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Structural changes in the skin and muscle tissue of rats with a model of diabetes

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Structural changes in the skin and skeletal muscles of rats with alloxan-induced diabetes were studied by scanning electron microscopy (SEM). Tissue samples were prepared for SEM using standard histological methods. The results of the study showed that diabetes causes a number of changes in the structure of the skin and muscles of the thigh, which was confirmed by histological examination. In particular, a decrease in the integrity of collagen fibrous structures was revealed. The severity of structural changes in skin and muscle tissues depended on the level of free glucose blood levels and the type of simulated diabetes.

Keywords: diabetes, changes in rat skin and muscles.

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Introduction

According to the International Diabetes Federation, the number of patients with diabetes mellitus (DM) is increasing annually and is projected to reach 643 million by 2030, and 783 million by 2045, with deaths from DM amounting to more than 1 million people per year [1]. Despite significant progress in the development of diagnostic methods for DM, early detection of complications caused by DM in various tissues and organs remains an urgent problem.

The studies of a number of authors has shown that significant changes occur in the skin and muscles during the development of DM [2,3]. It was found that there is a decrease in the content of lipids and enzymes in the stratum corneum, as well as moisture in the epidermis, and in the dermis there is a change in metalloprotein expression, suppression of collagen expression and degradation of fibrillar collagen proteins, in addition, the balance of proteolytic activity in the extracellular matrix is disrupted [4,5].

It is known that collagen structures determine the optical scattering and birefringence of the skin [6,7]. In the human body, collagen, other proteins, and lipids are constantly exposed to glucose in vascular and extravascular fluids. As a result, new potentially toxic molecules of proteins and lipids are formed in the form of so-called Advanced glycation end-products (AGEs) [8]. A number of studies have found that diabetes causes changes in the composition and structure of skin collagen, which is closely related to changes in its optical and diffusion characteristics [9,10].

The aim of this study was to study structural changes in skin and skeletal muscles in rats with various experimental models of alloxan diabetes.

Materials and methods

Experimental diabetes mellitus was modeled on outbred white male rats by intramuscular administration of alloxan in accordance with Ref. [11]. The type 2 diabetes mellitus model was reproduced using intramuscular administration of alloxan tetrahydrate („LaChema“ Czech Republic) at a dose of 65 mg/kg with prior intramuscular administration of nicotinamide (230 mg/kg). The type 1 diabetes mellitus model was reproduced by intramuscular administration of alloxan at a dose of 90 mg/kg 6 hours after fasting.

Animals without any exposure were used as a comparison group.

2 weeks after alloxan administration, blood glucose levels in rats were measured using a glucose meter (Accu-Check Performa Nano, Germany). The animals were kept in standard vivarium conditions, and the food regime was standard with the use of compound feed for rodents.

Tissue samples were fixed in a 10% neutral buffered formalin solution for 24 hours. Histological processing was performed in a series of solutions of 95–96% ethyl alcohol — 4 alcohol changes. After that, the tissue samples were soaked 1 h in 4 paraffin changes, and then the biopsies were poured into blocks using paraffin heated to 56–58 °. After the paraffin solidified, tissue sections with a thickness of about 4–5 μm were cut using a

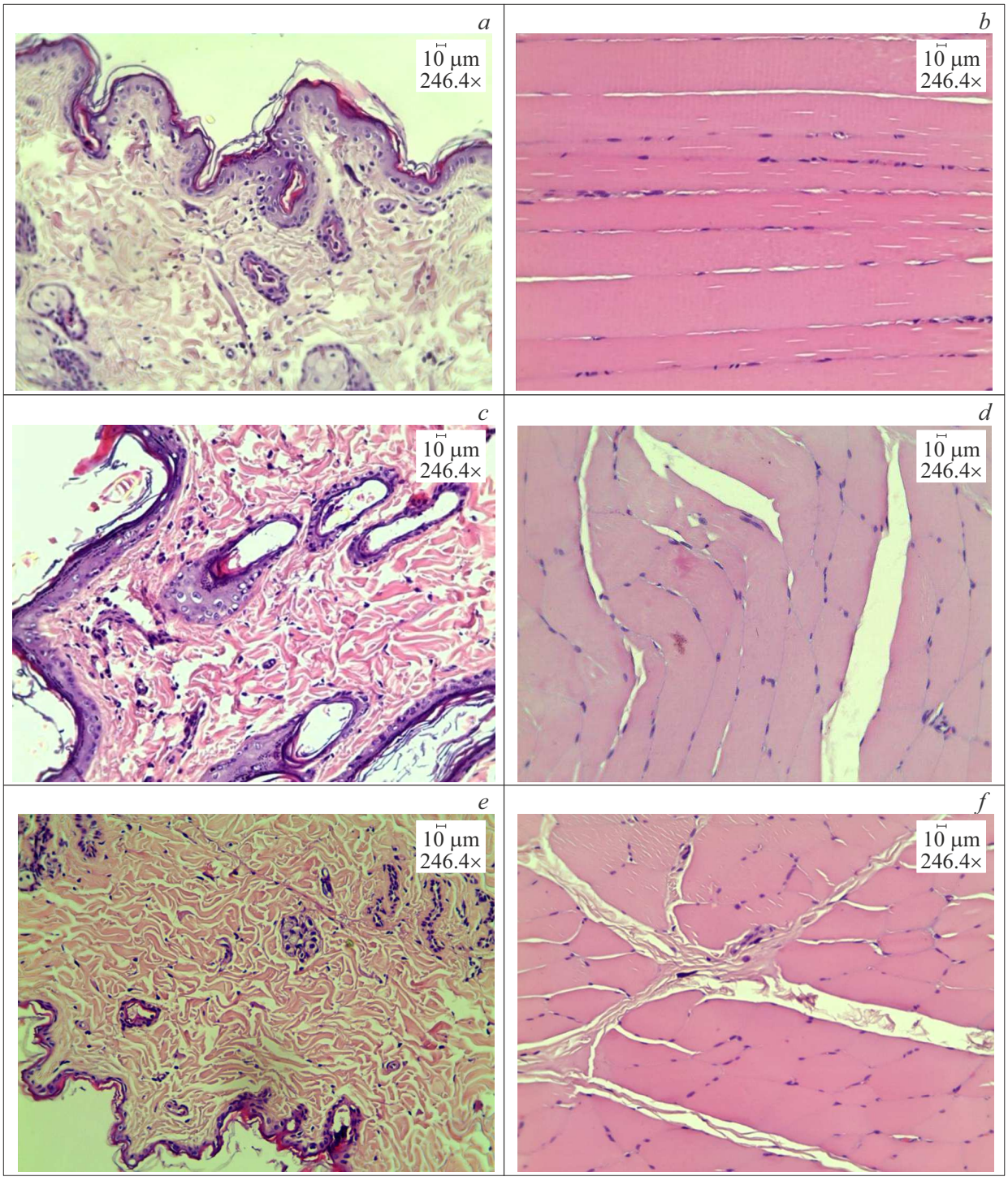

Figure 1. Micrographs of tissues. Rat from the control group: (*a*) skin, (*b*) muscle. A rat with type 2 diabetes: (*c*) skin, (*d*) muscle. A rat with type 1 diabetes: (*e*) skin, (*f*) muscle. Hematoxylin-eosin. Magnification 246.4.

rotary microtome. The tissue sections were then placed on slides and dried in a thermostat for 12 h at 37 °C. For morphological examination, sections were stained with standard histological methods, morphometric studies were performed using a medical transmitted light microimage mVizo-103.

Scanning electron microscopy (SEM) was performed using a Mira II LMU microscope (TESCAN, Czech Republic) to determine structural changes in the skin and skeletal muscles of rats. Before SEM, the transects were predewaxed in 3 xylene washes of 10–15 min and dehydrated in 3 shifts of 95–96 % ethyl alcohol solution of 10–15 min,

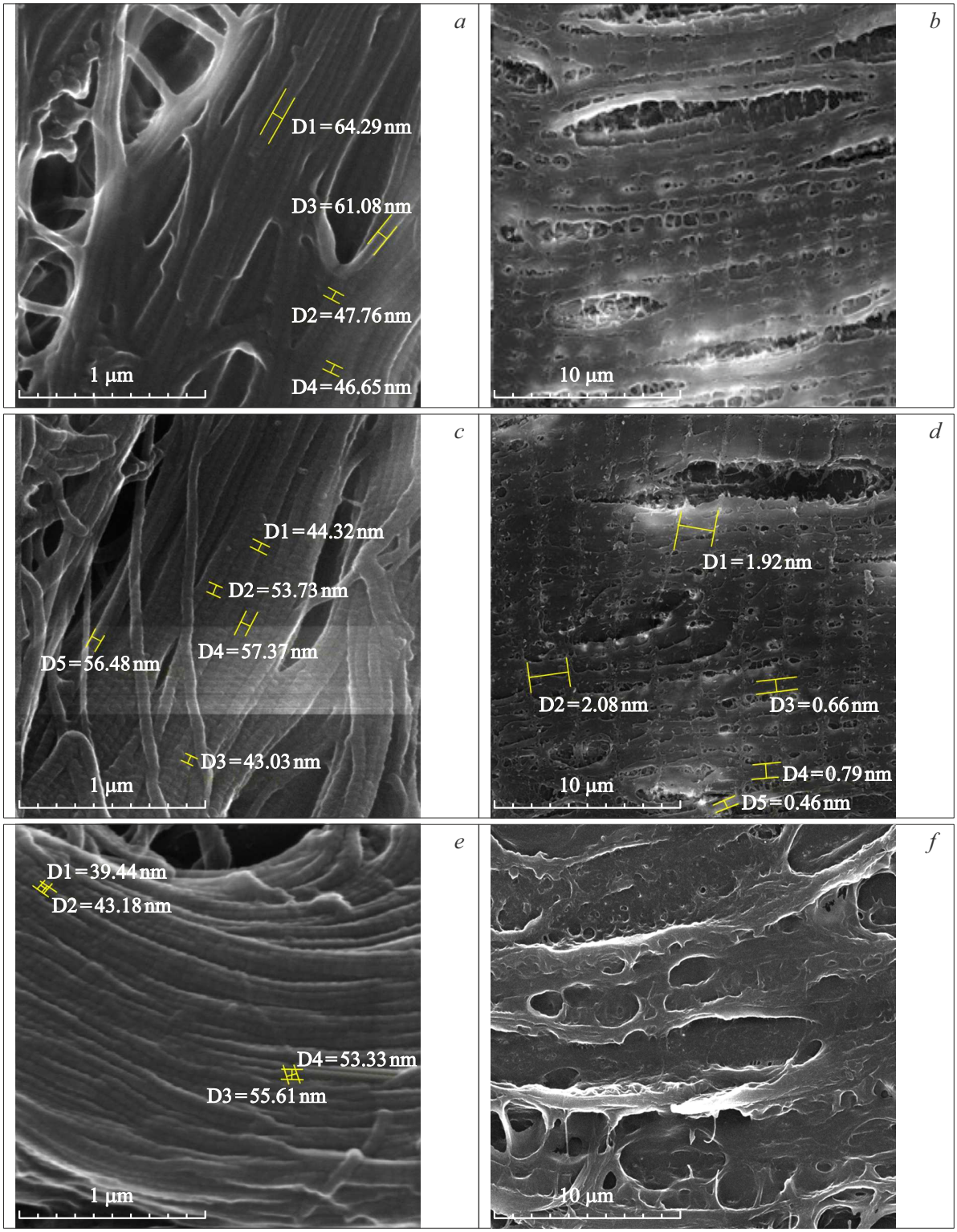

Figure 2. Scanning electron microscopy. Rat from the control group: (*a*) skin, collagen fibers; (*b*) muscle, transverse striation of muscle fibers is visible. A rat with type 2 diabetes: (*c*) skin, thickening of collagen fibers is noted; (*d*) muscle, the transverse striation of muscle fibers is preserved. A rat with type 1 diabetes: (*e*) skin, a decrease in diameter and a significant compaction of collagen fibers are observed (the fibers fit tightly to each other); (*f*) muscle, the transverse striation of muscle fibers is disrupted, tears appear, loosening of muscle tissue. D1, D2, D3, D4, D5 — sizes of fibers in fabrics.

then dried on in the air for 1 h. After drying, the transects were fixed to the sample table using vacuum double-sided carbon conductive adhesive tape. A thin conductive layer of metal was applied to them by sputtering in a vacuum chamber. To do this, the chamber was vacuumed to a pressure slightly less than 10^{-2} Pa, and the chamber was filled with argon until an equilibrium state was established inside the chamber. Then a plasma was created in the chamber, as a result of which the atoms released from the metal target were deposited on the surface of the sample. The thickness of the metal coating was controlled by the plasma current and the sputtering time and was usually 5–10 nm. This made it possible to obtain high-magnification electron micrographs without visualizing the sprayed substance.

Results

The normal structure of the epidermis and dermis was observed during histological examination of tissues in animals of the control group (Fig. 1, *a*). In muscle — fragments of muscle tissue with striated striation (Fig. 1, *b*).

In the group with simulated type 2 diabetes, the blood glucose level did not exceed 10 mmol/l, during histological examination, dermal edema was noted in the skin (Fig. 1,it c), dystrophic changes in myocytes were observed in the muscle, and swelling of muscle fibers was noted (Fig. 1, *d*).

In the group of animals with simulated type 1 diabetes, thinning of the epidermis and pronounced edema of the dermis were observed in the skin (Fig. 1, *e*), and areas of dystrophically altered muscles with swelling of fibers in the muscle (Fig. 1,*f*).

The studies using scanning microscopy (Fig. 2, *a*–*f*) found a decrease in the diameter and thickening of collagen fibers in both experimental groups, with the development of experimental diabetes in the skin and muscles probably due to the occurrence of cross-links. The severity of these changes depends on the level of free glucose and the model of experimental diabetes. These changes were more pronounced in rats with simulated type 1 diabetes.

Our results are consistent with research data from other authors. For instance, it was found in the study in Ref. [12] that changes in the biomechanical properties of the skin in healthy people during *ex vivo* glycation were similar to skin changes in patients with diabetes. This was probably attributable to an increase in the number of cross-links of collagen molecules during glycation [13,14]. The authors of Ref. [15] used polarizing optical coherence tomography (PS-OCT) and SEM to monitor and evaluate skin changes in mice at different stages of diabetes. A noticeable change in the structure of the skin, including the disruption of the organization of collagen fibers was observed with the development of experimental diabetes.

Conclusion

Thus, during the development of experimental alloxan diabetes, a change in the structural properties of the skin and muscles was revealed, probably due to a decrease in the integrity of collagen molecules. The severity of structural changes in the skin and muscles depended on the level of free glucose and the type of experimental diabetes.

Compliance with ethical standards

Experimental studies were conducted in accordance with the Recommendations of the EEC No.33 dated 14.11.23 on guidelines for working with laboratory (experimental) animals during preclinical (non-clinical) studies, as well as in accordance with the recommendations of the Ethics Committee of Saratov State Medical University named after V. I. Razumovsky. All applicable international, national, and/or institutional guidelines for animal care and management were observed.

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Conflict of interest

The authors declare that they have no conflict of interest.

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