Laser Doppler flowmetry to assess the function of the skin microvasculature in white outbred rats with diet-induced obesity

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Obesity is a global noncommunicable pandemic. Obesity is a heterogeneous multifactorial disease. It is a risk factor for cardiometabolic disorders, type 2 diabetes mellitus (DM), etc. Obesity in both humans and animals is based on excess nutrition and physical inactivity. Therefore, experimental animal models (rodents, mini-pigs, etc.) make it possible to fairly accurately simulate the picture of the disease. Taking into account the etiology of obesity, diet-induced obesity models are the closest to humans.

The impact of obesity on a living organism can be assessed using anthropometric indicators, functional diagnostics (for example, laser Doppler flowmetry, LDF) and biochemical indicators. In the last decade, special attention of the scientific community has been paid to the study of cause-and-effect relationships between obesity and dysfunction of microvasculature (MC) vessels. It is known that in obesity MC, disorders are observed in various age and sex groups, and it has also been demonstrated that regular excess nutritional (lipid) load leads to a metabolic inflammatory response and microvascular dysfunction. Despite the fact that MC blood flow and metabolic processes are closely related and interdependent, the question of the connection between obesity and microvascular dysfunction remains open.

In this regard**, the purpose of this study** was to use the LDF method to evaluate the effect of nutritional obesity and subclinical metabolic inflammation on the function of the MC bed of the skin of the distal hind limb in albino outbred rats.

Material and methods: the studies were performed on 20 white outbred rats, divided into groups: 1) control group (intact animals); 2) experimental group - animals with the diet-induced obesity. Obesity was induced by the cafeteria diet. The animals had free access to food and water. The cafeteria diet menu consisted of: fresh food, soft drinks, standard food and water. Food combinations were offered every other day to provide daily variety. All products were located on the grid of the living cell.

Obesity status in animals was determined based on body weight gain and Lee index, which is an indicator of obesity in rodents. Subclinical metabolic inflammation was assessed by serum levels of high-sensitivity(hs) CRP, monocyte chemoattractant protein-1 (MCP-1), and angiogenesis was assessed by vascular endothelial growth factor (VEGF) concentrations.

MC were studied using the LDF method using the LAKK-OP analyzer (Lazma, Russia). 10 minutes before the manipulations, the animals were anesthetized by intramuscular injection of Telazol (Zoetis Inc, Spain) at the rate of 0.1 ml/kg and Xylanit (Nita-Pharm LLC, Russia) at a dose of 1 mg/kg of animal weight. The recording of LDF-grams was carried out by fixing the light guide probe on the skin of the dorsum of the foot (distal hind limb) of the animal. The signal recording duration was 8 minutes. LDF-grams were recorded at the 6 месяцев after содержания на рационе «диета кафетерия». The perfusion index (M) was determined in perfusion units (PU) and its mean-square deviation were determined with the use of the LDF 3.0.2.395 software. Wavelet analysis was used to determine amplitudes of endothelial (0.01-0.076 Hz), neurogenic (0.076-0.2 Hz) and myogenic (0.2-0.74 Hz) oscillations normalized to the mean-square deviation (Fig. 1). Statistical processing of experimental data was performed using the program “Statistica 10” (StatSoft, USA).

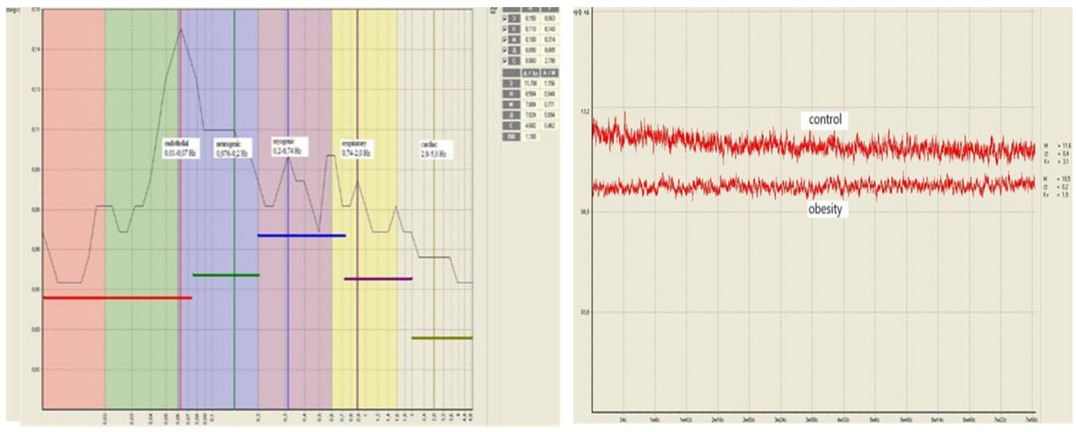

Figure 1. An example of working windows of the program “Lazma” of the analyzer “LAKK-OP” (“Lazma”, Russia).

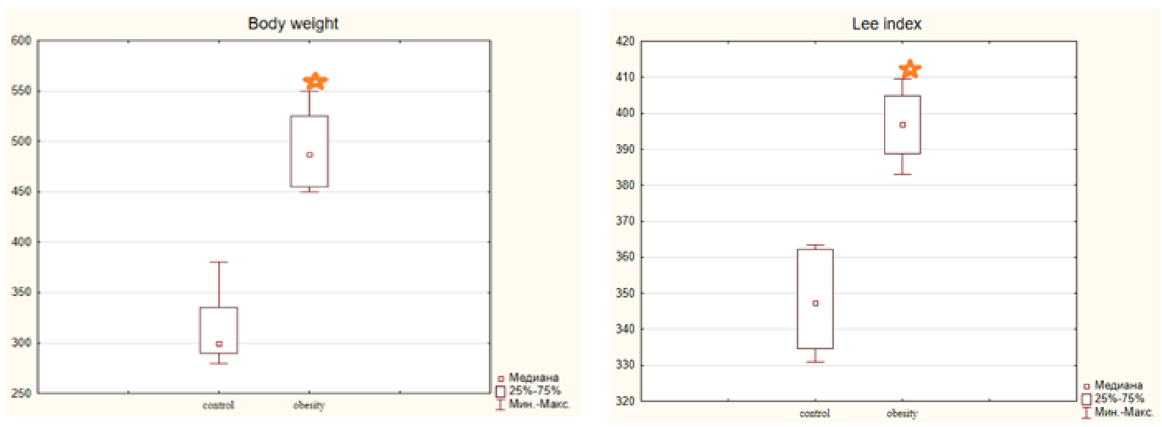

Figure 2. Changes in the body weight and Lee index in experimental animals with diet-induced obesity и контрольной группы. Significant differences (p<0,05) are marked: star – in comparison with control group.

The cafeteria diet is similar to the human diet because it provides food variety, products have high taste quality and calorie content. It reflects the main features of the human diet.

Cafeteria diet for 6 months caused in animals of the comparison group an increase in body weight by 1.5 times, as well as the Lee index by 33% relative to intact animals, which indicated the development of moderate obesity. It is known that the Lee index indicates obesity and its degree. In this regard, the Lee index can be used as a method for measuring obesity in animals, since it shows the correlation between adipose tissue and body weight.

The microcirculatory bed is a complex system that is in a state of constant regulation, carried out by vasomotions - continuous changes in the vascular diameter. The use of the LDF-gram wavelet transform makes it possible to analyze the amplitude-frequency characteristics of these vasomotions. It is believed that the frequencies of vessel oscillations lie within certain limits, depending on the origin of these oscillations. Thus, endothelial oscillations are considered the lowest frequency, followed by neurogenic, myogenic, respiratory and cardiac. A statistically significant decrease in endothelial oscillations was also observed.

When assessing the state of the microvasculature, it was found that in animals with the diet-induced obesity, a statistically significant decrease in the perfusion index (M) was observed compared to animals in the control group, indicating a reduction in tissue blood flow. The data of the present study indicate that with the diet-induced obesity, promotes the development of tissue hypoxia(Table 1).

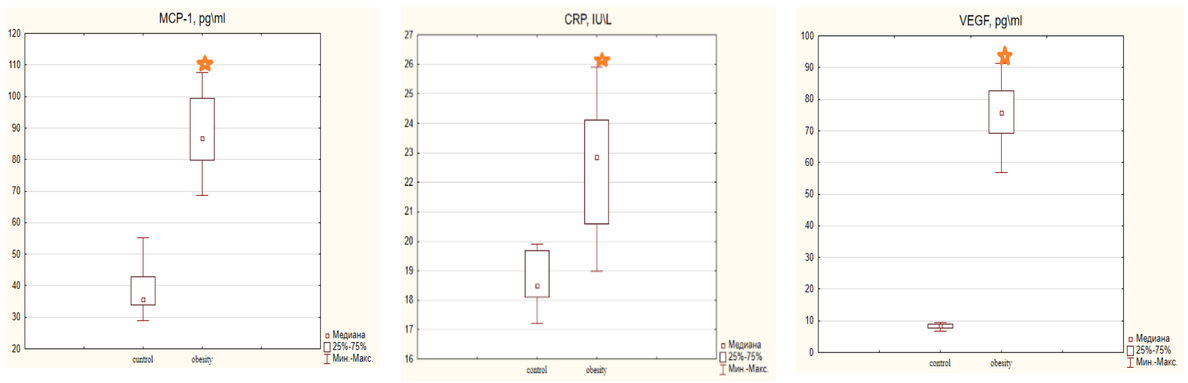

Figure 3. Changes in inflammation and angiogenesis indices during nutritional obesity in white outbred rats . Significant differences (p<0,05) are marked: star – in comparison with control group.

Table 1. Changes in perfusion and normalized amplitudes of blood flow oscillations in animals with the diet-induced obesity

Marker	Control	Experimental group (obesity)
M, (PU)	12,7 (12,0; 13,0)	10,1 (9,5;10,50) p_1 = 0,00014
А/3СКО э	17,7 (14,0; 20,0)	7,37 (6,87;11,35) p_1 = 0,002
А/3СКО н	11,2 (10,3; 14,3)	10,32 (9,18; 11,35) p_1 = 0,043
А/3СКО м	10,3 (9,9; 10,5)	10,66 (8,07; 12,22) p_1 = 0,908
А/3СКО д	8,8 (7,7; 11,5)	6,33 (4,61; 8,24) p_1 = 0,163
А/3СКО с	6,6 (5,5; 9,3)	5,13 (4,27; 6,81) p_1 = 0,373

Note: in each case the median and interquartile range are given; p1 – compared to control.

Overexpression of proinflammatory cytokines with subsequent reduction of anti-inflammatory markers in obesity is considered to link obesity-induced inflammation and ED. Infiltration of AT by macrophages is a major factor in the inflammation associated withendothelial dysfunction (ED). Inflammation can be detected by measuring proinflammatory markers such as hs-CRP and MCP-1.

In obesity, MCP-1 promotes the activation of pro-inflammatory monocytes, facilitates their migration into the subendothelium, where they, interacting with the oxidized form of low-density lipoproteins, form foam cells that participate in the formation of atherosclerotic plaque. In our study, a statistically significant increase in the content of CRP and MCP-1 in the blood serum was observed in animals of the experimental group on the background of the “cafeteria diet” (Fig. 3).

In obesity, there is a rapid increase in the mass of AT, which affects its vascularization. The absence of blood vessels causes a state of hypoxia, which promotes inflammation. Hypoxia induces increased expression of VEGF (Fig. 3), which triggers a cascade of biochemical reactions, which results in increased activity of metalloprotease enzymes, which disrupt the integrity of the endothelium and contribute to the formation of atherosclerotic plaque.

Conclusions:the “cafeteria diet” causes the development of obesity in albino rats, characterized by the occurrence of metabolic subclinical inflammation, as evidenced by increased levels of hs-CRP and MCP-1. Against the background of excessive development of AT, hypoxia occurs, stimulating excess production of VEGF, which in turn contributes to the formation of functionally immature blood vessels. Using the non-invasive LDF method, a decrease in the rate of tissue blood flow against the background of alimentary obesity was shown, and the mechanisms of its modulation were identified. Thus, the combined use of the LDF method and laboratory predictor markers made it possible to identify emerging ED against the background of alimentary obesity, which is still a reversible stage of atherosclerosis.

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