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Laser Doppler floumetry in assessing the function of the skin microvascular bed in white outbreed rats with diet-induced obesity

© E.B. Popyhova, T.E. Pylaev

Saratov State Medical University, Saratov, Russia

e-mail: PopyhovaEB@mail.ru

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Using the laser Doppler flowmetry method, the state of the microvasculature of the skin of the distal part of the hind limb of white outbred rats with nutritional obesity caused by a „cafeteria diet“was studied. It has been shown that nutritional obesity contributes to a decrease in perfusion, and also causes the development of subclinical metabolic inflammation and dyslipidemia, which are the pathogenetic basis of endothelial dysfunction and atherosclerosis, which increase the risk of cardiovascular diseases.

Keywords: nutritional obesity, microcirculation, laser Doppler flowmetry, inflammation, angiogenesis.

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Introduction

Obesity is a widespread multifactorial disease [1], the pathogenetic basis of which in both humans and animals is a genetic predisposition [2], as well as overeating and physical inactivity [3]. Due to the significant contribution of nutritional factors to the development of obesity, experimental animal models (rodents, mini-pigs, etc.) make it possible to accurately model the disease picture [4]. Given the etiology of nutritional obesity, its diet-induced patterns are closest to humans.

Currently, it has been proven that regular excessive dietary (lipid) load leads to a metabolic inflammatory reaction and microvascular dysfunction [5], causing the development of endothelial dysfunction (ED), underlying the development of cardiovascular diseases (CVD) [6]. The effect of obesity on a living organism can be assessed using anthropometric indicators [4], functional diagnostic methods (for example, laser Doppler fluorometry, LDF) [7] and biochemical parameters [8,9]. ED is characterized by functional changes in the vascular wall, which can be diagnosed using the noninvasive LDF method by examining the function of the endothelium of the microcirculatory (MC) bed of the skin [5,7].

Currently, researchers are showing increased interest in studying the pathogenetic mechanisms underlying vascular dysfunction of the MC bed against the background of nutritional obesity. Despite the fact that the effect of metabolic health on MC blood flow is known, the question of the relationship and interdependence of nutritional obesity and microvascular dysfunction remains open.

The purpose of this study was to evaluate the effect of alimentary obesity and subclinical metabolic inflammation on the function of the microcirculatory skin of the distal hindlimb of white outbred rats using the LDF method.

Materials and methods

Animal experiments are performed in accordance with the ethical standards set forth in the Declaration of Helsinki in Medical Ethics (1964) and the International Guiding Principles for Biomedical Research Involving Animals (2012), as well as the recommendations of the Ethical Committee of the Razumovsky Saratov State Medical University FSFEI HE of the Ministry of Health of the Russian Federation (minutes № 7 of 01.12.2022). The animals were kept under standard vivarium conditions, had *ad libitum* access to water and food, and were clinically healthy at the start of the study.

The study was conducted in 20 outbred female rats weighing 225 ± 25 g, which were randomly divided into the following groups of 10 animals each: 1) control group (intact animals), 2) experimental group included animals with alimentary obesity. The development of obesity in animals was induced using the „cafeteria diet“ [4.10].

The development of obesity in animals was determined by body weight gain and the Lee index, calculated using the formula [4]

$$1000 \times (3 \times (\text{body weight (g)})/(\text{lenght from the tip of the nose to the anus (cm)}))^{1/2},$$

which is an indicator of obesity in rodents. Lipid metabolism disorders were recorded by determining the serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) using Olvex reagent kits (Russia) on a ClimaMC-15 biochemical analyzer (Russia), and the atherogenicity coefficient (AC) was calculated by the formula

$$\text{AC} = (\text{TC} - \text{TC-HDL})/(\text{TC-HDL}).$$

Table 1. Changes in perfusion index and normalized amplitudes in animals with alimentary obesity

Indicator	Intact Control	Experimental group (obese)
M (PU)	12.7 [12.0; 13.0]	10.1 [9.5;10.5] $p_1 = 0.00014$
A/3 RMS endothelial	17.7 [14.0; 20.0]	7.37 [6.87;11.35] $p_1 = 0.002$
A/3 RMS nervous	11.2 [10.3; 14.3]	10.32 [9.18; 11.35] $p_1 = 0.043$
A/3 RMS muscle	10.3 [9.9; 10.5]	10.66 [8.07; 12.22] $p_1 = 0.908$
A/3 RMS respiratory	8.8 [7.7; 11.5]	6.33 [4.61; 8.24] $p_1 = 0.163$
A/3 RMS cordial	6.6 [5.5; 9.3]	5.13 [4.27; 6.81] $p_1 = 0.373$

In each case, the median and interquartile range are indicated, significant differences are noted ($p < 0.05$): p_1 — the differences are significant relative to the control.

Subclinical metabolic inflammation was assessed by the serum levels of highly sensitive C-reactive protein (hs-CRP) and monocyte chemoattractant protein-1 (MCP-1). Angiogenesis was assessed by the serum concentration of vascular endothelial growth factor (VEGF).

MC was studied by the LDF method using LAKK-OP analyzer („Lazma", Russia). The animals were anesthetized with intramuscular administration of Telazole (Zoetis Inc., Spain) at the rate of 0.1ml/kg and Xylanite („Nita-Pharm LLC" Russia) at a dose of 1 mg/kg of animal weight for 10 minutes before manipulation. LDF-grams were recorded by fixing a light guide probe on the skin of the back surface of the foot (distal part of the hindlimb) of the animal. The signal recording time was 8 min. LDF grams were recorded 6 months after keeping animals on the „cafeteria diet". 10 LDF grams of intact animals were used as a control. The perfusion index was determined in perfusion units (M, PU), and its standard deviation was calculated using the LDF 3.0.2.395 program. The wavelet analysis was used to determine the amplitudes of (A) endothelial (0.01−0.076 Hz), neurogenic (0.076−0.2 Hz), myogenic (0.2−0.74 Hz), respiratory (0.74−2.0 Hz) and cardiac (2.0−5.0 Hz) oscillations, normalized to the standard deviation.

The experimental data were statistically processed using the Statistica 10 software (StatSoft, USA).

Table 2. Parameters of lipid and carbohydrate metabolism in the studied animals

Group Parameter	Intact Control	Experimental group (obese)
TC, mmol/l	1.91 [1.89; 2.44]	282* [2.69; 3.00] $p_1 \leq 0.01$
TG, mmol/l	0.73 [0.53; 0.81]	1.55* [1.39; 1.63] $p_1 \leq 0.003$
LDL, mmol/l	0.22 [0.19; 0.28]	0.49* [0.40; 0.56] $p_1 \leq 0.004$
HDL, mmol/l	0.43 [0.37; 0.46]	0.64* [0.58; 0.68] $p_1 \leq 0.003$
AC, arb. units	2.45 [1.69; 3.93]	3.54* [3.01; 4.11] $p_1 \leq 0.005$

The median and interquartile range are given in each case; significant differences are noted ($p < 0.05$): p_1 — the differences are significant relative to the control; asterisk —- compared to the control.

Results and discussion

Keeping animals on the „cafeteria diet" for 6 months caused the development of nutritional obesity, as evidenced by a 1.5-fold increase in body weight and a 33% increase in the Li index (Fig. 1). Thus, the results of our study are consistent with the literature data on the use of the „cafeteria diet" for the induction of nutritional obesity in animals [4].

We noted a decrease in tissue blood flow in animals of the experimental group, as evidenced by a statistically significant decrease in M compared with the control (Table 1). The LDF-gram wavelet analysis revealed a decrease in the normalized amplitudes of endothelial and neurogenic oscillations in animals of the experimental group. The amplitude values of myogenic, respiratory, and cardiac oscillations did not change significantly (Table 1).

It was shown that a high-calorie diet in animals contributed to an increase in TC and TG levels by 1.5 and 2 times, respectively, relative to the control (Table 2), which indicated a violation of lipid metabolism and the development of dyslipidemia against the background of the use of the „cafeteria diet". In addition, the increase in AC (Table. 2) in animals of the experimental group, 1.4 times higher than in animals of the intact control demonstrated a high risk of atherosclerosis and CVD.

We noted a statistically significant increase in the content of hs-CRP, MCP-1 and VEGF in the blood serum of animals in the experimental group against the background of the „cafeteria diet" (Fig. 2).

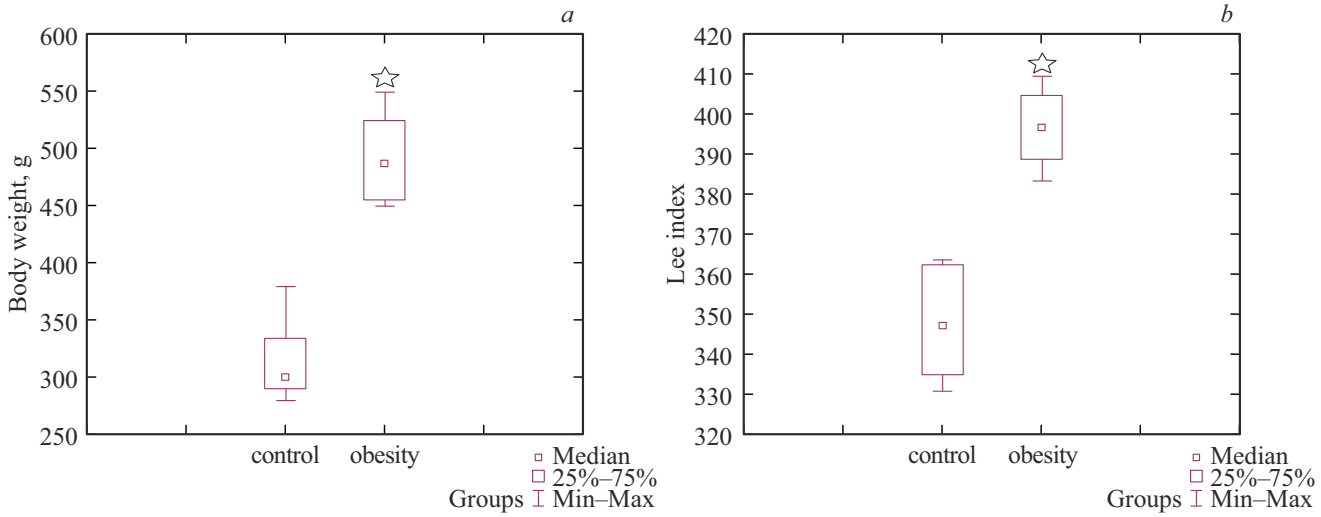

Figure 1. Change in body weight (a) and body mass index (b) in animals with alimentary obesity caused by the cafeteria diet. Significant differences were noted ($p < 0.05$): asterisk — compared to the control.

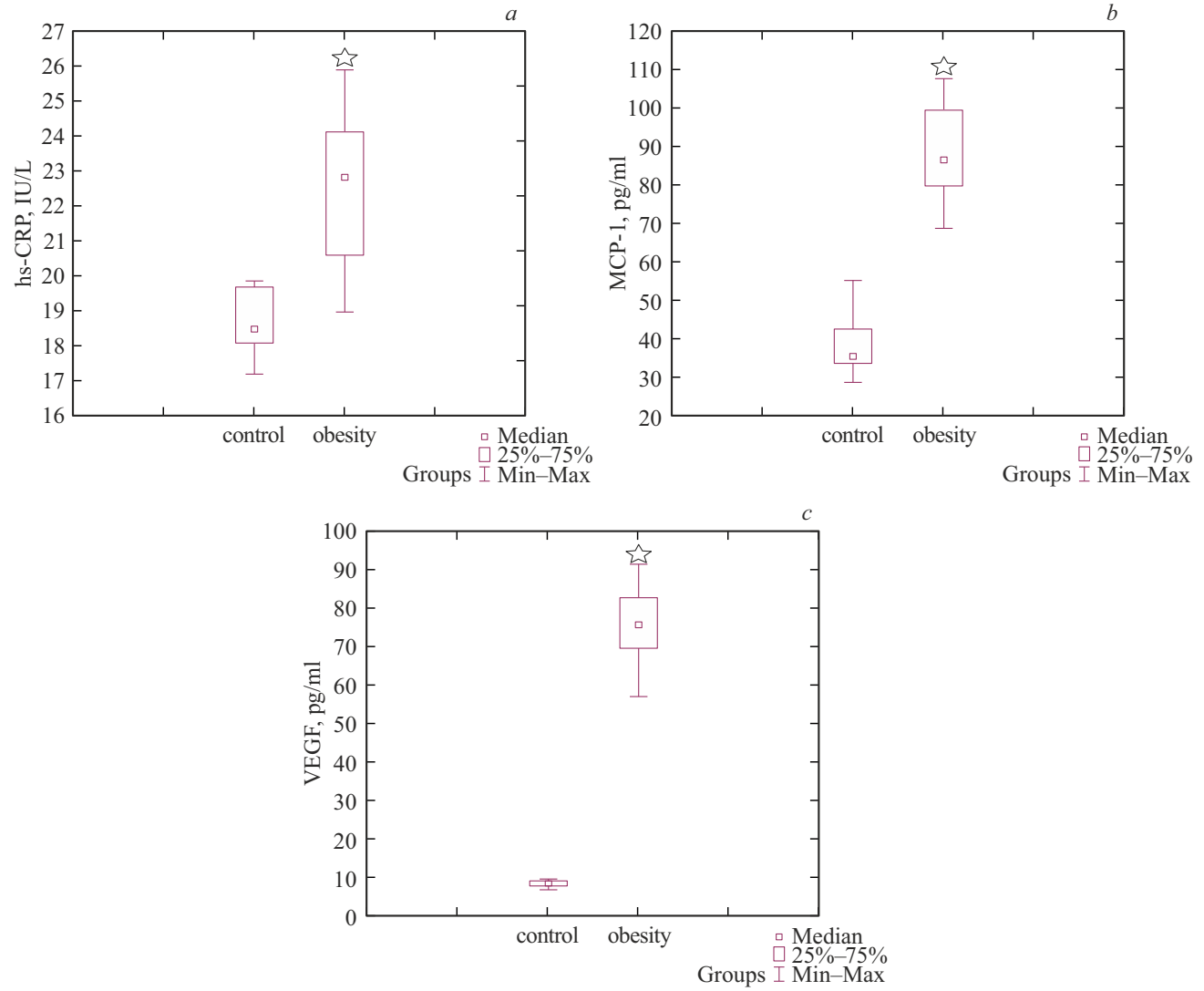

Figure 2. Modification of hs-CRP (a), MCP-1 (b) and VEGF (c) in animals with alimentary obesity caused by the cafeteria diet. Significant differences were noted ($p < 0.05$): asterisk — compared to the control.

Inflammation is a complex process that occurs during the alteration or action of a pathogen and is aimed at eliminating the factors causing it, as well as leading to maximum recovery in the area of damage. It is the result of an imbalance between pro- and anti-inflammatory factors [11]. A stable imbalance between pro- and anti-inflammatory factors occurs in case of obesity, leading to the development of chronic inflammation [12], which, in turn, is considered as the initial stage of vascular dysfunction, progressing to vascular diseases associated with obesity [6]. Overexpression of pro-inflammatory cytokines, followed by a decrease in anti-inflammatory markers in obesity, is considered to be a link between obesity-induced inflammation and ED [8]. Infiltration of the fat tissue by macrophages is the main factor of inflammation associated with ED [8,9]. The MCP-1 protein produced by macrophages promotes the activation of pro-inflammatory monocytes in case of obesity, facilitates their migration to the subendothelium, where they interact with the oxidized form of LDL to form foam cells involved in the formation of atherosclerotic plaque [13].

The body weight rapidly decreases in case of obesity, which affects its vascularization [14]. The absence of blood vessels causes a state of hypoxia, which also contributes to inflammation. Hypoxia induces increased VEGF expression (Fig. 2) and triggers a cascade of biochemical reactions, resulting in increased activity of metalloprotease enzymes [8,14], disrupting the integrity of the endothelium and contributing to the development of ED, followed by the formation of atherosclerotic plaques.

Conclusion

Alimentary obesity in white outbred rats leads to a violation of MC, which can be assessed using the noninvasive LDF method. Possible pathogenetic mechanisms MC dysfunctions are a violation of lipid metabolism, manifested by hypercholesterolemia, hypertriglyceridemia, high AC, chronic metabolic inflammation and hypoxia, as evidenced by elevated levels of hs-CRP, MCP-1 and VEGF.

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Conflict of interest

The authors declare no conflict of interest.

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