet group. Regarding the weight of offspring at birth (group 1: 5g, group 2: 2g, group 3: 5g, group 4: 4g, group 5: 5g) we can state that the high rate of lipid peroxidation placental causes a low weight at birth of fetuses. The lowest birth weight and also the highest peroxidation level were recorded in females with high-calorie high-fat diet during pregnancy associated with omega-6 fatty acids supplementation (Figures 5 and 6).

Similar results were obtained by studying the fetal tissue peroxidation on liver, pancreas and brain homogenates, thus confirming the direct effect of maternal obesity on the metabolism of the fetus. This confirmed the presence of lipid peroxidation higher for obese females and fetuses derived from them, which was improved by adopting anti-inflammatory therapeutic interventions such as food supplements (omega 3 fatty acids and fruits of sea buckthorn) or imposing a normocaloric normolipidic diet. Biohumoral usual samples from maternal blood showed no statistically significant differences between groups.

Discussion

It is known for decades that fat tissue is not inert, but a true endocrine organ, which responds by secreting adipokines to different energy and hormonal stimuli[13]. The low adiponectin and increased leptin levels as adipokines secreted by adipocytes of obese mothers were correlated with the level of placental and fetal lipid peroxidation (from the
liver, pancreas and brain) measured by elevated MDA and total thios and low levels of GSH.

Hypertrophied adipocytes of obese recruit macrophages in excess into the adipose tissue, which in turn, secrete cytokines(19). The link between obesity and inflammation appears to be represented precisely by the secretion of proinflammatory cytokines that are self-supporting. Proinflammatory adipokines and chemokines secretion of fat cells leads to the occurrence of a chronic subinflammatory state, which was defined in the literature as metainflammation(17). Obesity specific metainflammation is the main metabolic risk factor through the endothelial dysfunction caused by: oxidative stress generated by proinflammatory adipokines secreted by adipocytes, macrophages, lymphocytes and other cells present in hypertrophied adipose tissue (TNF and IL-6) and lipotoxicity mediated by the increased levels of free fatty acids, as a consequence of the alteration of carbohydrates and lipid metabolism(18).

Fetal exposure to chronic metainflammation caused by maternal obesity is a programming mediator of insulin resistance leading to long-term metabolic manifestations(19). Metabolic programming is an inducible phenomenon during critical periods of development that induce irreversible alterations through epigenetic changes with metabolic impact even to the next generation(20).

Leptin is secreted mainly by subcutaneous white adipose tissue, in higher proportion compared to the visceral one. It is positively correlated with BMI's increase. It decreases in periods of fasting and weight loss(21). Leptin stimulates the expression of other adipokines such as TNF, IL-6 and adiponectin and suppresses the expression of adipokines like resistin. It is the protein that signals satiety, having a major role in regulating food intake(22). Leptin promotes excess into the adipose tissue, which in turn, secret cytokines, modulating food intake and energy expenditure cell and having antiatherogenic effects(25).

2-3 times higher in women compared to men, being low in pathological conditions like obesity, peripheral insulin resistance and diabetes(24). Adiponectin suppresses the expression of other adipokines, such as TNF and IL-6. Stimulate insulin secretion, modulating food intake and energy expenditure cell and having antiatherogenic effects(25).

Until now there have been identified several hundred of molecules secreted by adipose tissue, which share both local (paracrine and autocrine) and systemic (endocrine) roles. But their study is just beginning; even intensely studied hormones secreted by adipose tissue such as the leptin require further study in order to fully elucidate their biological role. Besides the known genes in adipose tissue, 40% are new genes, while 20-30% are likely to synthesize proteins with roles to be discovered(10). Understanding the mechanisms of endocrine function of adipose tissue in regulating energy homeostasis will allow future development of targeted therapies for treating the negative consequences of both excess and deficiency, as our study tried to demonstrate.

Conclusions

Adipose tissue has been considered in the last two decades a true endocrine organ because of its response to various stimuli by secreting molecules with biological roles in regulating energy homeostasis and actively participating through its endocrine, paracrine and autocrine role. In pathological conditions like obesity, its biological role becomes crucial, as it promotes inflammation, atherogenicity and self-supports a pathogenic vicious circle.

Endocrine secretion of adipocyes from the adipocytes of obese mothers is positively correlated with placental and fetal lipid peroxidation level. Fetal metabolic programming as inducible phenomenon is explained partly by the influence of excess maternal adipokines secretion, causing a proinflammatory cascade which self-promotes adipogenesis. The present research suggests the possibility of a metabolic reprogramming process through prompt therapeutic intervention in order to redirect the metabolic phenotype of fetal alterations. ■

References