THE EFFECT OF A PROLONGED HIGH-FAT DIET ON THE PROCESSES OF CEREBRAL CIRCULATION

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ABSTRACT

In the current scientific work, violations of the cerebral circulation of laboratory animals with diabetes mellitus and metabolic syndrome have been studied. Changes in the density of the vascular network of the pial membrane of the sensorimotor cortex, dilation of the pial arteries, tissue perfusion, and oxygen saturation were considered. The studied rats were kept on a high-fat diet for 60 days, as a result of which the animals had an increase in blood glucose levels, the development of insulin resistance, and a significant set of visceral fat. The metabolic syndrome led to changes in cerebral circulation, tissue perfusion decreased by 22%, and oxygen saturation decreased by 6%. The development of type 2 diabetes mellitus leads to dysfunction of the endothelium of the cerebral arteries: the smaller the diameter of the vessel, the worse the expansion reaction to acetylcholine. It was found that the emptying of the vascular bed, and impaired reactivity of the cerebral arteries negatively affect the supply of oxygen to brain tissues.

Introduction

WHO experts have characterized metabolic syndrome (MS) as one of the most common and harmful diseases of the modern generation [1]. According to some studies, 22 to 35% of the adult population of different countries of the world suffers from metabolic syndrome [2]. More than 35% of residents of the Russian Federation have 2 MS symptoms, and 12% have 3 or more [3]. The main risk groups for metabolic syndrome include elderly patients (more than 30%), obese (almost 50%), impaired glucose tolerance (almost 50%), with diabetes mellitus (more than 80%) [4-7].

It is known that the timely detection of metabolic syndrome is of great importance for the successful relief and treatment of the disease and the obstacle to the development of symptoms. Cardiovascular morbidity and mortality in people with MS are significantly higher compared to people without it [8-10]. The presence of MS increases the risk of developing both type 2 diabetes mellitus (DM2) and arterial hypertension by 3-6 times [11-13]. The development of MS inevitably leads to significant subclinical damage to the main organs of the human body. It is possible to observe malfunctions of the kidneys, disorders of the cardiovascular system, thickening of the walls of the arteries, and loss of flexibility [14-18]. The development of hypertension significantly exacerbates the effects of MS. One of the serious manifestations of MS is a problem with the liver [19]. Almost all of these changes are reversible. The world's leading scientists dealing with the problem of MS tend to consider it as a pre-stage of atherosclerosis and DM2. Thus, MS isolation is based on the principle of primary prevention of diabetes, atherosclerosis, and its consequences.

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Currently, there is a steady trend towards an increase in the prevalence of metabolic syndrome in the world. Already, twice as many people are suffering from MS as there are patients with diabetes mellitus. Experts say that in 20 years there will be one and a half times more patients with MS. The main problem for society and the state is that metabolic syndrome is especially common among the able-bodied population [20]. At the same time, the average age of patients decreases every year, and the number of young patients increases significantly [21].

Increased consumption of high-calorie foods leads to the development of endocrine diseases, primarily type 2 diabetes mellitus, which is a direct path to the development of cerebrovascular pathologies [22]. The consequences of impaired lipid metabolism and high sugar levels are an increased risk of strokes (ischemic, hemorrhagic, lacunar) [23, 24], damage to small vessels and walls of large vessels of the brain [25, 26], the development of endothelial dysfunction [27].

To develop new approaches to the treatment of type 2 diabetes mellitus, detailed studies of the causes and mechanisms of cerebrovascular pathologies are necessary. Thus, the purpose of this scientific study is a comprehensive assessment of cerebral circulatory disorders in laboratory animals with metabolic syndrome and type 2 diabetes mellitus.

Materials and Methods

Experimental studies were conducted on 36 laboratory male Sprague Dawley rats. The animals were kept in 6 individuals in cages. At the beginning of the work, the age of the rats was 3 months, body weight was 290-340 g. Before the experiment, body weight, body length (without tail), abdominal circumference, and blood sugar levels were measured in animals of all groups. Animals of group 1 (n=12) were kept under standard conditions. The sleep and wakefulness regime directly depended on the cycle of natural illumination and was approximately 11 o'clock (at night) to 13 o'clock (daylight). The diet was generally accepted for rats of a particular line. Access to food and water was unlimited.

Group 2 rats (n=12) were kept in similar conditions, but their diet was significantly different for two months. Their high-calorie diet included: compound feed (360 g/kg), mutton fat (320 g/kg), whey protein (260 g/kg), and vitamin and mineral complex (40 g/kg). Thus, 57% of fat, 26% of protein, and 18% of carbohydrates from the total number of calories were formed. The animals of group 2 were assigned to the group with metabolic syndrome.

Group 3 rats (n=12) were kept in similar conditions, including diet, to group 2 animals. After 2 months, rats from group 3 were intraperitoneally injected with 38 mg/kg streptozotocin to cause type 2 diabetes.

Then, for another month, the animals of groups 2 and 3 were on a high-calorie diet. Once a month, blood was taken from the tail vein of all animals to measure glucose levels using a Roche Accu-Chek Active glucose meter (Roche Diabetes Care GmbH, Germany). 3 months after the start of the experiment, all rats were tested for insulin resistance and glucose tolerance.

Based on the basic principles of humanity, all surgical interventions were performed by animals under anesthesia. To do this, the drug Vesotil was administered intraperitoneally to rats at a dosage of 25 mg/kg of weight. For euthanasia, the dose of anesthesia was increased.

In rats, a 3.5 mm diameter hole was drilled in the parietal region of the skull, then the dura mater was removed. The brain surface was continuously irrigated with Krebs solution [28].

Throughout the experiment, the average blood pressure (BP) and body temperature of the animal were continuously monitored (BIO-THERMO-CIS laboratory thermometer (Bioseb SAS, France)).

During the experiment, more than 40 pial arteries were examined in each animal. All studied microvessels of the pial arteries were divided into groups depending on the initial diameter: 60-80 microns, 40-60 microns, 20-40 microns, and less than 20 microns. The results of acetylcholine (Ach) exposure were judged by the number of dilated arterial vessels [29]. Visualization of the pial arteries was performed using a stereoscopic microscope MC-2ZOOM (Micromed, Russia) and a video eyepiece DCM-510 (Scopetek, China).

The levels of perfusion (P) and oxygen saturation (SO2) in the tissues of the sensorimotor cortex of the brain were measured using the multifunctional laser diagnostic complex LACK-M (LAZMA, Russia) [30].

After euthanasia, the mass of visceral adipose tissue in rats was carefully measured, which is concentrated in the mesenteric (along the intestine), retroperitoneal (behind the kidneys), and appendage (along the testicles) zones [31].

The mathematical processing of the obtained data was carried out using the statistical software package Microsoft Excel 2010 and the Statistics 6.0 program.

Results and Discussion

According to the data obtained, the high-calorie diet of rats of groups 2 and 3 prompted to a serious surge in blood glucose levels, the build-up of insulin resistance, and a significant set of visceral fat **(Table 1)**. Blood pressure in all rats was within the normal range.

Table 1. Parameters of experimental animals

Indicator	Group 1	Group 2	Group 3
Increase in body weight relative to the initial, %	22.9±0.85	32.9±2.3, *p ≤ 0.05	103±12.4, ** p ≤ 0.001
The specific gravity of visceral fat, %	2.32±0.24	4.39±0.38, *p ≤ 0.05	5.34±0.28, ***p≤0.0001

Blood glucose level, mmol/l	5.4±0.12	10.6±0.75, **p≤ 0.001	15.8±0.85, ** p ≤ 0.001
Average blood pressure, mmHg	119.6±2.3	126.4±1.9, *p ≤ 0.05	129.2±1.8, *p ≤ 0.05
Glucose tolerance test*	100%	105%	201%
Insulin resistance test*	100%	114%	323%

Note: * The area under the curve "glucose concentration–time (120 min)" was measured. The result for group 1 was accepted as 100%. For groups 2 and 3, an increase in area relative to group 1 is shown, expressed in %. The levels of difference are indicated relative to group 1.

In rats with MS (group 2), a statistically crucial reduction in the density of the vascular network in the pial membrane of the sensorimotor cortex (on average 1.3 times), and in the number of arterial vessels per unit area of measurement (on average 1.4 times) were revealed. Almost the same narrowing of the vascular bed was found in group 3 (DM 2) **(Figure 1)**.
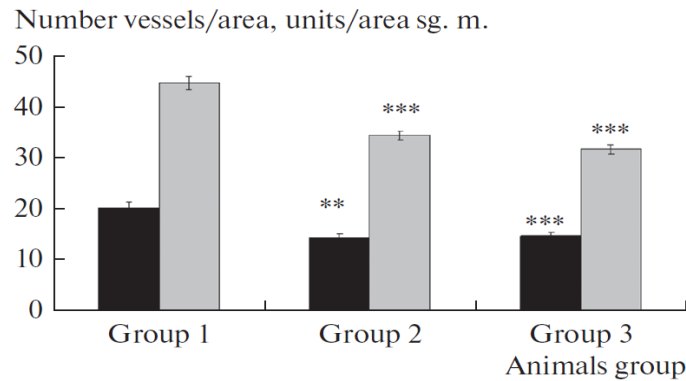

Figure 1. Microvascular density indices in rats. The dark color shows the number of pial arteries/measuring area (mm2); the light color shows the number of all vessels/ measuring area (mm^2). Along the abscissa axis – groups of experimental animals; along the ordinate axis – an indicator of the density of the microvascular bed.

The genesis of MS led to a statistically significant drop in the number of pial arterial vessels that responded with an increase in diameter to the effect of ACh solution on the brain surface **(Figure 2)** – by an average of 1.2–1.6 times, depending on the diameter of the vessels. In group 3 rats, the number of arteries dilating to the effects of ACh was significantly less than in control animals (on average 1.6–2.3 times) and than in group 2 rats (on average 1.2–1.9 times). Note: the changes are significant compared to the corresponding values in control animals (*p < 0.05, **p < 0.01, ***p < 0.001, Tukey criterion).
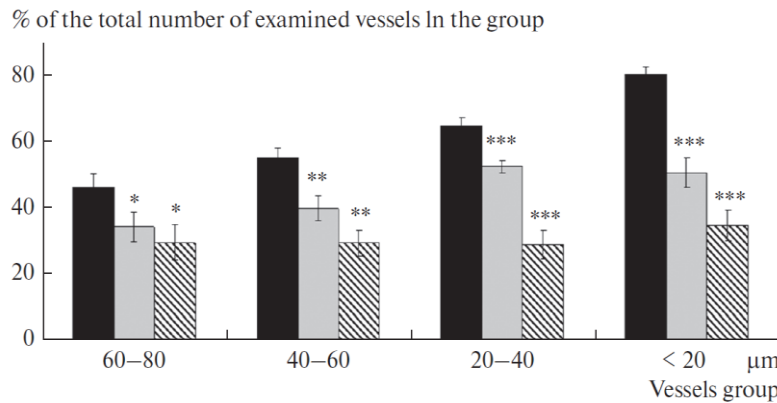

Figure 2. Dilatatory reaction of pial arterial vessels of various diameters to ACh exposure: – number of dilatations (% of the total number of examined vessels). Dark color shows animals of group 1, light color - animals of group 2, hatching - animals of group 3. Along the abscissa axis – groups of vessels of different diameters (microns); along the ordinate axis – the number of arteries expanded by the action of ACh relative to the total number of vessels in the group (%). Note: the changes are remarkable compared to the adjacent values in control animals (*p < 0.05, **p < 0.01, ***p < 0.001).

Figure 3 shows a shrinkage in tissue perfusion (a) and tissue saturation with oxygen SO2 (b) in the sensorimotor cortex of the brain in rats from group 3 compared with animals from groups 1 and 2. Thus, it can be concluded that a high-fat diet did not cause a statistically significant decrease in tissue perfusion. However, the development of type 2 diabetes mellitus led to a decrease in tissue perfusion by almost 22%. The level of CO_2 in tissues was reduced by 6-9% in rats of groups 2 and 3.
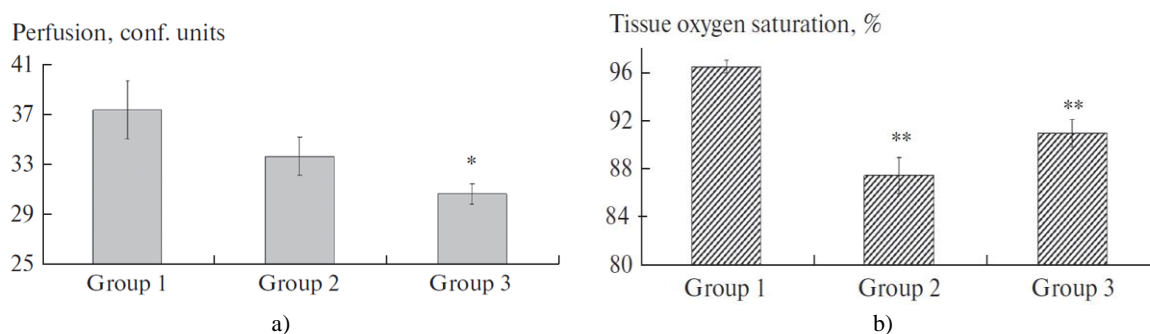
Figure 3. Changes in the level of perfusion. a) and oxygen saturation, b) of the sensorimotor cortex tissue in the studied rats: a – perfusion index, b – tissue oxygen saturation index SO_2 (%). The changes are significant compared with the corresponding values in control animals (**$p < 0.01$, Tukey criterion).

According to the results of the experiment, a high-calorie diet for three months led to the development of metabolic disorders. At the same time, the tested rats developed pathological changes in the body similar to those in humans with metabolic syndrome: there was a significant deterioration in blood circulation in the sensorimotor cortex of the brain, a decrease in the vascular network of the pial membrane was revealed **(Figure 1)**. Against the background of the development of diabetes mellitus in group 3, the architectonics of the vascular bed of the pial membrane of the sensorimotor cortex did not change the brain.

Currently, it is believed that damage to the walls of blood vessels, including cerebral ones, occurs due to an excess of insulin in body tissues against the background of insulin resistance, characteristic of both multiple sclerosis and DM2 [32-34].

Redudant insulin activates mitogen-activated protein kinase, which, in turn, vitalizes the yield of various growth factors, which triggers the proliferation and migration of smooth muscle cells in the vascular wall [35, 36]. Thus, the thickness of the vessel walls increases, and the inner diameter narrows accordingly until the passage is completely blocked. Nevertheless, our study showed that the insulin resistance of group 2 rats, although higher than normal, was less pronounced than in animals with type 2 diabetes mellitus.

The development of the metabolic syndrome led to a violation of lipid metabolism and the accumulation of visceral fat. According to some scientists, increased secretion of very low-density lipoproteins in visceral adipose tissue contributes to the accumulation of beta-amyloid in the brain [37, 38]. The presence of aggregated beta-amyloid in small vessels of the brain removes them from the circulatory system, and cerebral amyloid angiopathy develops [39].

The development of MS and DM2 led to suppression of ACh-mediated dilation of pial arterial vessels **(Figure 2)**. However, in rats with DM2, a pattern was violated: the smaller the diameters of the vessels, the more their number expands when exposed to ACh. So, normally, the difference between arteries of 60-80 microns in size and arteries less than 20 microns in this indicator was 1.7 times, whereas, with DM2, small arteries reacted in about the same way as large ones. In rats with multiple sclerosis, this pattern persisted: the number of expanding small arteries exceeded the number of the largest by 1.5 times. The data obtained confirm that with DM2, intimal cells in the arteries of the brain are damaged, and endothelial dysfunction develops [40, 41]. At the same time, small arteries suffer more, that is, those elements of the vascular network that are more involved in oxygen metabolism.

However, endothelial dysfunction has also been observed in animals with metabolic syndrome. That is, a violation of lipid metabolism leads to excessive amassment of saturated fatty acids, that leads to the production of pro-inflammatory factors IL-1β, TNF-α, IL-6 [42, 43], a decrease in the expression of close contact proteins and a violation of the integrity of the blood-brain barrier [44, 45]. With an increased permeability of the blood-brain barrier, penetration of various foreign bodies into the brain is observed: toxic metabolites, and immune cells. At the same time, in the brain. especially in his vascular system, inflammatory processes are triggered [46]. In animals of groups 2 and 3, a negative effect on perfusion and oxygen saturation of the sensorimotor cortex of the brain was found **(Figure 3)**. In group 3 rats, there was a decrease in the level of tissue perfusion, in groups 2 and 3 there was a decrease in SO_2 levels and cerebral blood flow rate, which is associated with impaired autoregulation of cerebral blood flow [47-49].

Conclusion

The results of the conducted studies demonstrate that the metabolic syndrome, using the example of laboratory animals, leads to a dwindle in the vascular network of the pial membrane of the brain, the formation of endothelial dysfunction of the pial arteries, and a collapse of their ACh-mediated dilation, a drop in tissue oxygen saturation. At the same time, normal blood pressure and moderate hyperglycemia are observed. With the development of diabetes mellitus, the progression of endothelial dysfunction can be noted, especially in small-diameter arteries. Emptying of the vascular bed and a compromised reactivity of the cerebral arteries effects the supply of oxygen to brain tissues.

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Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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