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# Method of laser Doppler flowmetry in assessing the effect of liraglutide on microcirculation in rats with diet-induced obesity

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The state of the microcirculatory bed in rats with obesity caused by the „cafeteria diet"was studied using laser Doppler flowmetry during therapy with liraglutide. It has been shown that obesity causes a decrease in the perfusion index and normalized amplitudes of endothelial and neurogenic oscillations on the skin of the dorsal foot of experimental animals. Therapy with liraglutide contributed to the reduction of excess body weight and the Lee index, which resulted in the restoration of disturbed microcirculation of the skin of the dorsal foot of rats, as well as the normalization of the functional activity of target organs.

**Keywords:** laser Doppler flowmetry, microcirculation, cafeteria diet, obesity, liraglutide.

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## Introduction

Obesity is a chronic disease whose prevalence is increasing worldwide. Factors contributing to its spread include unregulated diet, high-fat diet, and hypodynamia [1]. Obesity is characterized by excessive accumulation of adipose tissue (AT) in the body due to an imbalance between calorie intake and expenditure. Adipokines and cytokines produced by AT have pro- and anti-inflammatory activity and, getting through the systemic circulation to target organs (liver, heart, kidneys), they cause accumulation of excess fat in them, which contributes to a change in their functions [2,3].

In clinical practice, the Liraglutide medication is used to treat obesity. This is an analogue of glucagon-like peptide-1 (GLP-1), which is able to inhibit gastric motility and reduce the rate of gastric emptying [4], resulting in a decrease in food intake and a weight loss. The positive effect of liraglutide on microcirculation (MC) was also shown in experimental diabetes mellitus [5]. It has been proven that obesity contributes to the occurrence and development of cardiovascular diseases (CVD), therefore, researchers pay increased attention to the study of causal relationships between obesity and MC vessel disorders. It has been established that excessive nutritional load leads to an inflammatory reaction in the vessel wall [6], resulting in the development of microvascular dysfunction [7]. Modern non-invasive optical methods, one of which is laser Doppler flowmetry (LDF), open up opportunities for early diagnosis of MC disorders caused, in particular, by obesity. This is due to their ability to identify the most informative parameters both in the clinic and on experimental models.

In this context, the purpose of this study was to use the LDF method in investigate the state of the microcirculatory bloodstream in rats with alimentary obesity caused by the cafeteria diet during the liraglutide therapy, as well as the effect of therapy on the function of target organs (liver, heart, kidneys).

## 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 30 outbred female rats weighing $225 \pm 25$ g, which were randomly divided into the following groups of 10 animals each: 1) control (intact animals), 2) comparison group — animals with alimentary obesity, 3) experimental group — animals with alimentary obesity, which were administered liraglutide at a dose of 0.4 mg/kg for 21 days [8].

The development of obesity in animals caused by the cafeteria diet [2,9]. Diagnosis of its development, as well as assessment of the anti-obesity effect of liraglutide in rats, was performed by calculating the Lee index $(1000 \times (3\sqrt{}(\text{body weight (g)})/(\text{length from nose tip to anus (cm)}))$ [9] as it shows the correlation between AT and body weight [2]; also, body weight measurements were used. The functional activity of target organs (heart, liver, kidneys) was assessed by changes in their mass. During the study, body weight was determined in intact and obese animals at the beginning and at the end of the experiment, and in animals treated with liraglutide, body weight was additionally monitored before the start of therapy.

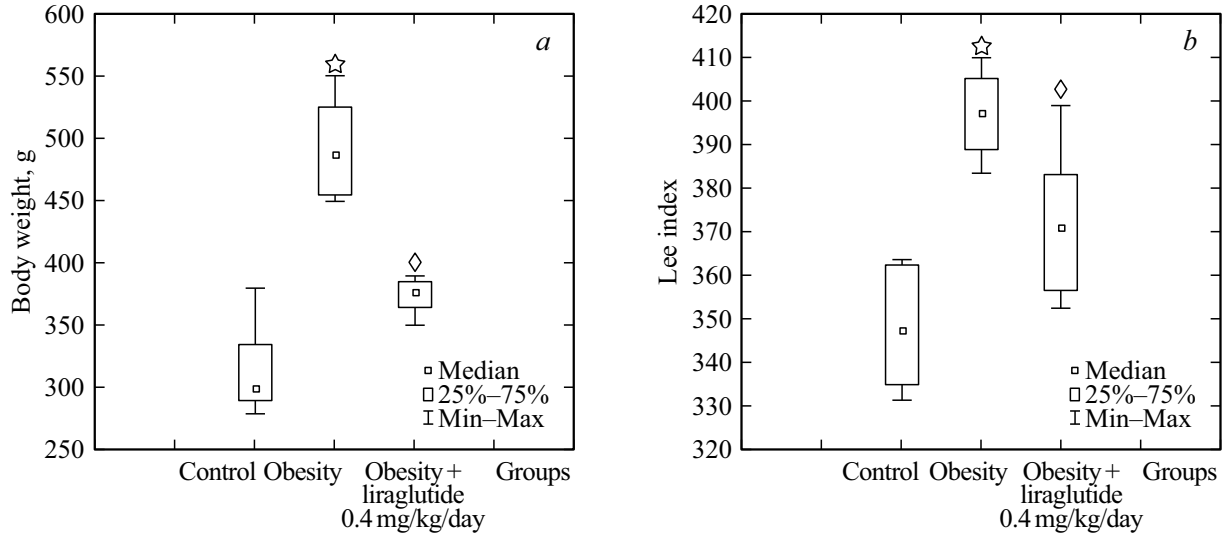


**Figure 1.** Changes in body weight (*a*) and Lee index (*b*) in animals with alimentary obesity caused by the cafeteria diet during the liraglutide therapy. Significant differences were noted ($p < 0.05$): star — compared with the control group, diamond — relative to the comparison group.

Microcirculation was studied by the LDF method on a LAKK-OP analyzer (Lazma LLC, Russia). For manipulations, the animals were anesthetized by intramuscular injection of 0.1 ml/kg Telazol (Zoetis Inc, Spain) and 1 mg/kg Xylanite (Nita-Pharm LLC, Russia). LDF-grams were registered in animals of all groups. Recording was carried out for 8 min by fixing the sensor on the skin of the distal segment of hind limb of the animal. The perfusion index was determined in perfusion units (M, PU) and its root-mean-square deviation was calculated using the LDF 3.0.2.395 program. Amplitude-frequency responses normalized to the root-mean-square deviation (RMSD) for endothelial (0.01–0.076 Hz), neurogenic (0.076–0.2 Hz) and myogenic (0.2–0.74 Hz) vasomotions were calculated using wavelet analysis and expressed in conventional units (CU). Normalized amplitudes of vasomotions were calculated by the following formula: $(A/3\text{RMSD}) \times 100$ [10].

The experimental data were statistically processed using the Statistica 10 software (StatSoft Inc., USA). In case of difference of values distribution in a sample from the normal distribution, median and interquartile range (Me [Q25; Q75]) were calculated. The nonparametric *p* Mann–Whitney test was used to determine differences between the groups. The differences were considered significant when $p < 0.05$.

## Results and discussion

It was shown that the cafeteria diet for 6 months caused in the animals of the comparison group 1.5 times increase in body weight and increase in Li index by 33% relative to the control group, which was indicative of the development of moderate obesity [2,9]. Subcutaneous administration of liraglutide to overweight rats for 21 days at a dose of 0.4 mg/kg/day caused a decrease in their body weight by 80% in relation to animals of the comparison group, however, their body weight exceeded that of intact animals by 23%. Accordingly, during the liraglutide therapy in animals, there was a statistically significant decrease in the Lee index relative to animals of the comparison group, and no statistically significant differences were observed relative to intact animals (Fig. 1).

Thus, the results of our study are consistent with the literature data on the ability of liraglutide to reduce body weight [4].

It was shown that obesity causes a statistically significant increase in heart weight by 1.4 times, in kidney weight by 1.5 times and in liver weight by 1.2 times compared with the control group. During with liraglutide therapy, a statistically significant decrease in the weight of the liver by 1.1 times and in the weight of the heart by 1.3 times in relation to the animals of the comparison group was noted, as a result, the weight of these organs almost did not differ from that in intact animals. Kidney weight tended to decrease, however this decrease was not statistically significant (Fig. 2).

It is known that obesity causes structural and functional changes in the heart muscle [11]. Adaptation of cardiac activity on top of obesity occurs due to the left ventricular hypertrophy, which is a predisposing factor to the occurrence of CVD [11,12]. The liraglutide therapy caused a decrease in body weight due to a decrease in the AT expansion. Normalization of body weight contributed to a decrease in the adaptive load on the left ventricle myocardium, as evidenced by a decrease in the mass of the target organ.

An increase in the mass of the liver during the experiment indicated the development of obesity of the first degree. An increase in organ mass is associated with an increase in the

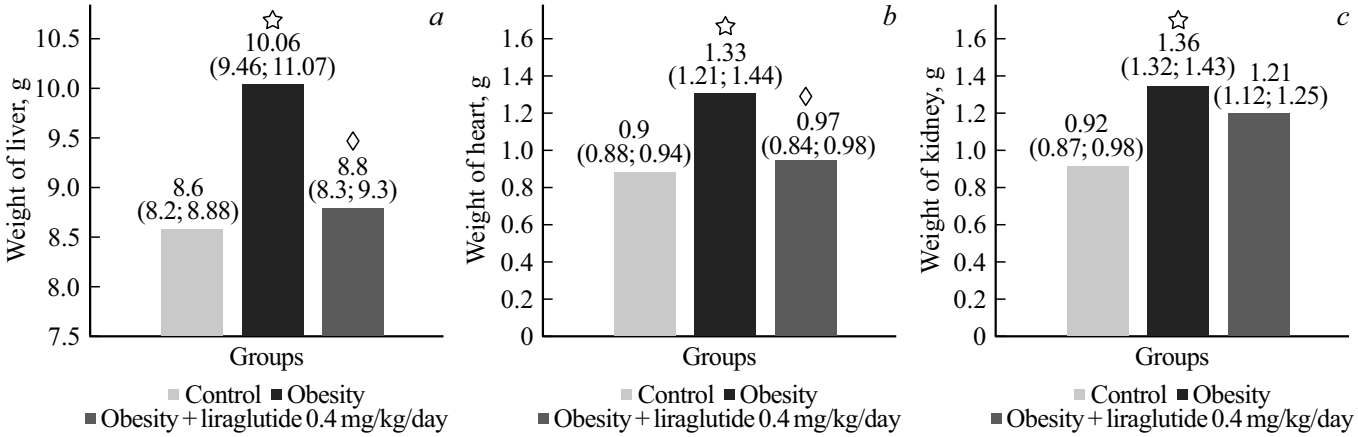


**Figure 2.** Changes in liver (*a*), heart (*b*) and kidney (*c*) weights in animals with alimentary obesity induced by the cafeteria diet during liraglutide therapy. Significant differences were noted ($p < 0.05$): star — compared with the control group, diamond — relative to the comparison group.

Changes in the perfusion index and normalized amplitudes of blood flow fluctuations in animals with alimentary obesity caused by the cafeteria diet during liraglutide therapy

| Parameter | Control | Comparison group (obesity) | Experimental group (obesity+liraglutide) |
|---|---|---|---|
| M, PU | 12,7<br>(12,0; 13,0) | 10,1<br>(9,5; 10,50)<br>$p_1 = 0, 00014$ | 14,61<br>(14,43; 15,94)<br>$p_1 = 0, 0018$<br>$p_2 = 0, 000003$ |
| *A*/3CKO E, CU | 17,7<br>(14,0; 20,0) | 7,37<br>(6,87; 11,35)<br>$p_1 = 0, 002$ | 17,09<br>(15,55; 17,83)<br>$p_1 = 0, 15$<br>$p_2 = 0, 00006$ |
| *A*/3CKO H, CU | 11,2<br>(10,3; 14,3) | 10,32<br>(9,18; 11,35)<br>$p_1 = 0, 043$ | 12,43<br>(10,71; 13,16)<br>$p_1 = 0, 983$<br>$p_2 = 0, 05$ |
| *A*/3CKO M, CU | 10,3<br>(9,9; 10,5) | 10,66<br>(8,07; 12,22)<br>$p_1 = 0, 908$ | 10,58<br>(6,85; 13,45)<br>$p_1 = 0, 65$<br>$p_2 = 0, 99$ |
| *A*/3CKO D, CU | 8,8<br>(7,7; 11,5) | 6,33<br>(4,61; 8,24)<br>$p_1 = 0, 163$ | 7,74<br>(5,61; 10,94)<br>$p_1 = 0, 448$<br>$p_2 = 0, 467$ |
| *A*/3CKO C, CU | 6,6<br>(5,5; 9,3) | 5,13<br>(4,27; 6,81)<br>$p_1 = 0, 373$ | 6,36<br>(4,90; 7,86)<br>$p_1 = 0, 437$<br>$p_2 = 0, 794$ |

*Note.* In each case, the median and interquartile range are given, $p_1$ — Mann–Whitney test compared to control, $p_2$ — Mann–Whitney test relative to the comparison group (obesity).

functional activity of hepatocytes, which is a compensatory reaction in response to the cafeteria diet [13].

It is known that obesity contributes to the occurrence and development of „metabolic “ nephropathies. AT expansion leads to the situation when the total nephron filtration surface area cannot permanently inactivate the excess of AT-produced biologically active substances. As a result, a condition of relative deficiency of nephron mass develops

in the kidneys, which causes hyperfiltration followed by hypertrophy and hyperplasia of glomerular cells. With continued expansion of AT, a gradual depletion of the functional renal reserve and fibrosis take place [14]. The liraglutide therapy causes a decrease in the expansion of AT and contributes to the keeping the functional activity of the renal parenchyma.

In animals with obesity a decrease in tissue blood flow was observed, as evidenced by a statistically significant decrease in M compared with the control group (see the Table). Wavelet analysis of LDF-grams has shown a decrease in the normalized amplitudes of endothelial and neurogenic oscillations in animals of the comparison group. The amplitude values of myogenic oscillations did not demonstrate statistically significant changes (see the Table).

The liraglutide therapy at a dose of 0.4 mg/kg/day caused an increase in *M* by 40% relative to the animals of the comparison group (table). Thus, a positive effect of liraglutide on the neurogenic component of vascular tone was experimentally shown, which is consistent with the previously obtained data presented in [5]. It has been established that in diet-induced obesity, liraglutide restores the disordered endothelium-dependent dilatation of the MC blood stream vessels, thus providing an endothelial protective effect (see the Table)

## Conclusion

Obesity has been shown to cause MC dysregulation and a decrease in tissue perfusion, as well as a functional load on target organs. The liraglutide therapy at a dose of 0.4 mg/kg/day contributes to a decrease in body weight and the Lee index in animals with alimentary obesity and, thus, leads to a decrease in the adaptation load on target organs. Using the LDF method, a positive effect of liraglutide therapy on endothelium-dependent vasodilation and on a decrease in the tone of the precapillary component of the MC blood stream was shown, which is indicative of the normalization of the MC. Thus, liraglutide has an endothelioprotective effect; therefore, it is advisable to use it in obese patients to prevent the development and progression of endothelial dysfunction.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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