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IMPACT OF PRENATAL EXPOSURE TO OBESOGENIC ENVIRONMENT ON LATER-LIFE BROWN AND WHITE ADIPOCYTES AND LIVER OUTCOMES

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Aims: *To analyze the link between prenatal obesogenic environment on brown and white adipose tissue (BAT and WAT) functions and its impact on liver activity in postnatal period. To identify biomarkers for predicting the risk of non-alcoholic fatty liver disease (NAFLD) in adulthood.*

Materials and methods: *The study evaluated female rats (n=20) and their male offspring (n=92). Pregnant rats were exposed to social stress (Pratt, 1989) during the first and third trimester and standard diet (StD, control), nutritional imbalance: high-sugar (Kozar, 2009), high-fat diets (Lintermans, 2011) or their combination – high-sugar and high-fat diet (HSFD). The effects of maternal stress and nutrition on the BAT, WAT and liver were evaluated in 3-month offspring during stress-exposure (Takagi, 1964) without and with abnormal cellular defence (treatment of indomethacin), along with the ELISA levels of IL-1β, IL-8, leptin (OB), adiponectin (AdipoQ) and its ratio (OB/AdipoQ index).*

Results: *Maternal exposure to stress in combination with the HSFD resulted in the most prominent histological changes in offspring's BAT and WAT inflammation and hepatocellular damage. Such effects were accompanied by most significant changes: increased IL (interleukin)-1β (178%, $p \leq 0.001$), IL-8 (57%, $p \leq 0.001$), leptin (OB) (34%, $p \leq 0.001$) and decrease of adiponectin (Adipoq) (53%, $p \leq 0.001$) vs control. In cases of combination of stress and indomethacin injury we noticed an increase of IL-1β (213%, $p \leq 0.001$), IL-8 (27%), OB (30%, $p \leq 0.001$) as well as decrease of AdipoQ (64%, $p \leq 0.001$) vs control.*

Conclusion: *The impairment in the BAT, WAT and liver function in the offspring was related to the sugar overload and stress during the prenatal period. Ectopic fat accumulation, hyperplastic and hypertrophic WAT changes, intracellular lipid and proinflammatory secretion, and the OB/AdipoQ index may be a predictive biomarkers of NAFLD.*

Key words: *liver, adipose tissue, inflammation, diet, stress, leptin, adiponectin, interleukin-1β, interleukin-8*

INTRODUCTION

Recent epidemiological studies have shown that the distribution of diseases related to lifestyle is often associated with a wide variety of chronic diseases leading to morbidity, disability and premature death [1, 2]. Moreover, the data suggest that lifestyle affects energy regulation not only intra- but also inter-generationally, and is thus associated with functional and organic disorders and long-term health problems in offspring [3]. One of these putative life-style generated conditions with inter-generational memory is obesity, a global disease rapidly increasing among world population over the past 30 years and is linked with several visceral disorders, including liver and gastro-intestinal co-morbidities [4, 5]. One of these diseases is non-alcoholic fat liver disease (NAFLD), which may include variable degrees of simple steatosis, non-alcoholic steatohepatitis, and cirrhosis, showing a worldwide prevalence in general population about 20-30% in Western Countries and 5-18% in Asia and 3-fold increasing in the USA over the last 30 years [6, 7]. Moreover, NAFLD, which is pathophysiologically related to hepatocarcinoma, has recently been recognized as one of the most destructive human cancer types with the fourth leading cause of cancer-related deaths [8, 9].

Obesity is the consequence of failure of the physiological activity of brown and white fat tissues (BAT and WAT, respectively). Obesity influences the function of all visceral organs as well as skeletal muscles. It impacts adipokine secretion and its associated signalling pathways. Other affected pathways may include adenosine monophosphate-dependent kinase, peroxisome proliferator-activated receptor alpha, Janus kinase, signal transducer and transcription activator [10, 11].

The proportion (in both quantity and quality) as well as specific activities of brown adipose tissue (BAT) and white adipose tissue (WAT) and their distribution (typical or ectopic) is important for ontogenesis [12, 13]. Recent research reflects that healthy adult human beings possess significant stores of metabolically-active BAT [14]. Having a significant capacity to control chemical energy, triglyceride and glucose metabolism, BAT could be a key factor in obesity [15]. In addition, BAT is a strong activator in a number of autoprotective mechanisms. This includes the proliferation and advancement of cell differentiation as well as apoptosis. BAT is also involved in lipolysis, the production of growth factors (NGF, VEGF), angiotensinogen, NO, IL-1 and IL-6, OB, AdipoQ, resistin. Its role with triiodothyronine type II and anti-obesity factor still remains hypothetical [16, 17]. It has been postulated that BAT may play the disproportional deposition of visceral fat, especially ectopic fat deposits in atypical locations and induce metabolic changes via the abnormal expression of adipokines, a large family of cytokines which participate in many systemic reactions. Among adipokines, changes of adipose tissue-derived hormone

OB and AdipoQ play prominent role in fat proliferation, insulin resistance, dyslipidemia and systemic immune response in human beings [18].

The pathogenesis of obesity is complex. More recent inquiry has been directed toward the environmental causes ("obesogenic environment") and gene-environment impacts on early fetal development [19]. According to "Barker's hypothesis" or "fetal programming hypothesis", the importance of maternal lifestyle and conditions for the early fetal development is undoubted, when changes in organism are caused by different environmental stimuli, including nutrition [20, 21, 22]. In addition, the predictive adaptive responses (PARs) hypothesis proposes that the degree of mismatch between the pre- and postnatal environments is a major determinant of subsequent disease risk [23]. Thus, the elucidation of new triggers and biomarkers of early stages of metabolic alterations induced by adipocytes is urgently needed.

The verification of the obesogenic environment effect via prenatal programming of adult physiological functions and diseases by maternal nutritional status has been simulated using a broad range of animal models [24, 25, 26]. However, these models have not yet identified: 1) the relationship between exposure to risk factors (e.g. stress, high caloric diet during early life-sensitive periods) for the development and functioning of BAT, WAT and liver in adulthood, 2) their subsequent postnatal-related outcomes which produced PARs, and 3) non-invasive biomarkers which can be used in assigning risk or identifying signs of early ectopic fat and intrahepatic fat accumulation. The approaches to evaluate the role of obesogenic environment *in utero* in humans are limited. We approached this problem by evaluating the impact of maternal stress and imbalanced nutrition via overload of sugar on changes in adipocyte physiological activity, hepatocellular organization in offspring, and their resistance to injury, related to stress and abnormal synthesis cytoprotective prostaglandins. We aim to identify a new non-invasive alternative biomarker to assess early liver changes which can be used for prognosis of ectopic and intrahepatic fat accumulation and compare this method to the invasive "gold standard" assessment of liver-biopsy.

Thus, this study has two **aims**. The first is to investigate the link between the combined effects of maternal stress and unbalanced hypercaloric feeding on BAT, WAT activity and hepatocellular organization and expression of pro-inflammatory activity of IL-1β and IL-8 and OB and anti-inflammatory AdipoQ in adult offspring during physiological conditions and extreme factors. Furthermore, this inquiry could provide new insights into PARs as well. The second aim is to introduce a novel diagnostic biomarker to assess the interplay between BAT, WAT and liver function, which can possibly have prognostic value for fat tissue alterations and NAFLD.

MATERIALS AND METHODS

Animals. All experiments were carried out on rats (N=112, body weight 211±24g; n=5-7) in accordance with the norms of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and in accordance with the Committee on Bioethics of Danylo Halytsky Lviv National Medical University (LNMU) agreement № 4, 11.04.2011. Animals were maintained under a constant 12 h light and dark cycle and an ambient temperature of 21–23°C with 50±10% relative humidity. All animals were kept in raised mesh-bottom cages to prevent coprophagy. Animal from the control group of dams and male rats were allowed free access to tap water.

Experimental model on prenatal obesogenic environment. Gestational rats were divided into 4 experimental groups. The control group was kept on a standard diet and with free tap water access; other three experimental groups were exposed to the social stress by Pratt [27]. This model of maternal stress was produced by daily regrouping of female rats in a cage. Each female rat was stressed for 6 hours per day in the first and third trimesters of their pregnancy. For the night period pregnant females were placed into individual cages.

For modification of the maternal diet we used different types of nutritional insults. Group 1 was kept on a high sugar diet (HSD) with unrestricted access to 30% solution of sucrose *ad libitum* within drinking-water [28]; group 2 was kept on a high fat diet (HFD) with 45% calories from fat [29]; group 3 was kept on a combined high sugar and fat diet (HSFD). The experimental diets were started after confirmation of pregnancy in female rats and stopped after offspring delivery.

Experimental model on acute stress. The second series of experiments studied effects of the extreme factors on BAT, WAT and liver functions. Concurrently, the experiment studied the impact of postnatal stress on their prenatally programmed activities which were used on 3-month aged 92 male offspring with different prenatal dietary history. Their dams were treated by the standard diet (control group) and HSD, HFD, HSFD diets. All offspring were fed with StD. For this study, cytoprotective responses in all offspring were induced by the restraint water-immersion stress (WIS) by Takagi et al., 1964 [30]. The animals were placed in restraint cages for 2 hours and immersed vertically to the level of the xiphoid process in a water bath of 23°C for 1,5 hours. To produce the cellular response we used blocking of prostaglandins (PGs)/cyclooxygenase (COX) activities by intraperitoneal (i.p.) administration of a non-selective inhibitor of cyclooxygenase, with a single 10 mg/kg dose of indomethacin (“Health”, Ukraine).

Weight measurement. The total body weight (TBW) in 3-month-old male rats was measured (PH 10C13U, 100g-10kg, ±5g, Ukraine). The rats consequently underwent

euthanasia under deep by ketamine anaesthesia (60 mkg/kg^{-1}) via intramuscular administration and sample of liver, BAT from interscapular region and visceral WAT were removed, washed with ice-cold saline, quickly blotted and weighed (Electronic balance, LT 1000B, 1000 g/0,1 g, China). The mass of each wet brown fat, liver and mesenteric WAT sample were measured.

Histopathological analysis. The samples of BAT, liver and WAT (mesenteric fat) were excised and rinsed in ice-cold saline. All excised organs were fixed with 10% neutral-buffered formalin and embedded in paraffin for histological analysis. The formalin-fixed, paraffin-embedded tissues were processed and 5-μm-thick serial sections 3 per animal per tissue were cut and stained with hematoxylin and eosin (HE). Histopathological imaging was performed using microscope Leica DM 750/4 and digital camera Leica DFC 420 (Germany). Video recording was performed using Leica Application Suit Version 3.8 (Germany), under x400 magnification. BAT histology was scored using semi-quantitative scoring system based on the recommendation proposed by Giordano [31]. Liver lesions were graded using original semi-quantitative scoring system based on the human histologic classification of the components of liver histopathology [32, 33]. Mesenteric fat tissue histology was described on the basis of the recommendation proposed by Nov [34]. BAT, liver and white mesenteric fat sections were histologically evaluated in double-blind approach by two individuals and their histological scores (HS) were averaged.

Visual analogue scales of histopathology of brown fat tissue. HS of BAT lesions are based on the sum of scores of the visual analogue scales grading by changes in each brown fat section in every group. Brown fat injury was examined according to the visual analogue scales grading: 0 – no changes; 1 – BAT with single unilocular adipocytes; 2 – BAT with plural unilocular adipocytes; 3 – BAT with unilocular adipocytes and leukocyte infiltration.

Visual analogue scales of the histopathology of mesenteric fat. White mesenteric fat tissue changes including inflammation was scored based on the following visual analogue scales grading: 0 – normal histological structure; 1 – mild polymorphism of adipocytes; 2 – severe polymorphism of adipocytes and focal mononuclear infiltration with the admixtures of leucocytes; 3 – severe polymorphism of adipocytes and multifocal mononuclear infiltration with the admixtures of leucocytes.

Morphometry of WAT adipocytes. Morphometric analyses of the adipocyte diameter (sectional area) were carried out by using the microscope Leica DM 750/4 with the digital camera Leica DFC 420 (Germany) and licensed program Image Pro Plus (Version 6, USA).

Visual analogue scales of the histopathology of liver tissue. HS of the liver tissue changes are based on the sum of the visual analogue scales grading by parenchymal and

vascular-stromal changes in each liver section in each group. Liver cell injury was examined according to the VAS grading: 0 – no changes; 1 – tissue structure is preserved; focal microvacuolar changes and moderate glycogen accumulation in the hepatocytes; 2 – cellular cords are normal, multiple foci of microvacuolar changes, single hepatocytes with the large intracytoplasmic vacuoles, irregular accumulation of glycogen in the nuclei and cytoplasm; 3 – severe discomplexation of hepatic cords due to the widespread micro/macrovacuolar changes of hepatocytes; single cells with foamy cytoplasm or signs of necrosis.

Liver vascular-stromal changes analyzed by visual analogue scales grading: 0 – no changes; 1 – dilatation and hyperemia of the central vein and centrolobular sinusoids; 2 – severe edema and mild infiltration of the portal tracts, periportal areas, hyperemia and dilatation of the sinusoids associated with the cord discomplexation; 3 – edema and severe mononuclear infiltration of the portal/periportal tracts.

Serum adipokines and interleukins levels. Immediately after the termination of the experiment, venous blood samples were drawn from the abdominal vein of the animals anaesthetized with the intramuscular administration of ketamine (60 mg kg^{-1}). Then, those samples were placed into the EDTA-containing vials and used for the measurement of levels of AdipoQ, OB, interleukin-1β (IL-1β) and growth-regulated oncogene/cytokine-induced neutrophil chemoattractant-1 (GRO/CINC-1), which is a rat chemokine with the structural and functional homology to human IL-8. OB and AdipoQ levels in the rat serum were assessed in duplicate using next ELISA kits Leptin (rat) («Enzo Life Sciences», United Kingdom), Adiponectin (rat) («Adipogen», Korea), respectively, interleukin-1β and interleukin-8: GRO/CINC-1 (rat) by ELISA kit («Enzo Life Sciences», United Kingdom) and IL-1b (rat), ELISA kit («Enzo Life Sciences», United Kingdom). The Leptin/Adiponectin (OB/AdipoQ) index was calculated as its ratio.

Statistical analyses. The data are presented as a mean ± standard deviations (SD) for 7-8 animals in each group and analyzed using the STATISTICA 8.0 software package (StatSoft Inc., USA). Differences among groups were assessed by the one-way analysis of variance (ANOVA), followed by a post hoc analysis using Newman-Keuls test. The level of statistical significance was $P < 0.05$.

RESULTS

Anthropometric measurements. The data of the mean body weight, mean brown fat, visceral fat pad and liver weight of the offspring from the rats fed by the standard (control group) or high-caloric diets HSD, HFD, HSFD and exposed to stress are represented in Fig. 1.

The mean body weight (Fig.1 A) increased in the group 1 by 4% ($p \leq 0.001$), in the groups 2 and 3 – by 7 % and 16%, respectively, vs control ($p \leq 0.001$). The BAT weight was significantly went up in the rats from the HSD group – by 54% and in the animals with

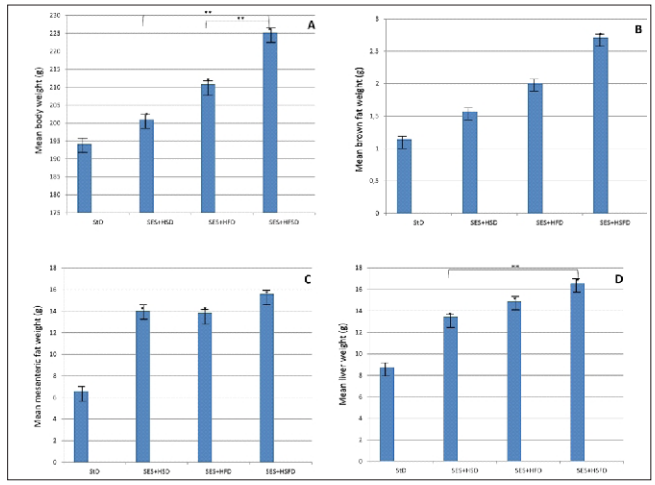

Fig. 1. Mean body weight (A), brown (B) and mesenteric (C) fat and liver (D) weights (g) of rats from experimental groups. Results are presented as means ± standard deviation of 7-8 rats per each group. Asterisks indicate a statistically significant difference * $p \leq 0.001$ vs control; ** $p \leq 0.001$ between groups. Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; SES – social emotional stress

HFD – by 71% vs control (Fig. 1 B). The greatest BAT weight gain was observed in the rats from the group with the combination of stress and nutritional insults; it appeared to be almost twice higher than the control ($p \leq 0.001$). The pairwise comparison of the groups showed that the mean mesenteric fat (Fig. 1 C) and liver (Fig. 1 D) weight in the rats with HFD increased more significantly comparatively not only to the control group, but also comparing to the rats with HSD. Data from the HSFD groups was accompanied by further elevation of all parameters.

Histopathology of the brown adipose tissue. The offspring of rats treated with the standard diet and free access to tap water (control group) exhibited normal BAT appearance (Fig. 2a). Their histology included multilocular or small lipid droplets in adipocytes, which are highly vascularized and innervated, and single leukocytes. BAT histopathology analysis in rats whose mothers were kept on a HSD (Fig. 2b) was characterized by a moderate fat accumulation: the plural unilocular adipocytes with the large (95% of the entire cellular volume) lipid-storage vacuoles in the cytoplasm, among the multilocular cells of BAT. The WIS-related changes of BAT in the offspring with HSD were noted with the significant mild perivascular leukocyte infiltration and plural unilocular changes (Fig. 2f). HFD have developed mild histological changes in the BAT of offspring, which was characterized by the consistent appearance of the single unilocular adipocytes (Fig. 2c) while stress-induced injury of BAT without and with indomethacin treatment was associated with the plural large adipocytes with lipid storage vacuoles

(Fig. 2g). The most severe changes in BAT were recognized in the offspring of rats kept on a HSFD. BAT histopathology analyses in this group showed islands of the unilocular adipocytes in addition to the perivascular moderate infiltration in BAT and ectopic WAT

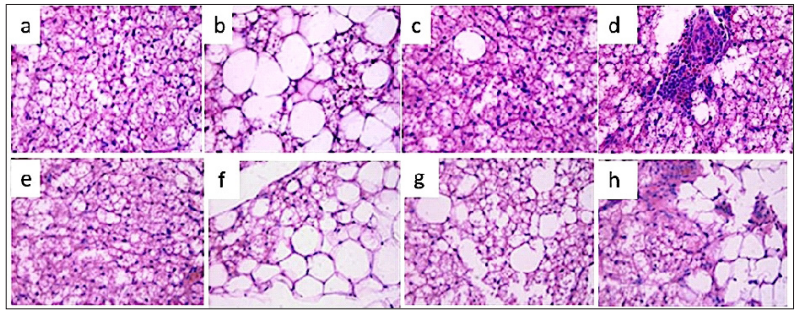

Fig. 2. Light microscopic histological appearance of brown adipose tissue obtained during experimental procedures from rats: a - control; b – SES+HSD; c – 3A SES+HFD; d – SES+HSFD; e – StD+WIS+I; f – SES+HSD+WIS+I; g – SES+HFD+WIS+I; h – SES+HSFD+WIS+I. H/E stain. Magnification ×400. Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; I – indomethacin treatment; SES – social emotional stress; StD – standard diet; WIS – restraint water-immersion stress

adipocytes (Fig. 2d). After the induction of stress and indomethacin treatment, BAT in rats from the HSD group was characterized by the intensive accumulation of WAT (Fig. 2h).

Histopathology of the mesenteric white adipose tissue. The histopathological appearance of the mesenteric WAT during the experimental procedures is shown in the Fig. 3.

The morphometric analysis of the mean diameter of adipocytes (nm) in the WAT was

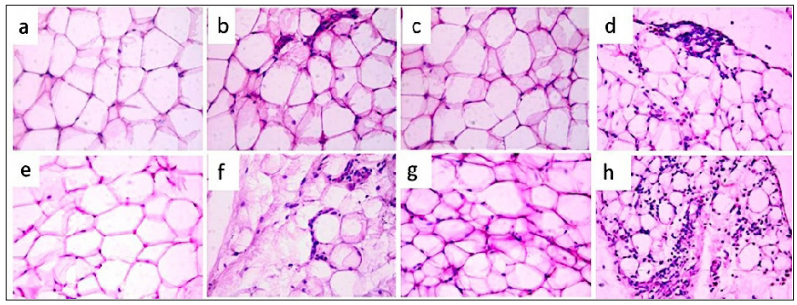

Fig. 3. Light microscopic histological appearance of mesenteric white adipose tissue obtained during experimental procedures from rats: a - control subgroup, b – SES+HSD, c – SES+HFD, d – SES+HSFD, e – StD+WIS+I, f – SES+HSD+WIS+I, g – SES+HFD+WIS+I, h – SES+HSFD+WIS+I. H/E stain. Magnification ×400 (a-c, e-g); ×200 (d, h). Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; I – indomethacin treatment; SES – social emotional stress; StD – standart diet; WIS – restraint water-immersion stress.

formulated to determine the character of histopathological changes. The results from all experimental groups are represented in the Fig. 4.

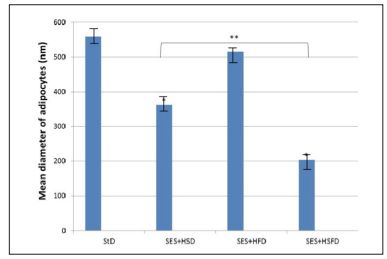

Fig. 4. Morphometry analysis of the mean diameter of adipocytes (nm) in the mesenteric white fat tissue in rats from the control group and groups with the fetal stress and nutritional insults. Results are presented as a mean±standard deviation of 7-8 rats per each group. Asterisks indicate a statistically significant difference * $p < 0.001$ vs control; ** $p < 0.001$ between groups. Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; SES – social emotional stress; StD – standard diet

The offspring of female rats treated with the standard diet and free access to tap water (control group) with or without the WIS induction exhibited normal mesenteric fat appearance (Fig. 3a). In the combined WIS and indomethacin injury, the signs of hemodynamic injury of insignificant degree were visualized in 25% of experimental animals (Fig. 3e). In the WAT obtained from the rats with HSD, there was noted a moderate polymorphism of the adipocytes with the islands of hyperplastic proliferation of the small adipocytes with the reticulated cytoplasm among the hypertrophic ones and single focuses of inflammatory infiltration (Fig. 3b and 4). The Fig. 3f represents the changes of adipocytes with the minor accumulation of leukocytes and hypertrophic-hyperplastic proliferation, which mimic lesions in the human visceral adipose tissue, which could trigger cellular mechanisms assisting the hepatic fibro-inflammatory lesions in obese patients [34, 35]. The structure of the mesenteric WAT of the rats with HFD in general was normal with solitary polymorphic hypertrophic adipocytes (Fig. 3c and 4). The stress-associated injury in this group was reflected by a greater population of polymorphic cells, which is shown in the Fig. 3g. The histological changes of the mesenteric WAT in the group with the mixed overnutrition (HSFD) were the most severe and characterized by the multiple hyperplastic proliferative changes and intensive leukocyte infiltration (Fig. 3d and 3h).

These findings together with liver histopathology histologically correspond to the extent of ectopic lipid accumulation in a human [35].

Histopathology of the liver tissue. The offspring of dams treated with the standard diet and free access to tap water (control) exhibited normal liver appearance and normal histological structure (Fig. 5a; Fig. 6a). Acute stress and cytotoxic influence of

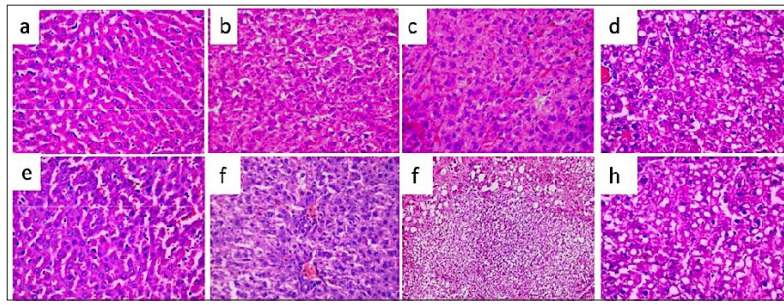

Fig. 5. Light microscopic histological appearance of liver parenchyma obtained from obtained during experimental procedures from rats: a - control, b – SES+HSD, c – 3A SES+HFD, d – SES+HSFD, e – StD+WIS+I, f – SES+HSD+WIS+I, g –SES+HFD+WIS+I, h – SES+HSFD+WIS+I. H/E stain. Magnification ×200. Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; I – indomethacin treatment; SES – social emotional stress; StD – standard diet; WIS – restraint water-immersion stress

indomethacin were represented by minor dilatation of sinusoids with hepatocytes with the single microvacuolar change, irregular cellular accumulations and hyperemia of periportal sinusoids, which were visualized in 75% of animals. The histopathological analysis of liver damage in the rats that were kept on a HSD during the fetal period revealed moderate changes (Fig. 5b; Fig. 6b). The lobular structure was disrupted due to the irregular cellular accumulations and abnormalities in the tissue; hepatocytes were swollen with the focal microvacuolar changes and irregular glycogen accumulations in the nuclei and cytoplasm. Discomplexation of hepatic cords developed due to the accumulation of extravascular fluid and focal diapedesis of red blood cells in the Disse space. These data were similar to the histological findings of steatohepatitis in human beings. The WIS-related injury in these rats was characterized by the severe dilatation and hyperemia of periportal sinusoids. We

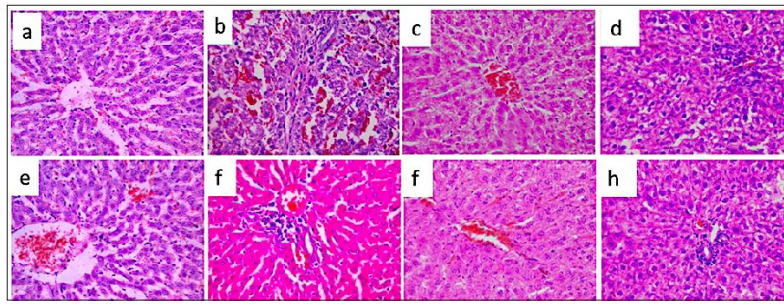

Fig. 6. Light microscopic histological appearance of liver microvasculature obtained during experimental procedures from rats: a - control subgroup, b – SES+HSD, c – SES+HFD, d – SES+HSFD, e – StD+WIS+I, f – SES+HSD+WIS+I, g – SES+HFD+WIS+I, h – SES+HSFD+WIS+I. H/E stain. Magnification ×200. Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; I – indomethacin treatment; SES – social emotional stress; StD – standard diet; WIS – restraint water-immersion stress

have also revealed necrosis of the single hepatocytes in the rats with the WIS-associated injury and indomethacin treatment; moreover, portal tracts were noticed to be swollen with leukocyte infiltration (Fig. 5f; Fig. 6f).

Mild histological liver changes in the offspring of the rats fed with HFD from the intact subgroup were consistent with the preserved parenchymal cells with rare hepatocytes with the clearing of cytoplasm and insignificant polymorphism (Fig. 5c; Fig. 6c). After the WIS induction, these rats displayed parenchymal changes similar to the subgroup of intact rats with the additional manifestation of central vein hyperemia. In the rats with the WIS+I injury these was noted as single hepatocytes with the clearing of cytoplasm and insignificant polymorphism, hyperemia of central vein and insignificant dilatation of centrolobular sinusoids. The findings of low-level parenchymal involvement are similar to those observed in the human beings with the low-power parenchymal involvement and would be assigned a grading of possible/borderline steatohepatitis, in accordance with the NASH clinical research network scoring system [33].

The most severe liver changes were noted in the offspring of rats kept on a HSFD. Histopathological studies in this group showed preserved lobular structure, discomplexation of hepatic cords; hepatocytes were noticed to be with the signs of the moderate damage –

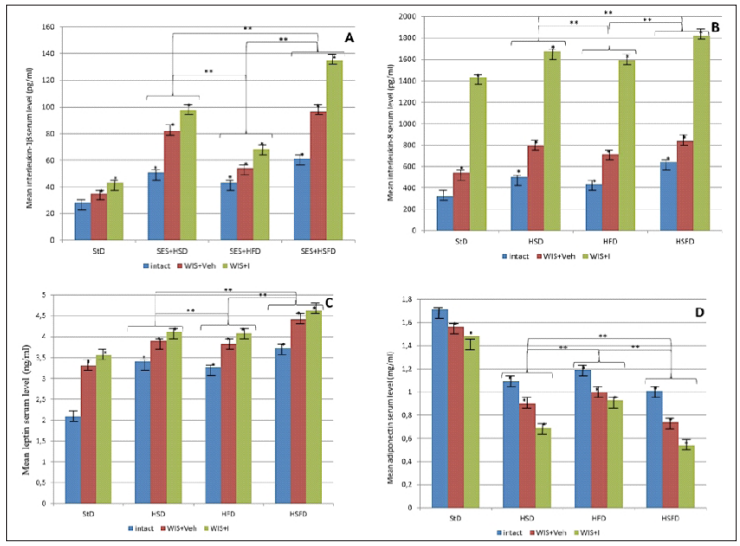

Fig. 7. Serum IL-1β (A), IL-8 (B), leptin (C), adiponectin (D) levels of rats from experimental groups. Results are presented as a mean±standard deviation of 7-8 rats per each group. Asterisks indicate a statistically significant difference between groups * $p < 0.001$ vs control; ** $p < 0.001$ between groups. Abbreviations: HFD – high fat diet; HSD – high sugar diet; HSFD – combined high sugar and fat diet; I – indomethacin; SES – social emotional stress; Veh – vehicle; WIS – restraint water-immersion stress

micro- and macrovacuolar changes in 25% of hepatocytes, irregular accumulation of glycogen in 75% of cells (Fig. 5d; Fig. 6d). The signs of interstitial oedema were observed mainly in the centrolobular compartments, and leucocyte infiltration was determined in 25% of portal tracts. Combining stress and administration of indomethacin has led to more expressed changes of liver injury such as steatohepatitis (Fig. 5h, 6h).

Serum adipokines and interleukins levels. The levels of IL-1β (28,29 ± 2,06 pg/ ml), IL-8 (321 ± 2,85 pg/ml), OB (2,08±0,05 ng/ml) and AdipoQ (1,71±0,13 mg/ml) in the control group were considered as a baseline. Changes in the serum cytokine levels under the stress and different nutritional insults during the fetal development and their reaction during the acute stress and indomethacin treatment are showed in the Fig. 7 A-D.

Significant changes were noted in the serum ILs and adipokines levels in all experimental groups. The most significant IL-8 elevation was detected in the offspring of the rats treated with HSFD (99%, $p \leq 0.001$) vs HSD (56%, $p \leq 0.001$) and HFD (46%, $p \leq 0.001$). Similar trend was noticed in terms of IL-1β, which reached 217% ($p \leq 0.001$) (HSFD), 100% ($p \leq 0.001$) (HSD), and 57% ($p \leq 0.001$) (HFD) vs control. The highest elevation of OB developed in HSFD of 79% ($p \leq 0.001$); therefore, lower in HSD – 64% ($p \leq 0.001$); in HFD – 60% vs control. At the same time, the serum AdipoQ level was reduced in rats: noted to 41% in HSFD; 36% ($p \leq 0.001$) in HSD; 32% ($p \leq 0.001$) in HFD vs control. The subgroups of animals exposed to stress revealed increased cytokine levels and OB and decreasing AdipoQ levels, reflecting the progression of the inflammatory process noted in the feathers in BAT, WAT and liver histopathology of Figs 2-5.

To have full appreciation of the disbalance between pro- and anti-inflammatory reactions, we calculated OB/AdipoQ index. The mean index in the control group was 1,22±0,11 and considered as a baseline level. The OB/AdipoQ index rose in the offspring mostly from the groups with sugar overload during the fetal period: in HSD – on 1,5 times, in HSFD – twice vs control. The leptin/adiponectin index may be a novel, non-invasive and more sensitive biomarker for the ectopic fat accumulation and low-grade inflammation in the liver and fat tissue to separate values of adipokines levels.

DISCUSSION

This paper described that the BAT and WAT of the offspring from the dams with the obesogenic environment (unbalanced and overloaded by sugar or fat nutrition or their combination, stress exposure) during the pregnancy period have displayed modified adipocyte tissues activities. This is reflected by the destructive, proliferative and secretory changes, which are associated with the liver histopathology signs of damage and inflammation, as well as worsening resistance against extreme factors.

Obesity is a complex research area, which interfaces with the epidemiological, biomedical and clinical studies of the modern biomedical research, discovering numerous fundamental, translational, epidemiological and clinical studies. As an extension of this, the etiology and pathophysiology broadly addresses genetic profiling and the study of the obesogenic environment. Genetic models of obesity and diabetes include db/db mice, ob/ob mice, Zucker diabetic fatty rats and Otsuka Long-Evans Tokushima Fatty rats while Goto-Kakizaki rats are diabetic but non-obese [26, 36]. These models are useful in evaluating specific molecular mechanisms that may be involved in obesity development of rodents. A diet high in carbohydrates together with fat, either of animal or plant origin, mimics the human diet more closely. A diet with the different mixtures and amounts of carbohydrates and fats has been used in different studies of the metabolic alterations in rodents [37, 38, 39]. Since high-carbohydrate, high-fat diet-fed rodents develop all the complications present in human diseases with the metabolic alterations and similar to human diets (sometimes called a "cafeteria diet"), that is why we used this model in order to reflect the human obesogenic environment in the most accurate way. Little is known about the effect of the obesogenic environment on the prenatal programming of adult BAT and WAT functional activities. Moreover, the accumulation of essential factors such as lipids and triglycerides may become toxic via activation of free radical formation and inflammatory response, mitochondrial dysfunction, apoptosis, and metabolic alteration [40, 41, 42]. This underlines the importance of using this approach in our study for mimicking human fetal obesogenic environment in programming BAT, WAT and liver activity in adulthood. We have proved that the combination of maternal stress and nutritional insults produce changes in anthropometric parameters. In addition, we noticed increased adiposity and changes in the morpho-functional activity of adipose tissue in the offspring. It should be also mentioned that those changes initiate the hepatocellular reorganization and disbalance of pro- and anti-inflammatory cytokines.

Our pathohistologic results have shown that the most severe changes in BAT, WAT and liver are present in the offspring of rats kept on HSFD. BAT in this group have displayed fat accumulation and perivascular inflammation. WAT was characterized by numerous hyperplastic proliferative changes and intensive leukocyte infiltrations. Interestingly, HSD induced a hypertrophic-hyperplastic type of the proliferation of white adipocytes and minor inflammation, contrary to our pathohistologic data of WAT from the rats with HFD. Moreover, excess sugar feeding during the fetal period in both groups HSD, HSFD was also a key trigger for a marked increase in the serum levels of pro-inflammatory cytokines (IL-1β, IL-8 and OB), and decrease of AdipoQ. Similar human's results were associated with the expansion in adiposity, insulin resistance, dyslipidaemia,

and atherosclerosis [42]. In addition to the presented data, a number of other studies have shown that the hypothalamus-adipose axis is a crucial system for programming systemic behavioural and defence reactions via central and peripheral signals [43]. In more recent years, researchers have shown that adipose tissues are important endocrine organs, which contribute to pro- and anti-inflammatory reactions [44, 45, 46]. There is substantial evidence that BAT has functions beyond childhood, and it participates in many physiological processes in adulthood in addition to the control of thermogenesis. Moreover, it synthesizes a unique mitochondrial uncoupling protein 1 (UCP1) and control of glucose metabolism [15]. Our data showed that the appearance of WAT in BAT in the offspring from dams fed HSD and HSFD resulted in mean BAT weight gain and visceral adiposity, which indirectly confirmed the impaired control of energy metabolism in these rats. Similar fat accumulation was noted in the liver, as in BAT, which can be analogous to human hepatosteatosis [47].

The histologic damage by acute stress in BAT and liver, which was diminished by the administration of indomethacin in both offspring with the fetal sugar-overloaded diet, can be explained by PARs. This change in the functioning of BAT, WAT and liver was confirmed by the significant rise of secretion of proinflammatory IL-1β, IL-8 in the rats fed by HSD, and HSFD. This may indicate that prenatal stress and excess of sugar caused modification of the functional activity of BAT and WAT in adulthood. Our data is supported by other researchers who indicate that ectopic deposits of fat in the intracellular form are the real risk factor of insulin resistance and induction type II diabetes [35, 48]. There is growing evidence that over-consumption of sugar contributes to postprandial hyperglycemia, and, in turn, to the increase of metabolic disorders, appetite alterations, and induced over-weight and obesity [49]. Also, the over-loading of sugar in nutrition has been implicated as a regulator of both nitric oxide and eicosanoid synthesis, the key mechanisms of cellular cytoprotective system in the gastro-intestinal tract [50, 51]. Overeating to sweet foods and sugar is a typical response to stress; taste preference to sweets is one of the physiological changes due to homeostasis- and non-homeostasis-related transformations of behavior [52]. Moreover, hyperglycemia triggers other pro-inflammatory reactions. This includes a shift in redox systems, characterized by cellular membrane injury by free radicals, as well as by the reduced circulating homocysteine levels, which follows raised oxidative stress and cellular damage [53, 54].

In the present study, we established that induction of the obesogenic environment could be represented in the laboratory by inducing stress and hyperglycemia in rats through the supplementation of drinking sucrose *ad libitum*, or by its combination with the HFD during the prenatal period. Fetal nutrition and adaptation to the obesogenic environment

has an impact on BAT in adulthood, and is characterized by abnormal quantities and proportion of BAT and WAT. These changes are attributed to the transformation of the normal WAT to its hyperplastic modification, alteration of BAT, and decrease of its anti-inflammatory properties [55]. Moreover, the liver displayed changes consistent with steatosis, a primary stage of steatohepatitis, a pre-malignant precursor of hepatocellular carcinoma [9].

CONCLUSION

Dietary insults of offspring from the dams fed by HSD, HFD or HSFD and stress induction were shown to exacerbate experimental stress resistance of BAT, WAT and liver, changing its pathophysiology and stimulating pro-inflammatory activities. The OB/AdipoQ index may be a novel, non-invasive and more sensitive biomarker for the ectopic fat accumulation and low-grade inflammation in liver and fat tissue compared with the separate values of those adipokines levels. Ectopic accumulation in BAT, intra-hepatocellular lipid and hyperplastic and hypertrophic cellular changes in the mesenteric WAT can be evaluated early by the expression of OB/AdipoQ index and levels of cytokines IL-1β and IL-8. Our data suggests that the obesogenic environment, in the early-life period, plays an important role in the severe hepatocellular injury and inflammation associated with mimicking similar to that observed in the NAFLD.

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Limitation of study: Any investigations of dams during pregnancy related to monitoring of body weight, biochemical analysis etc. are absent in the present study. Using manual or other procedures during the gestation period could create additional alterations and pathophysiological changes in the offspring. We did not study offspring from the dams exposed to stress with the standard diet for two reasons: 1) these conditions cannot adequately reproduce the obesogenic environment and 2) technical limitation with ELISA testing to 92-94 tests.

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РЕЗЮМЕ

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ВПЛИВ ПРЕНАТАЛЬНОГО СЕРЕДОВИЩА, ЩО СПРИЯЄ ОЖИРІННЮ, НА АКТИВНІСТЬ БУРОЇ ТА БІЛОЇ ЖИРОВОЇ ТКАНИНИ ТА ПЕЧІНКИ В ДОРОСЛОМУ ВІЦІ

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***Мета**: Вивчити вплив пренатального стресу та високовуглеводної материнської дієти на морфо-функціональну активність білої (WAT) і бурої (BAT) жирової тканини і печінки в щурів-нащадків першого покоління. Визначити біомаркери предикторів неалкогольної жирової хвороби печінки (НАЖХП).*

***Методи**: У дослідженні використано щурів: гестаційних самок (n=20) та їх нащадків чоловічої статі (n=92). В першому та третьому триместрі вагітності гестаційним самицям індукували соціально-емоційний стрес за моделлю Pratt, 1989 та модифікували харчування: стандартну дієту (контроль), високовуглеводну дієту (Козар, 2009), високожирову дієту (Lintermans, 2011) та їх поєднання – високовуглеводну-жирову дієту (ВВЖД). Для дослідження ефектів впливу пренатального стресу та харчування на морфо-функціональну активність BAT, WAT і печінки 3-місячним нащадкам індукували водно-іммобілізаційний стрес за Takagi, 1964 без та з модифікацією природньої цитопротекції шляхом введення індометацину. Після виведення тварин з експерименту проводили забір BAT, WAT та печінки для масометричних та патогістологічних досліджень, а також крові – для визначення вмісту IL-1β, IL-8, лептину (OB), адипонектину (AdipoQ) та індексу співвідношення лептину до адипонектину (OB/AdipoQ index).*

***Результати**: Пренатальний стрес та ВВЖД ініціювали у нащадків найбільш виражені патогістологічні зміни, що проявлялись вираженою адипоцитарною реорганізацією, запаленням та гепатоцелюлярною альтерацією, які супроводжувались гіперсекрецією IL-1β (178%, $p \leq 0.001$), IL-8 (57%, $p \leq 0.001$), гіперлептинемією (34%, $p \leq 0.001$) та гіпоадипонектинемією (AdipoQ) (53%, $p \leq 0.001$) порівняно з контролем. Поєднання стресу з введенням індометацину призвело до подальшого збільшення вмісту IL-1β (213%, $p \leq 0.001$), IL-8 (27%), OB (30%, $p \leq 0.001$) та зменшення AdipoQ (64%, $p \leq 0.001$) порівняно з відповідною підгрупою контролю.*

***Висновки**: Пренатальний стрес та високовуглеводний раціон вагітної викликають у нащадків порушення морфо-функціональної активності BAT, WAT та печінки, що проявляється перерозподілом жирової тканини в організмі за вісцеральним типом, ектопічним нагромадженням ліпідів, гіперпластично-гіпертрофічними змінами адипоцитів, хронічним системним запаленням та адипокіновим дисбалансом. Індекс OB/AdipoQ є чутливим неінвазивним біомаркером предикторів НАЖХП і може використовуватись для метаболічного скринінгу.*

***Ключові слова**: печінка, жирова тканина, запалення, харчування, стрес, лептин, адипонектин, інтерлейкін-1β, інтерлейкін-8.*

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