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Correction of metabolic disturbances by functional food compositions in experimental obesity in CD-1 and agouti-yellow mice

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ABSTRACT

Background: To determine the most promising composition, the possibility of correction of metabolic disturbances was investigated with the use of functional food compositions in CD1 and agouti-yellow mice in experimental obesity.

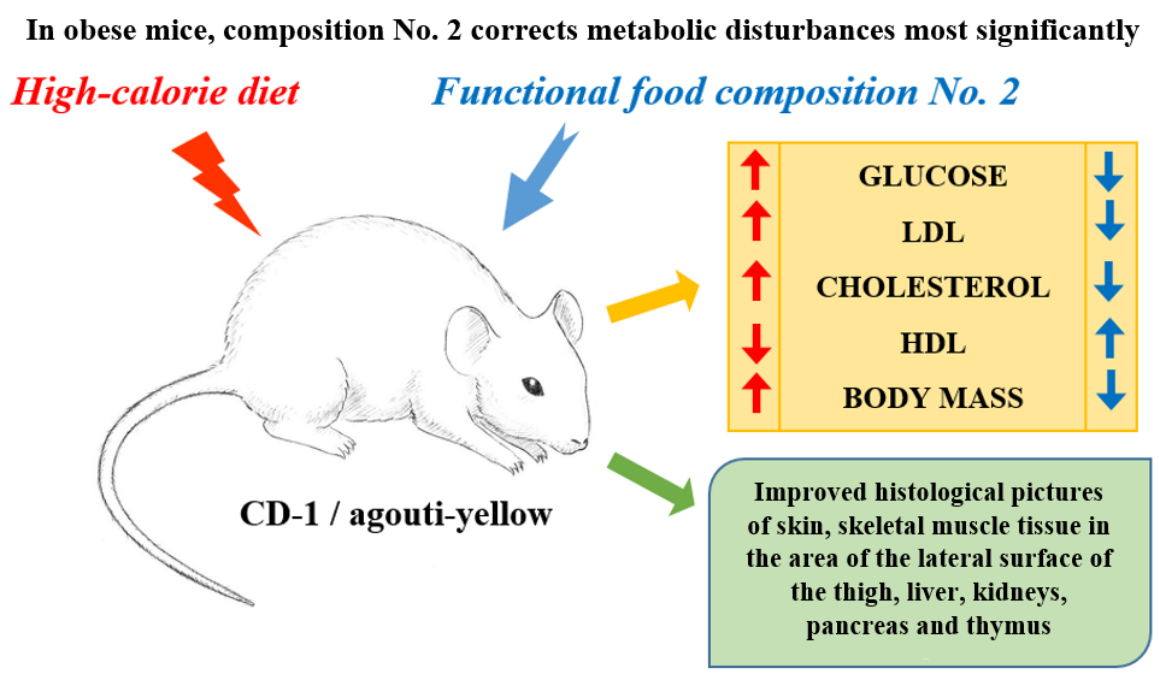
Methods: Agouti-yellow and CD1 mice were divided into 10 groups (control, 1/1, 1/9, 2/1, 2/9 in each line) according to diets. For 4 weeks, the groups ate a high-calorie diet. Starting with week 5th, composition 1 was added to the diet in groups 1/1 (60% lard + 39% standard laboratory feed + 1% composition 1 + 10% fructose solution), 1/9 (60% lard + 31% standard laboratory feed + 9% composition 1 + 10% fructose solution); composition 2 was added to the diet in groups 2/1 (60% lard + 39% standard laboratory feed + 1% composition 2 + 10% fructose solution), 2/9 (60% lard + 39% standard laboratory feed + 9% composition 2 + 10% fructose solution). On the day 56th, each animal was weighed, blood was taken for biochemical analysis (glucose, LDL, cholesterol and HDL) and organs (the skin of the anterior abdominal wall, skeletal muscle tissue in the area of the lateral surface of the thigh, liver, kidneys with perirenal tissue, pancreas and thymus) were taken for histological examination.

Results: In agouti-yellow mice, group 2/9, the greatest decrease in blood glucose level was observed compared to the

control group by 34.6% ($p < 0.05$). Consumption of compositions in groups 1/9 and 2/9 led to a significant decrease in LDL and a significant decrease in total cholesterol level by 21.9% ($p < 0.05$) and 18.8% ($p < 0.05$) respectively compared to the control group. HDL was significantly higher in groups 1/9, 2/9 than in the control group. In CD-1 mice, group 2/9 showed the best result in glucose level (mean 5.78 mmol/l) among all groups receiving functional food compositions and the control group. The most pronounced decrease in LDL was observed in groups 1/9, 2/9 compared to the control group ($p < 0.05$). HDL value in group 2/9 significantly exceeded the mean value in the control group, by 54.8% ($p < 0.05$). Body mass of agouti-yellow mice and CD-1 mice in groups 1/1, 2/1, 1/9 and 2/9 significantly decreased compared to control groups. According to the results of histological examination of organs and tissues, functional food composition 2 turned out to be the most promising.

Conclusion: According to the results of the study, the most promising composition was selected (composition 2). Feeding animals with this composition improved histological pictures of studied organs and tissues, biochemical blood parameters and effectively reduced body mass in both experimental animal lines, CD-1, and agouti-yellow mice, compared to the control groups.

Keywords: CD-1 mice, agouti-yellow mice, experimental obesity, LDL, cholesterol



INTRODUCTION

Currently, obesity is one of the most common problems in human life. Obesity can be categorized either as total obesity (body mass index ≥ 25.0 kg/m^2) and severe obesity (body mass index ≥ 30.0 kg/m^2) [1]. Obesity is the result of complex relationships between genetic, socio-economic and cultural factors. This condition can be a manifestation of various diseases, their complications, pharmacological treatment, bad habits, improper lifestyle, etc. [2-3]. Obesity may also be a risk factor for the development of concomitant diseases. The fundamental approach is to reduce weight and increase

physical activity; however, alternative methods of correction of such syndromes may be suitable for the prevention and reduction of the risk of complications of diabetes and cardiovascular diseases [4-5].

In the experiment, agouti-yellow mice with a dominant allele were used, which is the result of insertions, causing the expression of chimeric transcription encoding the wild-type agouti protein. Dominant agouti alleles increase the amount of yellow pigment in the coat and are associated with pleiotropic effects, including obesity, diabetes [6-7]. The agouti protein acts as an antagonist at melanocortin receptors. The á-melanocyte-stimulating hormone (MSH) normally acts on melanocortin-4 receptors in the hypothalamus to inhibit feeding and promote thermogenic metabolism. Blockade of á-MSH effects on melanocortin-4 receptors causes obesity in the agouti syndrome [8-9]. á-MSH has been reported to act in the hypothalamus to decrease blood pressure and heart rate in rats. It has been demonstrated that agouti-yellow obese mice have higher arterial pressure than their lean controls [10]. In this work, the effects of two functional food compositions in CD-1 and agouti-yellow mice in obesity simulation were investigated. Agouti-yellow mice were selected for the experiment because of their predisposition to obesity due to the "agouti" peptide and its effect on metabolic processes in the animal's body [11]. CD-1 mice were selected as the second experimental line since this line is standard for conducting preclinical studies, as well as not predisposed to obesity when kept in normal conditions [12]. Based on literature data, a high-fat diet induces the development of metabolic disorders in outbred CD-1 mice. Compared to mice on regular chow, high-fat diet-fed CD-1 mice gradually gained more fat mass and consequently exhibited accelerated body weight gain, which was associated with adipocyte hypertrophy and up-regulated expression of adipose inflammatory chemokines and cytokines such as Mcp-1 and Tnf-α [13].

The various component included in the definition of metabolic syndrome include measures of obesity, high-density lipoprotein, cholesterol, triglycerides, systolic or diastolic hypertension, and fasting hyperglycemia [14-15]. According to the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria, metabolic syndrome is diagnosed when three or more of the following risk factors are present: abdominal obesity (>102 cm in men, and >88 cm in women), hypertension ≥130/≥85 mmHg or specific medication, level of triglycerides ≥1.7 mmol/L or specific medication, low HDL cholesterol: in men <1.03 mmol/L, and in women <1.29 mmol/L or specific medication, and fasting plasma glucose ≥5.6 mmol/L or history of diabetes mellitus or taking antidiabetic drugs [16]. The mortality and morbidity of cardiovascular diseases associated with each of these criteria components in isolation or as metabolic syndrome make this problem one of the most important in modern society and medicine.

Today there is a shortage of vitamins, minerals and other biologically active substances, which is directly related to the lifestyle of modern man. The lack of vital nutrients leads to a decrease in working capacity, a decrease in the immune resistance to infections, metabolic disorders, as well as to the development of chronic diseases [17-18]. Thus, the development of functional food compositions for the correction of metabolic disturbances is very relevant.

METHODS

Animals: The animals were housed in an animal facility with a 12-h day/12-h night cycle and provided water. Ethical principles of conducting experiments on laboratory animals were observed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [Directive 2010/63/EU]. The experiments were approved by the Local Ethics Committee of Belgorod State National Research University, Belgorod, Russia (Protocol#05/21). The animals were kept in standard vivarium conditions with a natural light regime at a relative humidity of 40-50% and a temperature of 22-24 °C.

The study included 30 CD-1male mice and 30 agouti-yellow male mice. The agouti-yellow line is heterozygous for the dominant mutation Agouti viable yellow (A^{vy}/a), having a golden coat color. The study of obesity correction effectiveness with the studied compositions was planned to be carried out on mice of specified lines in combination with a high-calorie diet containing lard in an amount of 60% of the total ration, and a 10% solution of fructose, since obesity with such a combination of genetics and alimentary causes is expressed as much as possible, with the development of metabolic disturbances [19-20].

For a diet using excess fats of animal origin, it is supposed to use melted pork fat (lard) for 8 weeks as a percentage of crushed laboratory feed lard: feed = 60: 40. For feeding groups of animals on such a diet, granules of standard laboratory feed are crushed in advance and placed in a glass container with a wide neck, then heated to a liquid state (melted) lard is poured into this container. The mixture of liquid fat and crushed feed pellets is evenly mixed and cools, when cooled, the fat hardens, and the mixture takes on a sticky plastic consistency. This mixture is laid out in excess in the feeding compartments in the cages and replenished daily. In addition to a high-calorie diet, mice receive a 10% solution of fructose as a drink daily without a limit on the amount. At week 5, functional food compositions were added to the high-calorie diet.

Study subjects: Composition No. 1: dried grapes, dried apple, almond kernels, natural honey, flax seeds, dried cranberries, ground cinnamon, vanilla flavor, ground cloves. 100 g of composition No. 1 contains 1.9 g of PUFAs, including 1.48 g of ω-3; vitamin A <0.01 mg; β-carotene 0.05 mg; vitamin C 5.6 mg; vitamin PP 1.3 mg; vitamin B1 0.22 mg; vitamin B2 0.04 mg.

Composition No. 2: dried apricot, dried grapes, dried apple, almond kernels, natural honey, flax seeds, dried cranberries, vanilla flavor, ground cinnamon, ground cloves. 100 g of composition No.2 contains 2.9 g of PUFAs, including 1.6 g of ω-3; vitamin A <0.01 mg; β-carotene 0.6 mg; vitamin C 15.5 mg; vitamin PP 2.3 mg; vitamin B1 0.3 mg; vitamin B2 0.2 mg.

Both compositions were provided by VITBIOKOR LLC (Vitebsk region, Republic of Belarus) by order of ARIDA LLC (Smolensk, Russian Federation).

Study design: The experiment included 10 groups of animals, 6 mice in each group. Agouti-yellow and CD1 mice were divided into 5 groups (control, 1/1, 1/9, 2/1, 2/9) according to diets:

1) Control group: high-calorie diet = 60% lard + 40% standard laboratory food + 10% fructose solution;
2) Group 1/1: 60% lard + 39% standard laboratory feed + 1% "Composition No. 1" + 10% fructose solution;
3) Group 1/9: 60% lard + 31% standard laboratory feed + 9% "Composition No. 1" + 10% fructose solution;
4) Group 2/1: 60% lard + 39% standard laboratory feed + 1% "Composition No. 2" + 10% fructose solution;
5) Group 2/9: 60% lard + 31% standard laboratory feed + 9% "Composition No. 2" + 10% fructose solution.

For 4 weeks, the groups ate a high-calorie diet. Starting with week 5, food composition No. 1 was added to the diet in groups 1/1, 1/9; food composition No. 2 was added to the diet in groups 2/1, 2/9.

On the euthanasia day (day 56^{th}), blood was taken for biochemical analysis, and organs were taken for morphological examination. Euthanasia of animals was carried out according to the requirements set out in the "International Recommendations for conducting Biomedical Research using Animals" (1997). Euthanasia was performed by dislocation of the cervical vertebrae, and blood samples were immediately taken by heart puncture. Each animal was weighed and removed from the experiment for histological examination of internal organs.

Analyzed biochemical blood parameters: On the 56^{th} day of the experiment, blood samples were taken from all mice on an empty stomach to assess biochemical parameters. The serum was separated by centrifugation at 3000 g for 10 min at 4°C after full clotting was ensured.

The separated serum was used to analyze clinical biochemical parameters, glucose, total cholesterol, high-density lipoproteins, and low-density lipoproteins.

Histological examination: After blood was taken from the mice, their internal organs of interest, namely the skin of the anterior abdominal wall, the skeletal muscle tissue of the lateral surface of the thigh, liver, kidneys with perirenal tissue, pancreas, and thymus, taken for biopsy. The organs were placed in a 10% formalin solution for histopathological analysis with hematoxylin and eosin staining. Organs and tissues were examined by light microscopy.

To compare tissue samples, the following metrics were used:

- Skin:
  1. Increase in the number and density of adipocytes in the hypoderm to the skin muscle (between the hair follicles).
  2. Increase in the number and density of adipocytes in the hypodermis under the skin muscle.
  3. Increase in the thickness of the hypodermis.
  4. Change in the shape and size of adipocytes.
  5. The presence of a tendency to merge into segments.
- Skeletal muscle:
  1. Accumulation of adipocytes between individual symplasts.
  2. Accumulation of adipocytes in the outer covering fascia.
- Liver:
  1. The change in the color of the hepatocytes' cytoplasm – light foamy, which indicates small-drop fatty dystrophy.
  2. Vasodilation – either the central vein or the interlobular vein in triads.
  3. Increase in the number of multinucleated hepatocytes.
  4. Accumulation of adipocytes in the stroma of the organ.
  5. Accumulation of adipocytes in the capsule of the organ.
  6. Infiltration of the organ stroma.
- Pancreas:
  1. Accumulation of adipocytes in the organ capsule.
  2. Accumulation of adipocytes in trabeculae.
  3. Change in zonality in the acinus – a blurring of contours between zymogenic and homogeneous zones.
  4. Increase in the size of the islets of Langerhans.
  5. Infiltration of the organ stroma.
- Thymus:
  1. Change in the lobule structure – change in the ratio of the thickness of the cortical and medullary substances, change in the shape of the lobule.
  2. Erasure of the boundary between the cortical and medullary substances.
  3. An increase in the Ghassal corpuscles in the medullary substance.
  4. Accumulation of adipocytes in trabeculae.
  5. Accumulation of adipocytes in the capsule of the organ.
- Kidneys:
  1. The size of the glomerulus.
  1. Infiltration of the glomerulus.
  2. Change in the width of the subcapsular space.
  3. Change in the height of the tubules' epithelium (proximal or distal).
  4. Infiltration of the organ stroma.
  5. Dilation of blood vessels.
  6. Accumulation of adipocytes in the stroma of the organ.
  7. The content of adipocytes in the capsule of the organ.

Statistical analysis: The data were checked for the normality of the distribution using the Shapiro-Wilk criterion. Data with normal distribution were compared using ordinary one-way analysis of variance (ANOVA) with a Tukey's post-hoc test. Data with abnormal distribution were compared with the Kruskal-Wallace

test and Dunn's post hoc test. Differences were determined at a 0.05 significance level. Statistical analyses were performed using GraphPad Prism 9.2.0 software.

RESULTS

Histology of agouti-yellow mice groups: In the analysis of qualitative histological data in agouti-yellow mice, it was revealed that the use of compositions 1 and 2 (1% of the diet) did not lead to improvements in histological pictures of studied organs and tissues (Fig.1-3).

In agouti-yellow mice, the use of compositions 1 and 2 (9% of the diet) led to the improvement of histological pictures in comparison with the control group that received a high-calorie diet (Fig. 4, 5). Microscopy of skin sections of group 1/9 (Fig. 4a) visualizes white adipose tissue in the mesh layer of the dermis between the hair follicles and the hypoderm under the "skin's own muscle", the thickness of which is less than in the group 1/1 (Fig. 1b).

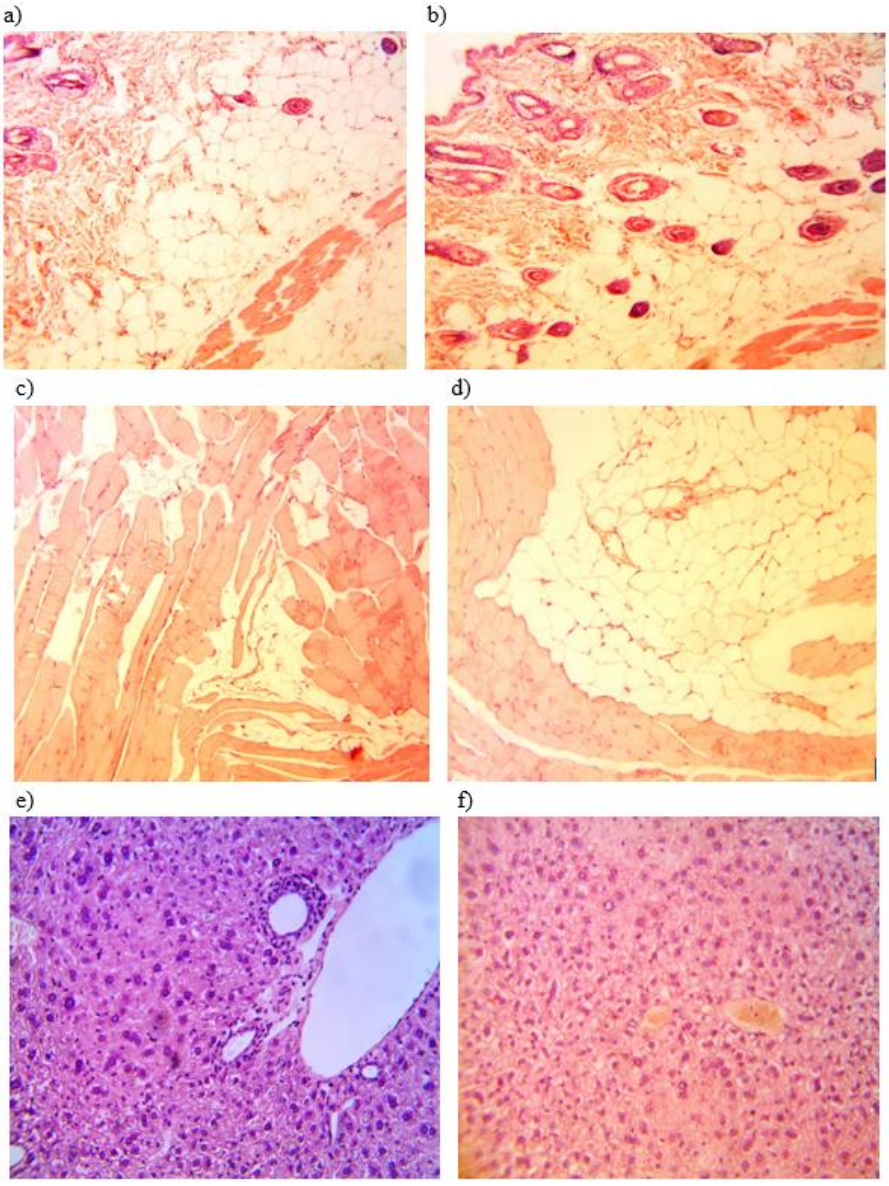


Figure 1. Comparative histology of the control group and group 1/1 (agouti-yellow mice) **a)** Skin section (control). Magnification of ×200. **b)** Skin section (1/1). Magnification of ×200. **c)** Skeletal muscle (control). Magnification of ×200. **d)** Skeletal muscle (1/1). Magnification of ×200. **e)** Liver (control). Magnification of ×200. **f)** Liver (1/1). Magnification of ×200.

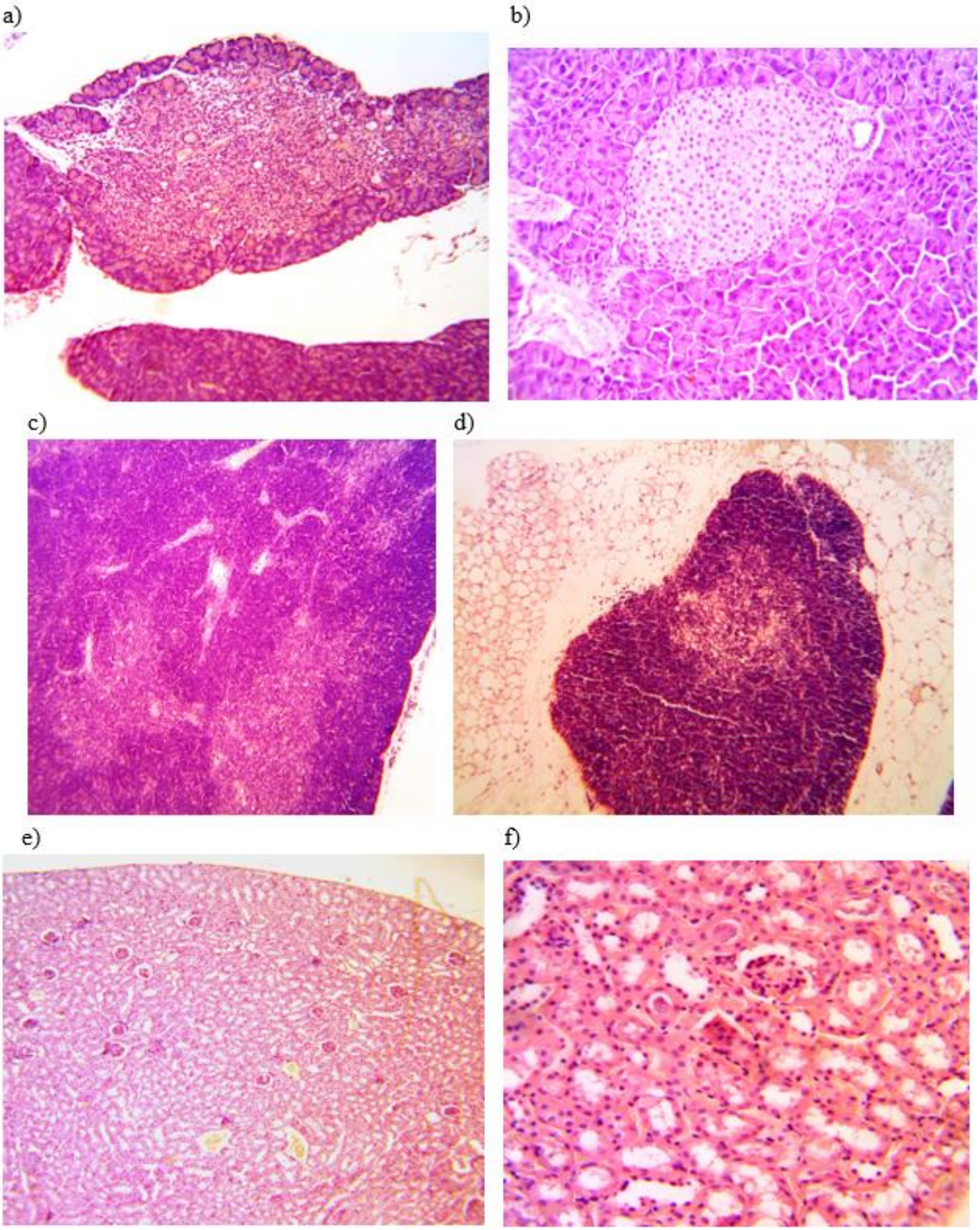


Figure 2. Comparative histology of the control group and group 1/1 (agouti-yellow mice) **a)** Pancreas (control). Magnification of ×100. **b)** Pancreas (1/1). Magnification of ×400. **c)** Thymus (control). Magnification of ×100. **d)** Thymus (1/1). Magnification of ×100. **e)** Kidney (control). Magnification of ×100 **f)** Kidney (1/1). Magnification of ×200.

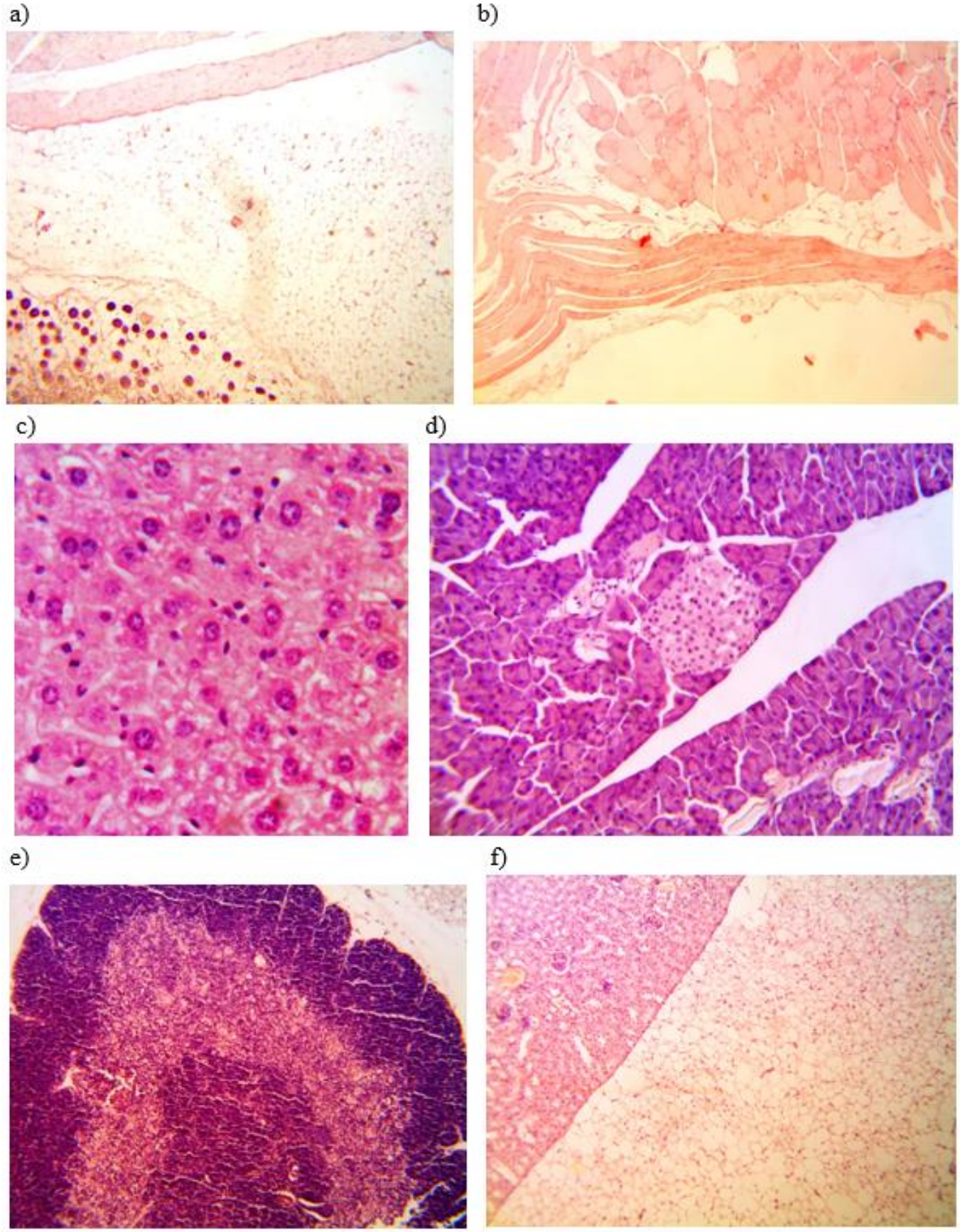


Figure 3. Histology of the group 2/1 (agouti-yellow mice) **a)** Skin section. Magnification of ×100. **b)** Skeletal muscle. Magnification of ×200. **c)** Liver. Magnification of ×400. **d)** Pancreas. Magnification of ×400. **e)** Thymus. Magnification of ×100. **f)** Kidney. Magnification of ×100.

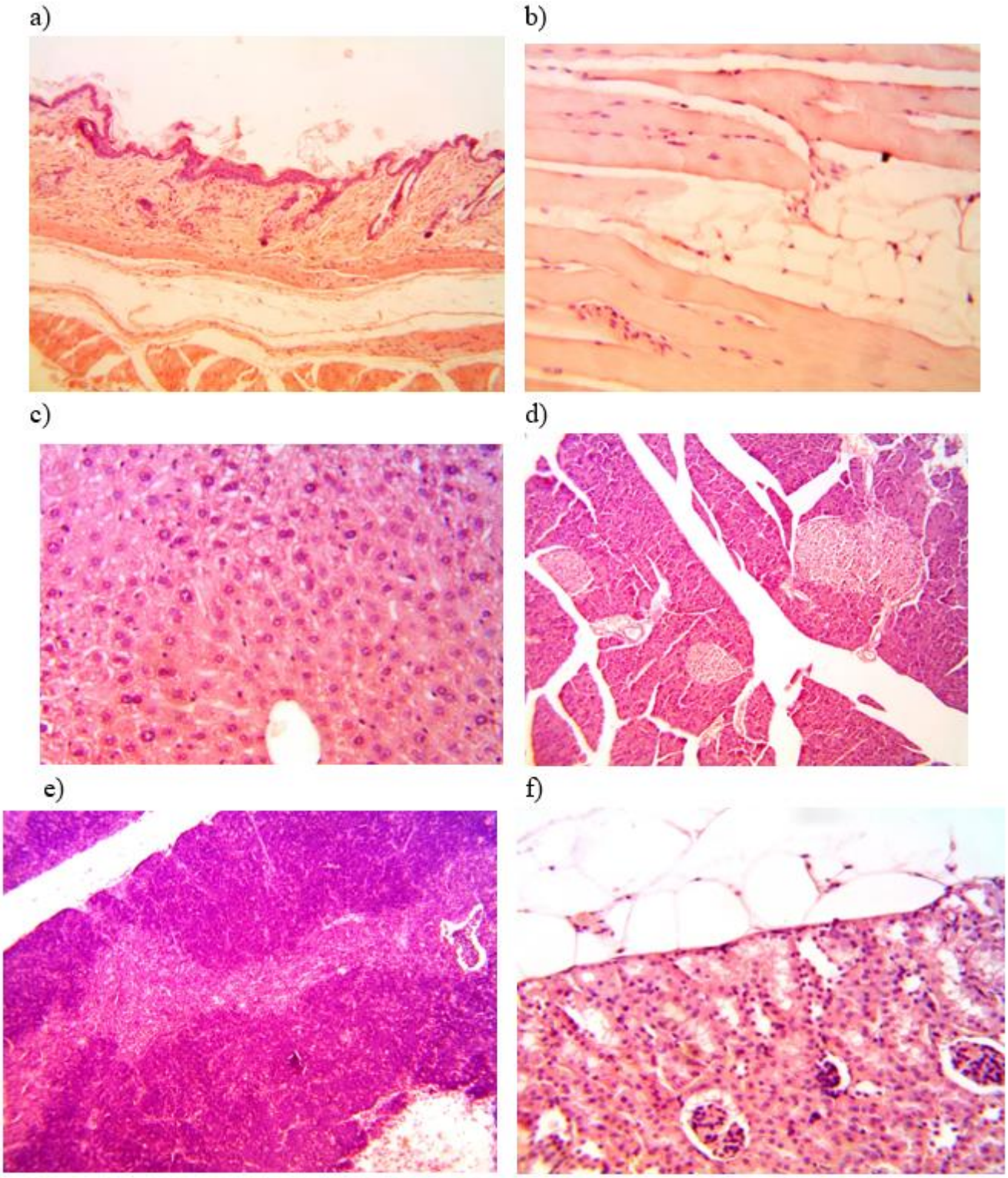


Figure 4. Histology of the group 1/9 (agouti-yellow mice) **a)** Skin section. Magnification of ×200. **b)** Skeletal muscle. Magnification of ×400. **c)** Liver. Magnification of ×200. **d)** Pancreas. Magnification of ×200. **e)** Thymus. Magnification of ×200. **f)** Kidney. Magnification of ×400.

During microscopy of skeletal muscle tissue in group 1/9, agouti-yellow mice, white adipose tissue is localized around the muscle and in the layers between the symplasts (Fig. 4b), but its amount is less than in the group 1/1 (Fig. 1d).

When microscopy of liver sections, the histological picture is much better – there are no dilated central veins, more uniform cell density, hepatocytes with fatty

degeneration are isolated with localization along the periphery of the lobules. There is no infiltration of the stroma (Fig. 4c).

Microscopy of pancreas sections shows that the number of large islets of Langerhans is less than in the group 1/1, but in some preparations even giant ones are visualized. The exocrine part is without destructive changes (Fig. 4d).

Microscopy of thymus sections shows a change of the lobules structure, manifested by a change in the thickness and ratio of the cortex and medulla. In the thickness of the cortical substance, small areas of the medulla are visualized, as well as a large number of reticuloepithelial cells. At the same time, the degree of severity of reactive changes is less than in group 1/1 (Fig. 4e).

Microscopy of kidney sections shows single wrinkled glomeruli, mostly unchanged renal corpuscles in the field of view. The arc vessels are dilated. The outside of the kidney is covered with a capsule, the bulk of which is white and brown adipose tissue, well-structured and forming lobules. Adipose tissue is visualized in the area of the kidney gate (Fig. 4f).

Microscopy of skin sections of group 2/9 (Fig. 5a) visualizes a small amount of white adipose tissue in the hypoderm, between the hair follicles. In the hypoderm, under the "skin's own muscle" up to the muscles of the anterior abdominal wall, a small fat layer is localized, the thickness of which is less than in the group 2/1. Adipocytes are large, irregular in shape.

Microscopy of skeletal muscle – between the symplasts the adipose tissue was not revealed, small clusters of adipocytes around the muscle (Fig. 5b).

Microscopy of liver sections shows a different density of cells in the center and on the periphery of the lobule, there are hepatocytes with fatty degeneration, mainly around the central vein. The density of hepatic macrophages is high. A large number of binuclear hepatocytes is visualized in the field of vision (Fig. 5c).

Microscopy of pancreas sections revealed no structural abnormalities. Endocrine islets of usual shape and size with clear contours without deformation. The exocrine part without features (Fig. 5d).

When microscopy of sections, structural deformation of the thymus lobule is observed. The thickness of the cortical layer is greater than the cerebral. Single adipocytes are visualized in thickened septa. There is a large number of Ghassal bodies in the cerebral substance (Fig. 5e).

In microscopy of kidney sections, the severity degree of reactive changes is minimal. The infiltration of stroma vessels persists. The capsule is thick, formed mainly of white and brown adipose tissue, the adipocytes of which are hypertrophied with uneven contours (Fig. 5f).

Histology of CD-1 mice groups: It was found that the addition of compositions 1 and 2 (1% of the diet) to the diet of CD-1 mice did not have a significant effect on the histological pictures of the studied organs and tissues compared to the control group (Fig.6, 7).

In the tissues and organs structure of group 1/9 (Fig.8), there was a noticeable improvement in the condition compared to the control group, as well as groups 1/1 and 2/1, but it turned out to be less pronounced compared to group 2/9 (Fig. 9).

In microscopy of skin sections of group 1/9, in the hypoderm, there are no fat deposits between the hair roots, or single adipocytes are visualized. There is also no adipose tissue under the "skin's own muscle" to the muscles of the anterior abdominal wall (Fig. 8a).

Microscopy of skeletal muscle tissue shows no adipose tissue in the area of the lateral surface of the thigh between the muscle fibers. At the same time, a large number of adipocytes with a tendency to merge

into lobules are visualized around the muscles from the outside (Fig. 8c).

Microscopy of liver sections visualizes normal lobules, lobules with fatty dystrophy are single. There is a round-cell infiltration of the stroma with predominant localization in the triad region, in proximity to the bile duct (Fig. 8b).

There is no infiltration of the stroma by microscopy of the pancreas. Islands of the usual shape and size (Fig. 8e).

In microscopy of the thymus, there is no disruption in the structure of the lobules, but the ratio of the cortical and cerebral matter is changed. The stromal component is strongly pronounced, the layered epithelial corpuscles are large (Fig. 8d).

Microscopy of kidney sections shows an accumulation of lymphocytes in the area of the adventitia of the arc and interlobular vessels. All blood vessels are full. The arc vessels are dilated. The density of mesangium cells is high. The renal corpuscle is without changes (Fig. 8f).

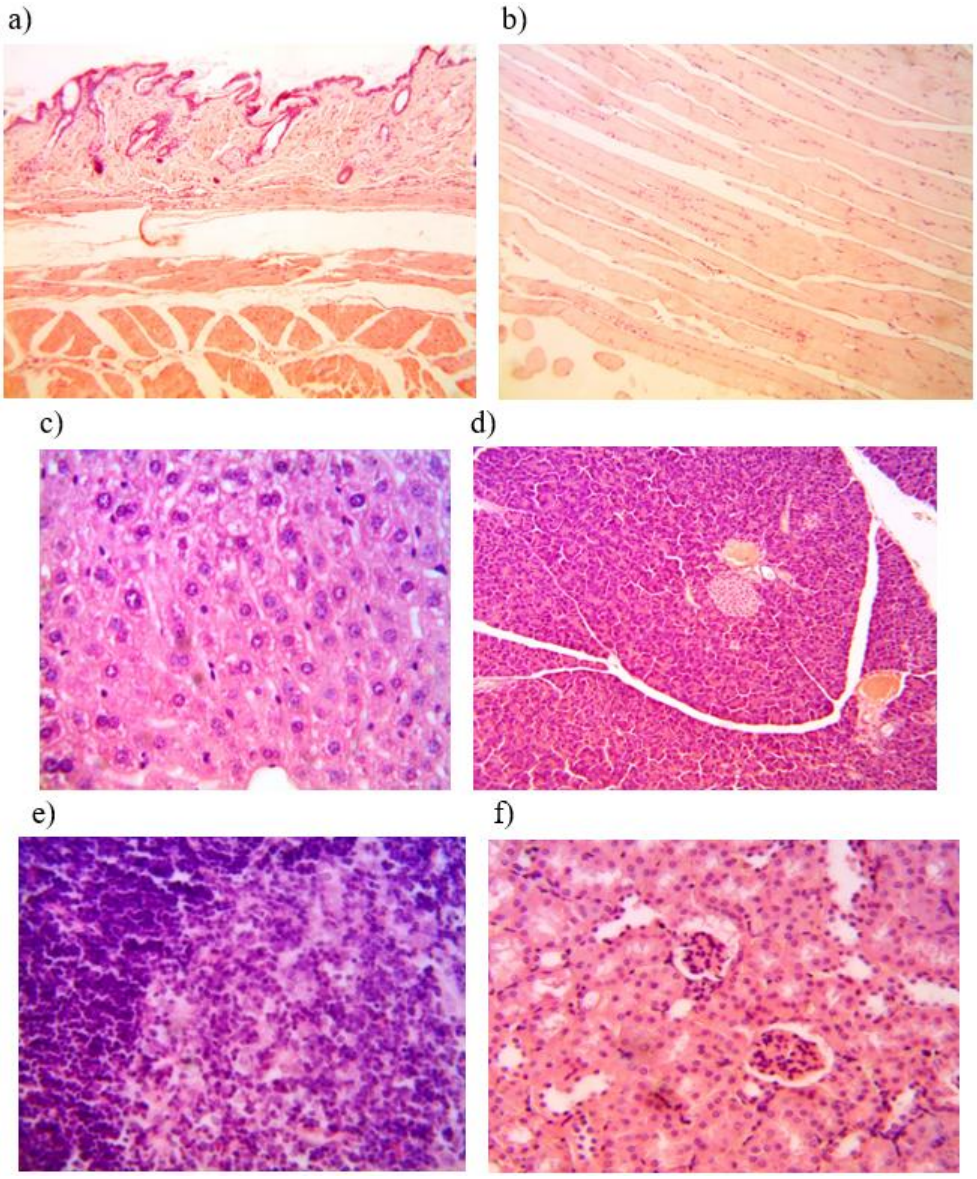


Figure 5. Histology of the group 2/9 (agouti-yellow mice) **a)** Skin section. Magnification of ×100. **b)** Skeletal muscle. Magnification of ×200. **c)** Liver. Magnification of ×400. **d)** Pancreas. Magnification of ×200. **e)** Thymus. Magnification of ×400. **f)** Kidney. Magnification of ×200.

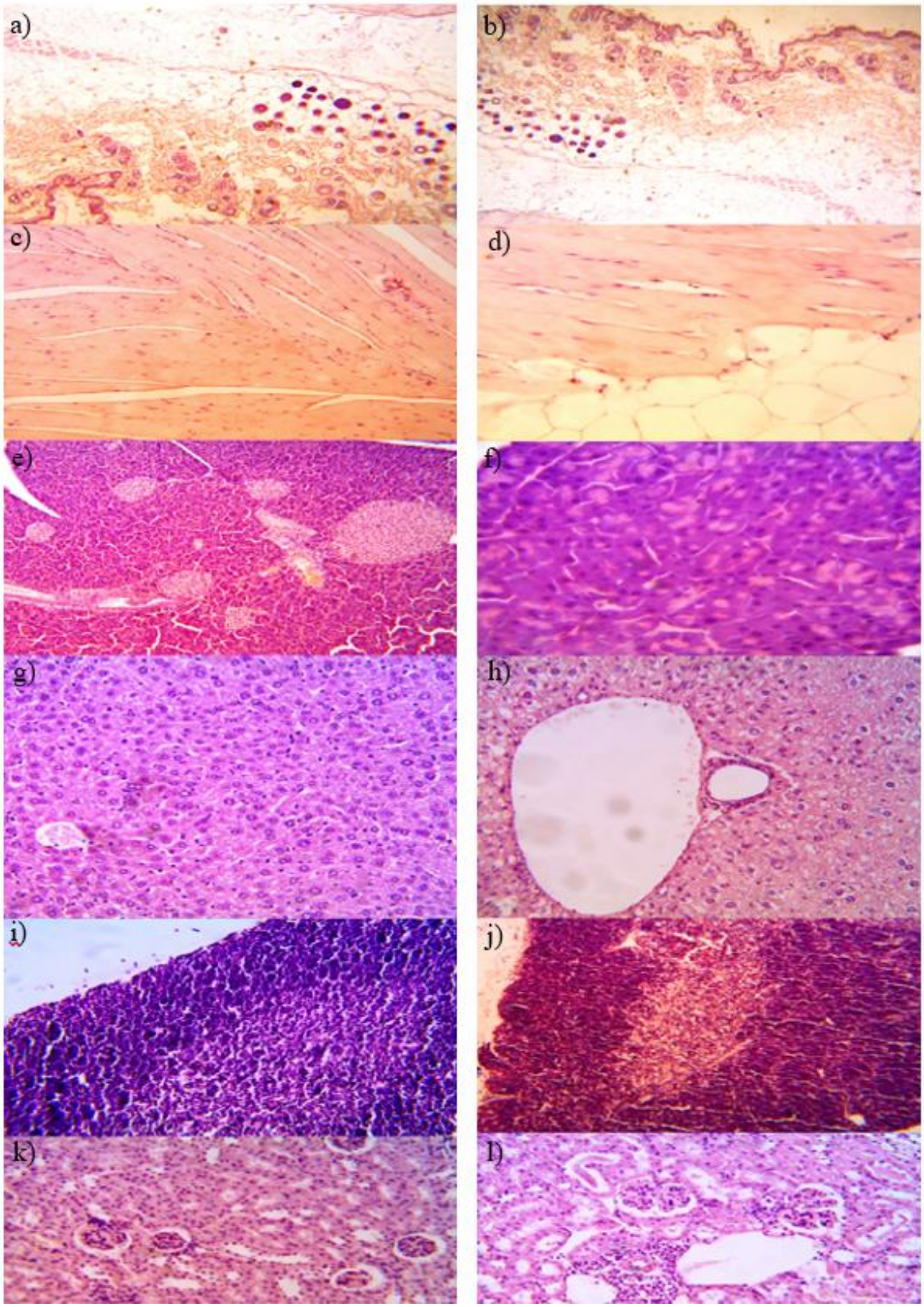


Figure 6. Comparative histology of the control group and group 1/1 (CD-1 mice) **a)** Skin section (control). Magnification of ×200. **b)** Skin section (1/1). Magnification of ×200. **c)** Skeletal muscle (control). Magnification of ×200. **d)** Skeletal muscle (1/1). Magnification of ×200. **e)** Pancreas (control). Magnification of ×100. **f)** Pancreas (1/1). Magnification of ×400. **g)** Liver (control). Magnification of ×200. **h)** Liver (1/1). Magnification of ×200. **i)** Thymus (control). Magnification of ×200. **j)** Thymus (1/1). Magnification of ×100. **k)** Kidney (control). Magnification of ×200 **l)** Kidney (1/1). Magnification of ×200.

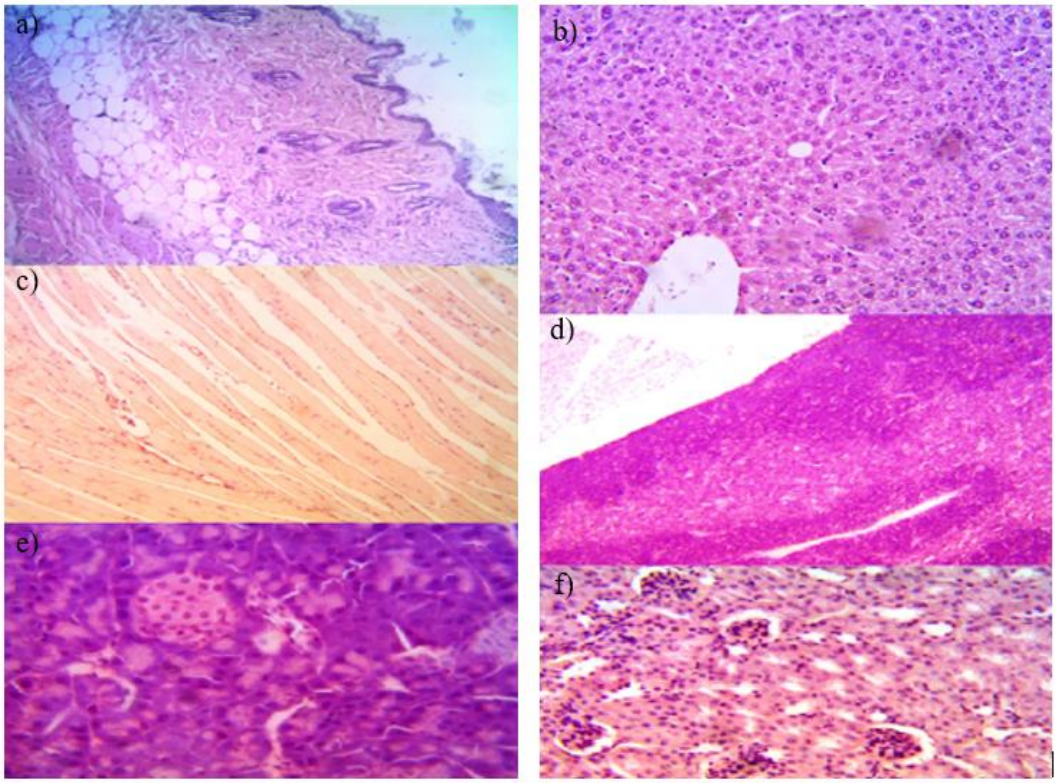


Figure 7. Histology of the group 2/1 (CD-1 mice) **a)** Skin section. Magnification of ×100. **b)** Liver. Magnification of ×200. **c)** Skeletal muscle. Magnification of ×200. **d)** Thymus. Magnification of ×100. **e)** Pancreas. Magnification of ×400. **f)** Kidney. Magnification of ×200.

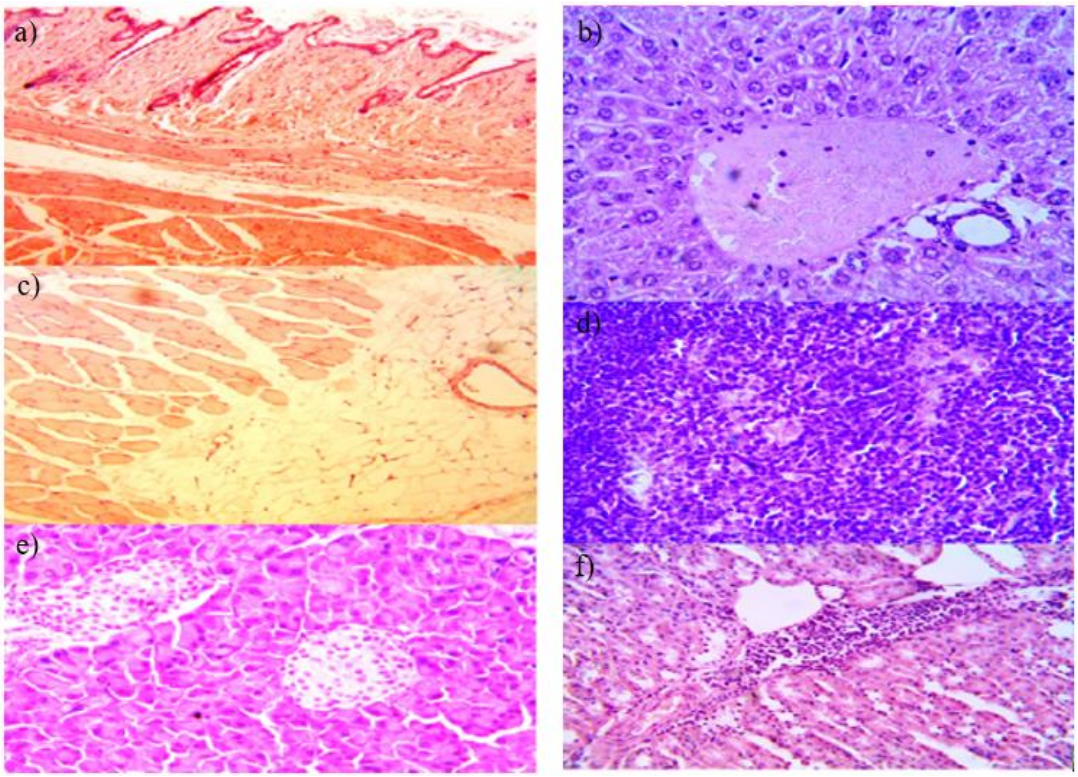


Figure 8. Histology of the group 1/9 (CD-1 mice) **a)** Skin section. Magnification of ×100. **b)** Liver. Magnification of ×400. **c)** Skeletal muscle. Magnification of ×200. **d)** Thymus. Magnification of ×400. **e)** Pancreas. Magnification of ×400. **f)** Kidney. Magnification of ×400.

In microscopy of skin sections of group 1/9, in the hypoderm, there are no fat deposits between the hair roots. In the hypoderm, there is also no adipose tissue under the "skin's own muscle" to the muscles of the anterior abdominal wall (Fig. 9a).

Microscopy shows no adipose tissue in skeletal muscle between the muscle fibers (Fig.9c).

In group 2/9, the degree of reactive changes in the liver is the lowest in comparison with all experimental groups. Microscopy of liver sections visualizes ordinary lobules, hepatocytes without signs of fatty dystrophy. Stroma infiltration is weakly expressed. The interlobular veins in the triads are dilated (Fig. 9b).

There is no infiltration of the stroma by microscopy of the pancreas. Islands of the usual shape and size (Fig. 9e).

In microscopy of thymus sections, there are no pathological changes in the structure of the lobules, but the boundary between the cortical and cerebral substance is blurred (Fig. 9d).

Microscopy of kidney sections shows a weakly pronounced infiltration of the stroma. Renal corpuscles of the usual shape and size. Adipose tissue is present only in the area of the kidney gate and in the capsule (Fig. 9f).

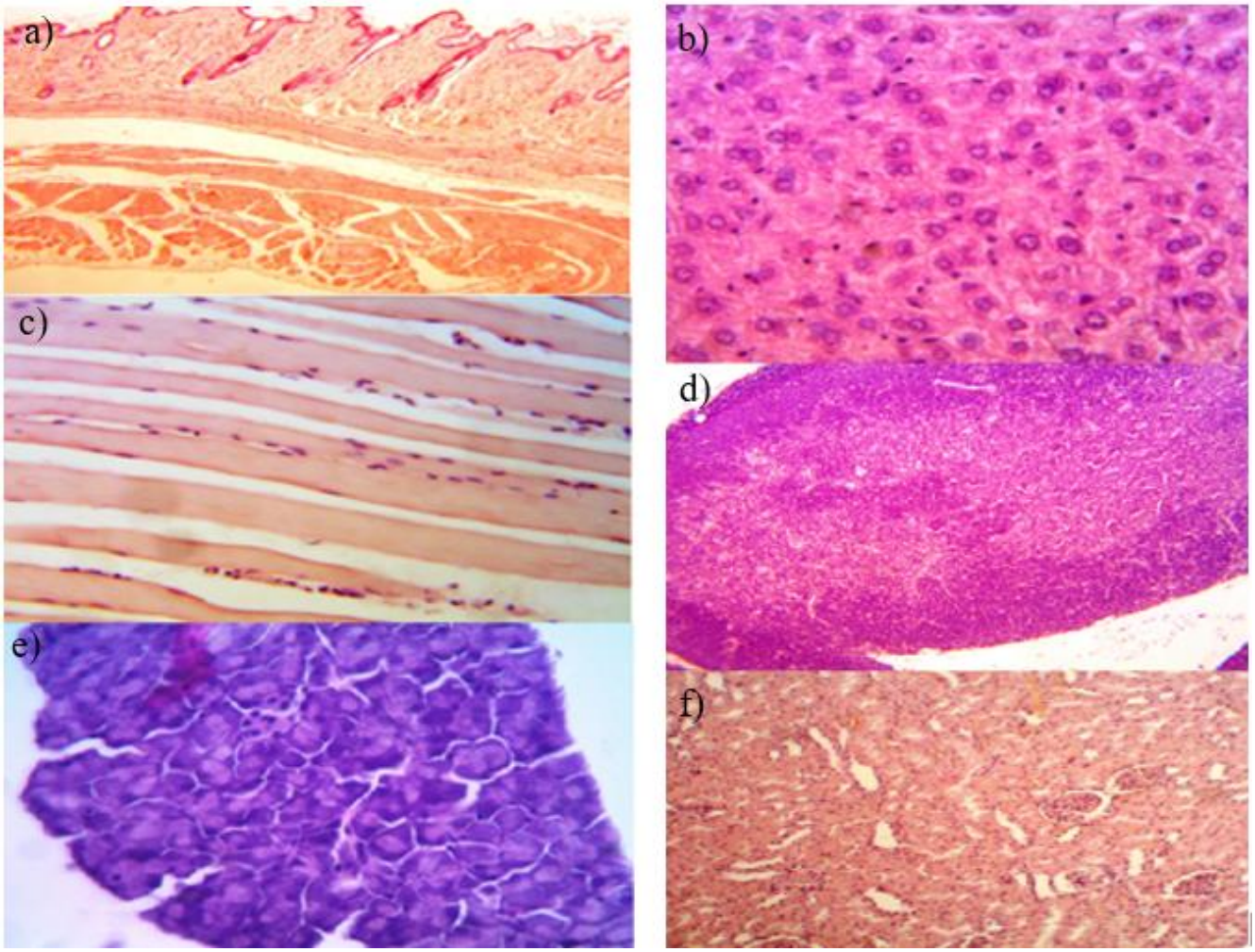


Figure 9. Histology of the group 2/9 (CD-1 mice) **a)** Skin section. Magnification of ×200. **b)** Liver. Magnification of ×400. **c)** Skeletal muscle. Magnification of ×400. **d)** Thymus. Magnification of ×200. **e)** Pancreas. Magnification of ×400. **f)** Kidney. Magnification of ×100.

Biochemical blood markers in agouti-yellow mice: Glucose level was the highest in mice fed a high-calorie diet. In all groups that received compositions to the diet, it was possible to reduce this marker. In group 2/9, the greatest decrease in blood glucose level was observed compared to the control group, by 34.6% ($p < 0.05$) (Fig. 10a).

The LDL parameter was significantly higher in mice receiving a high-calorie diet. Consumption of compositions in groups 1/9 and 2/9 led to a significant decrease in LDL, more pronounced than in groups 1/1, 2/1 (Fig. 10b).

The total cholesterol was significantly higher in mice that received a high-calorie diet. Consumption of compositions 1 and 2 in groups 1/9 and 2/9 led to a significant decrease in total cholesterol level by 21.9% ($p < 0.05$) and 18.8% ($p < 0.05$) respectively compared to the control group (Fig. 10c).

HDL data were significantly higher in groups 1/1 and 2/1 than in the control group and were significantly higher in groups 1/9, 2/9 than in the control group also (Fig. 10d).

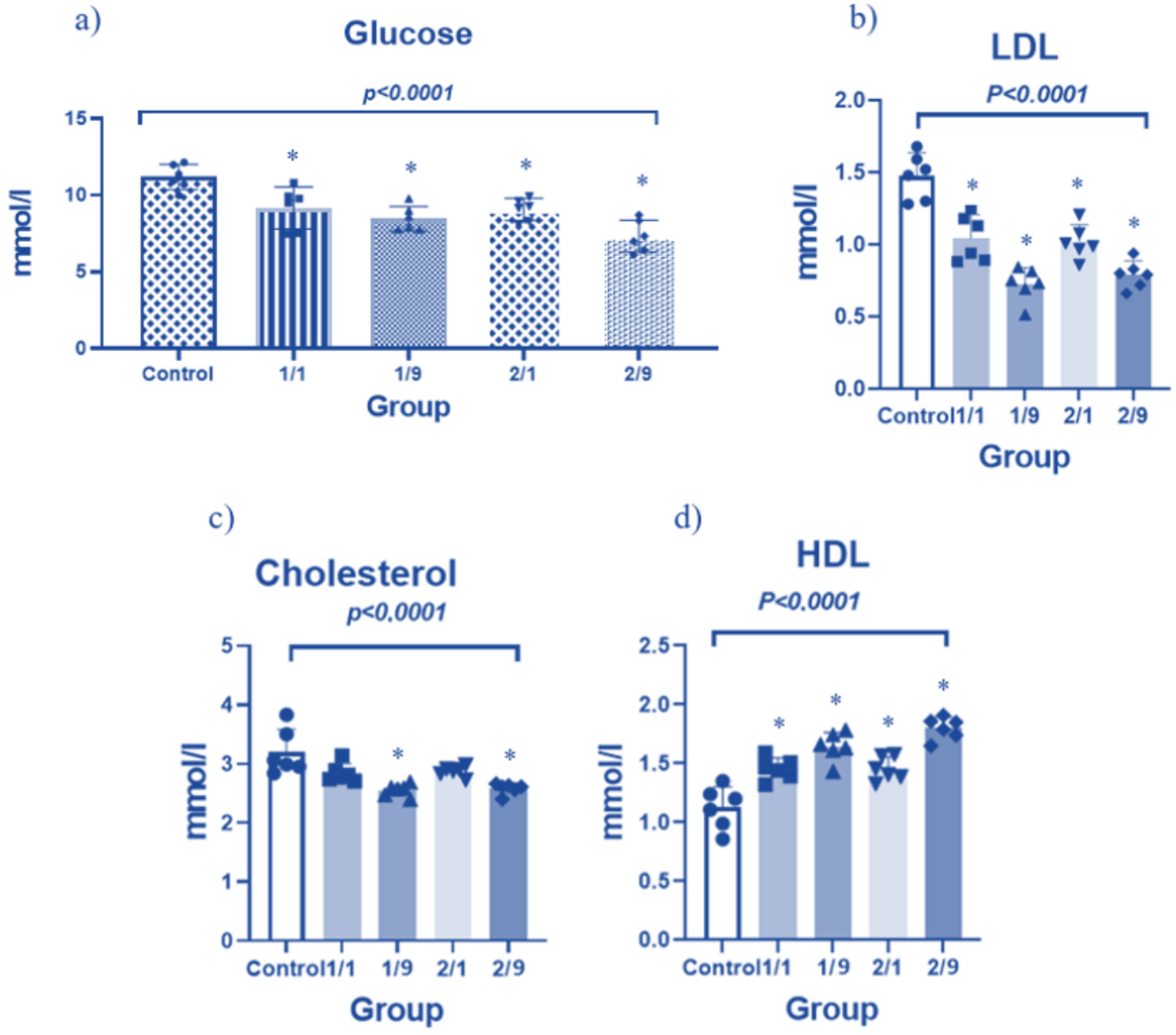


Figure 10. The glucose, LDL, cholesterol, and HDL levels in blood plasma in experimental groups (agouti-yellow mice) (M ± SD; n = 6), mmol/l. *Statistically significant, p < 0.05 compared to the control group.

Biochemical blood markers in CD-1 mice: The highest glucose level was observed in the control group. Approximately the same decrease in glucose levels compared to the control group was observed in groups 1/1 and 2/1. Group 2/9 showed the best result (mean 5.78 mmol/l) among all groups receiving functional food compositions and the control group (Fig. 11a).

The values of total cholesterol (Fig. 11c) and LDL (Fig. 11b) were significantly higher in mice receiving a high-calorie diet. Consumption of compositions 1 and 2 in groups 1/1 and 2/1 led to a decrease in LDL compared to the control group, but the most pronounced decrease in LDL was observed in groups 1/9, 2/9.

HDL values in groups 1/9 and 2/9 significantly exceeded the mean value in the control group (Fig. 11d).

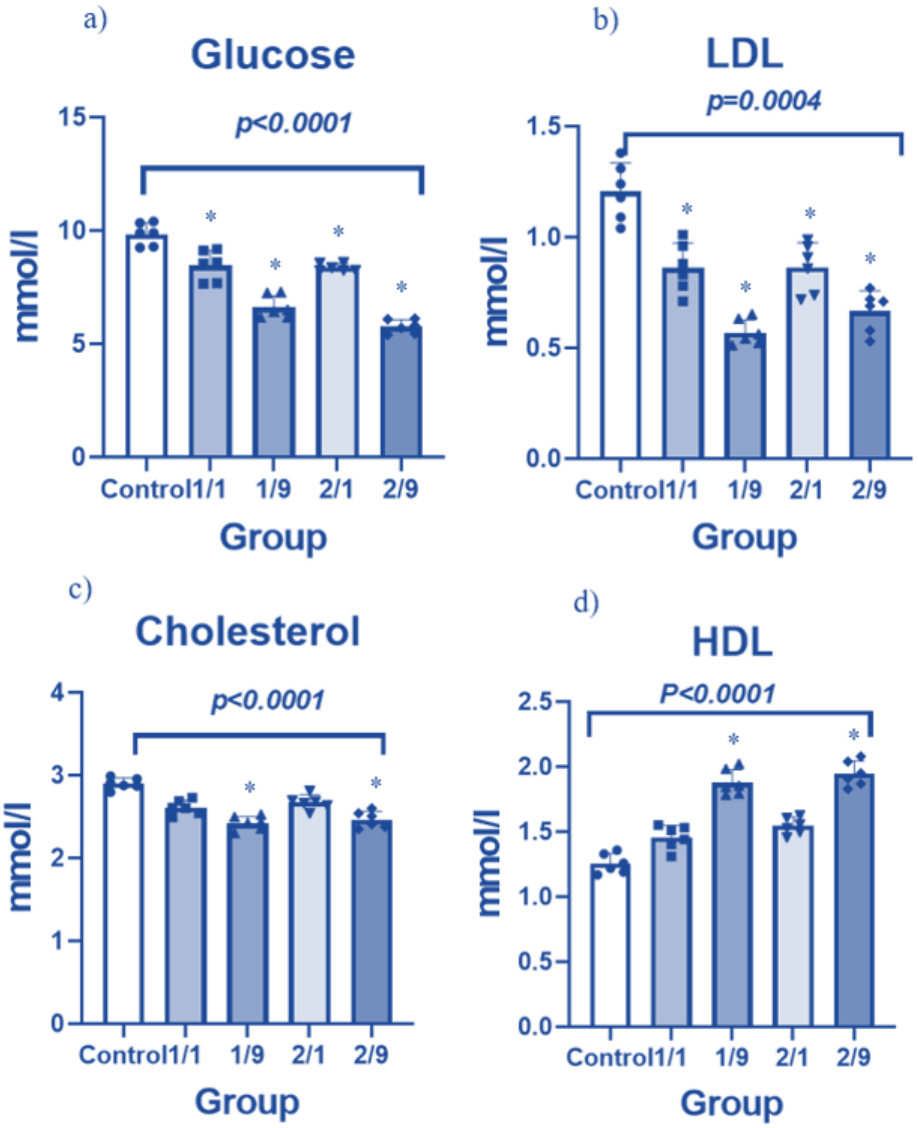


Figure 11. The glucose, LDL, cholesterol, and HDL levels in blood plasma in experimental groups (CD-1 mice) (M ± SD; n = 6), mmol/l. *Statistically significant, p < 0.05 compared to the control group.

The body mass change of agouti-yellow mice: After 4 weeks of feeding all groups with a high-calorie diet, the mass of the animals averaged 43.5 grams, and no significant difference was found between the masses in different groups (p=0.0793) (Fig. 12a). At the end of the experiment, a significant difference was observed between all groups (p<0.0001) (Fig. 12b). Body mass of mice in groups 1/1, 2/1, 1/9 and 2/9 significantly decreased compared to the control group.

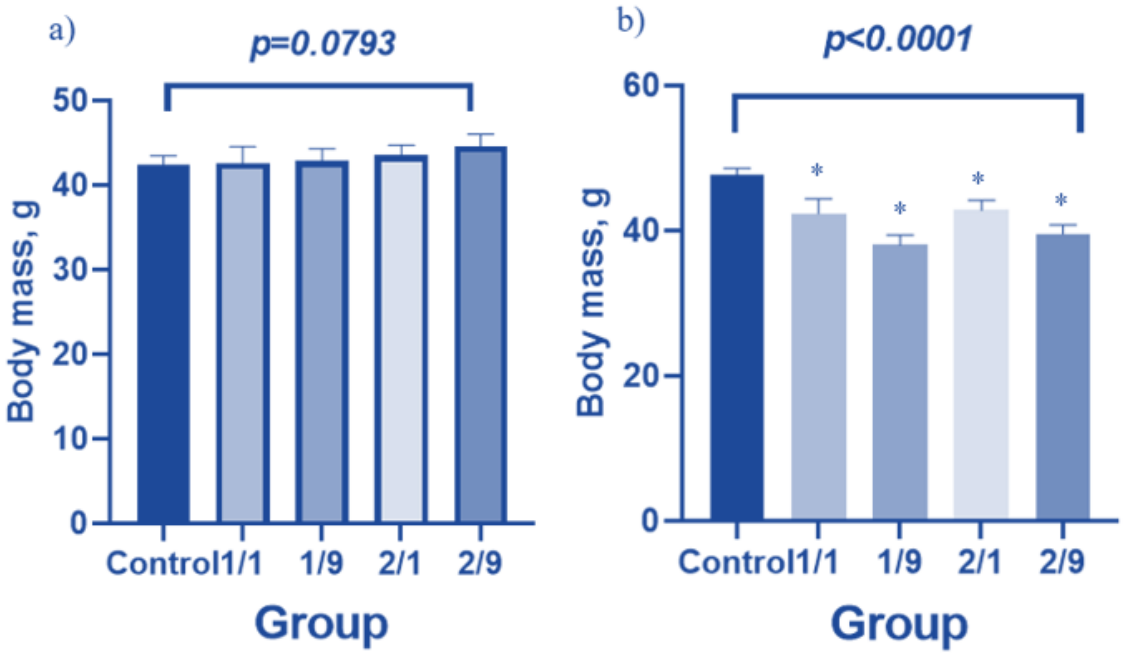


Figure 12. The effect of functional food compositions on the body mass in experimental obesity (agouti-yellow mice) (M ± SD; n = 6), g **a)** After 4 weeks of feeding all groups with a high-calorie diet (without compositions 1, 2). **b)** After 4 weeks of using compositions 1, 2 (day 56th). *Statistically significant, $p < 0.05$ compared to the control group.

The body mass of CD-1 mice: After 4 weeks of feeding all groups with a high-calorie diet, the body mass of all animals averaged 35.15 grams, and no significant difference was found between the masses in different groups (p=0.361) (Fig. 13a). At the end of the experiment, a significant difference was reliably observed between all experimental groups and the control group (Fig. 13b).

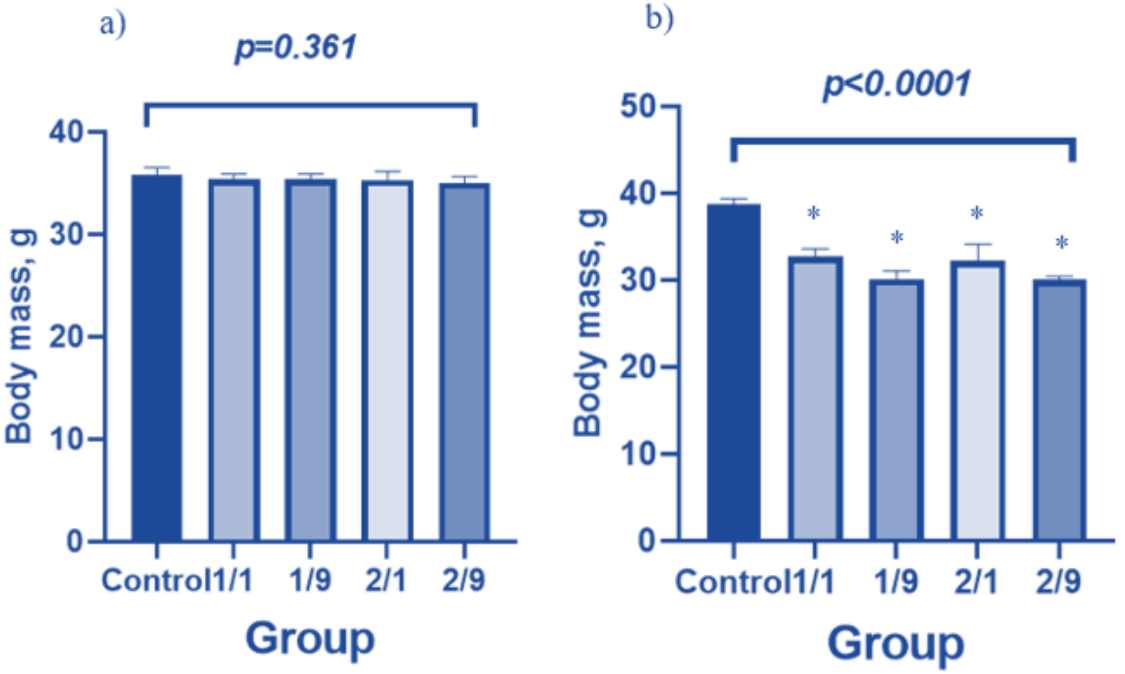


Figure 13. The effect of functional food compositions on the body mass in experimental obesity (CD-1 mice) (M ± SD; n = 6), g **a)** After 4 weeks of feeding all groups with a high-calorie diet (without compositions 1, 2). **b)** After 4 weeks of using compositions 1, 2 (day 56th). *Statistically significant, $p < 0.05$ compared to the control group.

DISCUSSION

Obesity represents a major problem because it increases the risk of cardiovascular disease, hypertension, diabetes, dyslipidemia, obstructive sleep apnea, and cancers, thus contributing to a decline in life quality and expectancy [21].

The use of medicinal plants and nutraceuticals have always functioned as a key source in prevention and treatment of chronic diseases like diabetes, obesity, or diabesity, and are also considered to be the most cost-effective natural source of health care [22]. Both studied functional food compositions contain PUFAs, including ω-3, vitamin A, β-carotene, vitamin C, vitamin PP, vitamin B1, vitamin B2, but in different amounts. As well known, the nutraceuticals with anti-obesity properties are ω-3 polyunsaturated fatty acids (PUFAs), which can act on inflammation in adipose tissue. Both ω-3 and ω-6 PUFAs contribute to the production of endocannabinoids, which are notably involved in the control of food intake, energy sensing, food-related disorders, stress response, etc. [23-25]. Based on the literature data, mice fed the lowest ω-6/ω-3 ratio had the lowest non-HDL (i.e., atherogenic lipoproteins) and IL-6. Mice fed lower ω-6/ω-3 ratio diets also had less macrophage cholesterol increase and fewer aortic atherosclerotic lesions [26]. Yang et al. reported that β-carotene improved the viability of hepatocytes, and increased catalase activities and glutathione levels in hepatocytes from chronically ethanol-fed rats, which confirms the presence of antioxidant activity in β-carotene [27]. Based on the literature data, most vitamins are deficient in obese patients, especially the fat-soluble vitamins, vitamin B9, vitamin B12 and vitamin C [28]. Inadequate eating behavior must be the main cause of lower vitamin C levels in obese patients, besides being a common antioxidant added to many processed foods. This relation is verified in data where fruits consumption associated with higher vitamin C concentrations in obese individuals [29] and low vitamin C intake, associated with larger central adiposity in 926 women [30]. The administration of vitamin PP (nicotinic acid) has long been known to promote beneficial effects on blood lipid and cholesterol profiles [31]. Besides, vitamin PP is a “scavenger” of the superoxide radical from which hydroxyl is subsequently formed, which has a powerful destructive potential against cell structures and enzymes [32].

In this work, the effects of two functional food compositions in CD-1 and agouti-yellow mice in obesity simulation were investigated. It is assumed that composition 2 is the most balanced and when adding 9% of this composition to the diet of animals, it improves metabolic processes disrupted in obesity, presumably reducing oxidative stress, normalizing the balance of nutrients and vitamins.

CONCLUSION

According to the results of the study, the most promising composition was selected (composition 2). Feeding animals with this composition improved histological pictures of studied organs and tissues, biochemical blood parameters and effectively reduced body mass in both experimental animal lines, CD-1, and agouti-yellow mice, compared to the control groups.

List of Abbreviations: MSH: á-melanocyte-stimulating hormone, LDL: low-density lipoproteins, HDL: high-density lipoproteins, M: mean, SD: standard deviation, PUFAs: polyunsaturated fatty acids, IL-6: interleukin 6

Competing Interests: There are no conflicts of interest to declare.

Author Contributions: VaG, OG, and VlG designed the research; PL and AG performed the experimental study; MZ performed the histological study; AP and MP analyzed the data; PL and AP prepared the manuscript.

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