ORIGINAL ARTICLE

CONTENTS

Morphological features of brown adipose tissue in an experimental polycystic ovary syndrome under intermittent cold exposure

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ABSTRACT

Aim: The aim of the study was to identify the morphological features of brown adipose tissue in an experimental model of PCOS under intermittent cold exposure.

Materials and Methods: The study was conducted on 40 immature female WAG rats, aged 27 days and weighing 80-90 g, which were divided into five groups (eight animals per group). Group 1 consisted of intact rats. Group 2 included rats that received daily subcutaneous injections of 0.2 ml of purified and sterilized olive oil for 25 days. Group 3 consisted of rats subjected to intermittent cold exposure for 25 days. Group 4 included rats in which dehydroepiandrosterone (DHEA)-induced PCOS was modeled. Group 5 consisted of rats that, in addition to intermittent cold exposure, received DHEA administration. Rats in groups 1-5 were removed from the experiment on day 26 by cervical dislocation. The material for morphological study was brown adipose tissue, which was cut from the interscapular region during the autopsy of animals. Histological, morphometric and statistical research methods were used.

Results: During the survey microscopy of the slides stained with hematoxylin and eosin, brown adipose tissue from the interscapular region of rats in all groups was characterized by the presence of parenchyma and stroma. Depending on the number of lipid droplets, all adipocytes were classified into three categories: adipocytes containing up to 5 lipid droplets (type 1 adipocytes); adipocytes containing 5-10 lipid droplets (type 2 adipocytes); and adipocytes containing more than 10 lipid droplets (type 3 adipocytes). Brown adipose tissue in DHEA-induced PCOS was characterized by a decrease in the number of type 3 adipocytes and an increase in the number of type 1 adipocytes; a reduction in adipocyte area, and an increase in adipocyte nuclear area. Under intermittent cold exposure and during DHEA administration combined with intermittent cold exposure, brown adipose tissue was characterized by a decrease in the number of type 2 and 3 adipocytes and an increase in the number of type 1 adipocytes; a reduction in adipocyte area; an increase in adipocyte nuclear area; and an increase in the number of blood vessels in the stroma.

Conclusions: In DHEA-induced PCOS, under intermittent cold exposure, and during DHEA administration combined with intermittent cold exposure, activation of brown adipose tissue in the interscapular region is observed, with the degree of activation being minimally, moderately, and maximally expressed, respectively.

KEY WORDS: morphology, brown adipose tissue, experiment, polycystic ovary syndrome, intermittent cold exposure

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most prevalent hormonal and metabolic disorders, affecting 8-13% of women of reproductive age and 3-11% of adolescent girls [1, 2]. It is a heterogeneous condition with varying clinical manifestations and health impacts across the lifespan [3].

Understanding the disease's pathogenesis could lead to novel treatments and better patient outcomes [4]. At present, there is no single fundamental concept explaining the mechanism of PCOS development; therefore, researchers continue to investigate the etiopathogenesis of the specified pathology.

Adipose tissue is a complex endocrine organ that produces various hormones, adipokines, and cytokines, which are involved in a wide range of physiological functions and in the pathogenesis of multiple diseases [5, 6]. Adipose tissue is classified into white and brown types, which differ in function, anatomical distribution, and morphofunctional properties. A third, beige type, is recognized as an intermediate form between white and brown adipose tissues [7].

The role of obesity and adipose tissue dysfunction in the pathogenesis of PCOS has been well established. Interestingly, adipose tissue dysfunction appears to play a greater role in the pathogenesis of PCOS than obesity itself [8].

Brown adipose tissue is a specialized tissue involved in non-shivering thermogenesis, which dissipates energy in the form of heat. Moreover, it secretes various paracrine and endocrine factors that exert effects on other peripheral tissues and regulate systemic metabolic homeostasis [9]. Women with PCOS exhibit reduced brown adipose tissue activity. Numerous studies have shown that brown adipose tissue may regulate the pathophysiological features of PCOS. Studies by numerous researchers, along with our own findings, have demonstrated that increasing brown adipose tissue mass through its transplantation, or stimulating brown adipose tissue via cold exposure, dietary interventions, physical activity, and other factors, is effective in the treatment and prevention of PCOS. It has been shown that activation of brown adipose tissue normalizes the metabolic alterations and the morphofunctional state of the ovaries characteristic of PCOS [10, 11]. Our analysis of the literature revealed no studies specifically aimed at investigating the morphofunctional characteristics of brown adipose tissue in PCOS under conditions of intermittent cold exposure.

AIM

The aim of the study was to identify the morphological features of brown adipose tissue in an experimental model of PCOS under intermittent cold exposure.

MATERIALS AND METHODS

The study was conducted on 40 immature female WAG rats, aged 27 days and weighing 80-90 g, which were divided into five groups (eight animals per group). Group 1 consisted of intact rats. Group 2 included rats that received daily subcutaneous injections of 0.2 ml of purified and sterilized olive oil for 25 days. Group 3 consisted of rats subjected to intermittent cold exposure for 25 days. Group 4 included rats in which dehydroepiandrosterone (DHEA)-induced PCOS was modeled. Group 5 consisted of rats that, in addition to intermittent cold exposure, received DHEA administration.

In rats of groups 3 and 5, intermittent cold exposure was modeled by placing the animals daily for 4 hours in a chamber with a controlled light regime and a temperature maintained at +4 °C. For the remaining 20 hours of the day, the animals were kept under standard housing conditions.

In rats of groups 4 and 5, PCOS was induced by daily subcutaneous administration of DHEA for 25 days at a dose of 8 mg per 100 g of body weight, dissolved in 0.2 ml of purified and sterilized olive oil. The effectiveness of this method was confirmed by morphological examination, which revealed cystically changed follicles, thecal cell hyperplasia, a reduced number of mature follicles and corpora lutea, which form the microscopic findings characteristic of polycystic ovarian morphology [11].

Rats in groups 1-5 were removed from the experiment on day 26 by cervical dislocation. The material for morphological study was brown adipose tissue, which was cut from the interscapular region during the autopsy of animals. The tissue samples were fixed in 10% formalin solution. Tissue consolidation after formalin fixation was achieved by passing the samples through a graded series of ethanol solutions, Nikiforov's solution (96% ethanol and diethyl ether in a 1:1 ratio), chloroform, and subsequent embedding in paraffin. Serial sections with a thickness of $4\text{-}5\times10^{-6}$ m were prepared from the paraffin blocks and stained with hematoxylin and eosin.

Microscope slides were examined using a ZEISS Primostar 3 microscope (Carl Zeiss, Germany) equipped with an Axiocam 208 color camera. Morphometric analysis was performed with the ZEISS ZEN 3.6 software (blue edition). In each case, at ×100 magnification, the following parameters were determined: the relative volumes (%) of adipocytes containing different numbers of lipid droplets, the area of adipocytes, the area of adipocyte nuclei, the absolute number of blood vessels in the stromal component.

Data in the groups were statistically processed using the PAST software (version 4.15, Natural History Museum, University of Oslo, Norway). Mean values between groups were compared using Student's t-test and the Mann-Whitney U-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

During the survey microscopy of the slides stained with hematoxylin and eosin, brown adipose tissue from the interscapular region of rats in groups 1-5 was characterized by the presence of parenchyma and stroma (Fig. 1). The parenchymal component consisted of adipocytes of oval, round, or elongated shape, with centrally located nuclei surrounded by lipid droplets. Due to the presence of these droplets, brown adipose tissue adipocytes acquired a multi-chambered appearance.

Depending on the number of lipid droplets, all adipocytes were classified into three categories: adipocytes containing up to 5 lipid droplets (type 1 adipocytes); adipocytes containing 5-10 lipid droplets (type 2 adipocytes); and adipocytes containing more than 10 lipid droplets (type 3 adipocytes). When assessing the relative number of adipocytes in groups 1-5, a predominance ($p<0.05$) of type 3 adipocytes was observed (Fig. 2). Compared with group 1, the relative number of type 3 adipocytes was reduced ($p<0.05$) in groups

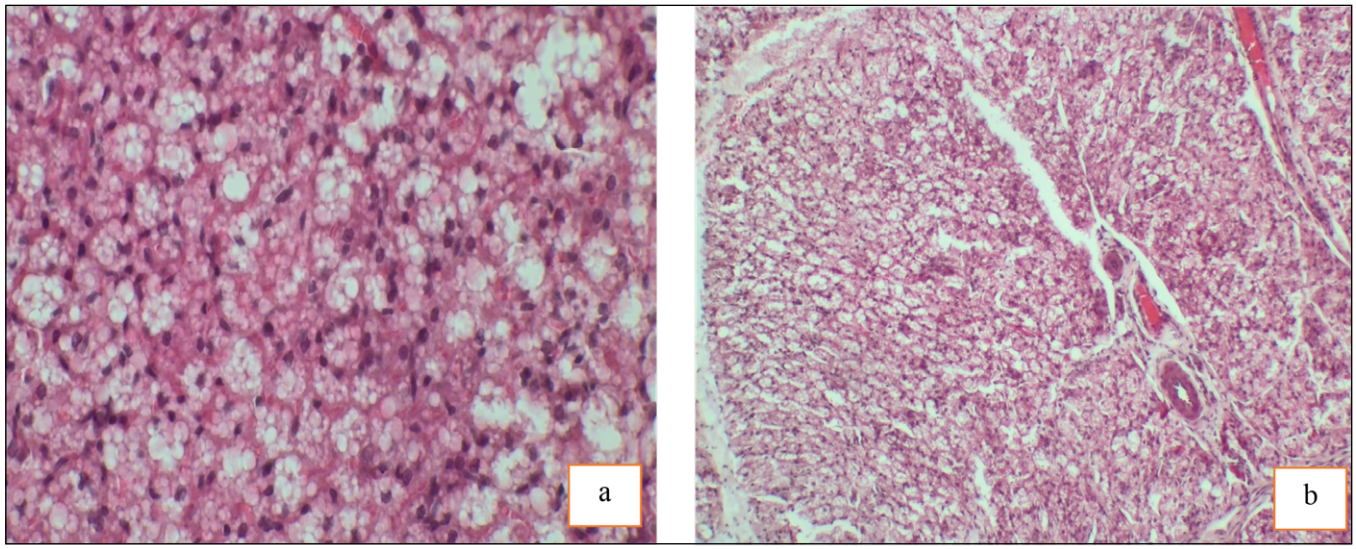

Fig. 1. Parenchymal and stromal components of brown adipose tissue from the interscapular region of a rat from group 3 (a) and group 4 (b). Hematoxylin and eosin staining, a) ×100, b) ×40.

2-5. The reduction in the relative number of type 3 adipocytes was most pronounced ($p<0.05$) in group 5 compared with group 3, and moderate ($p<0.05$) in group 3 compared with groups 2 and 4. In the latter two groups, the relative number of adipocytes showed no significant differences ($p>0.05$).

Compared with group 1, the relative number of type 2 adipocytes decreased ($p<0.05$) in groups 3 and 5, and showed no change ($p>0.05$) in groups 2 and 4. The number of type 2 adipocytes did not differ significantly ($p>0.05$) between group 2 and group 4, or between group 3 and group 5. In groups 2 and 4, the relative number of type 2 adipocytes was higher ($p<0.05$) compared with groups 3 and 5.

The relative number of type 1 adipocytes increased ($p<0.05$) in groups 2-5 compared with group 1, with the highest increase observed in groups 3 and 5 and a moderate increase in groups 2 and 4. The relative number of type 1 adipocytes did not differ significantly ($p>0.05$) between groups 2 and 4, but was higher ($p<0.05$) in group 5 compared with group 3.

Intergroup comparative analysis of adipocyte counts indicated activation of brown adipose tissue and, consequently, thermogenesis in groups 2-5. This process was most pronounced in group 5, moderately expressed in group 3, and minimally expressed in groups 2 and 4. Activation of brown adipose tissue was manifested by a reduction in the number of lipid droplets within adipocytes, leading to an increase in the number of type 1 adipocytes and a decrease in the number of type 2 and 3 adipocytes. It is known that activation of brown adipose tissue involves the breakdown of fatty acids stored in lipid droplets, resulting in a reduction in both the number and size of these droplets.

The area of brown adipose tissue adipocytes decreased ($p<0.05$) in groups 2-5 compared with group 1 (Fig. 3). This parameter was lowest in group 5, moderately reduced in group 3, and minimally reduced in groups 2 and 4. In the latter groups, adipocyte area did not differ significantly ($p>0.05$). The decrease in adipocyte area was, in our opinion, due to a reduction in the number of lipid droplets as a result of brown adipose tissue activation.

The size of the cell nucleus is an important indicator of its morphofunctional state. In the present study, the nuclear area of adipocytes was measured in groups 1-5 (Fig. 4). This parameter increased ($p<0.05$) in groups 2-5 compared with group 1, representing a morphological manifestation of brown adipose tissue activation. The increase in adipocyte nuclear area was most pronounced in group 5, moderately expressed in group 3, and minimally expressed in groups 2 and 4. In the latter groups, the adipocyte nuclear area did not differ significantly ($p>0.05$).

In the stroma of brown adipose tissue in groups 1-5, connective tissue fibers, blood vessels of various calibers, and nerve fibers were identified. In group 4, compared with the other groups, the stromal component was characterized by a higher content of connective tissue fibers, indicating the development of sclerotic changes.

An important component of the stroma is the vasculature, which is responsible for maintaining adequate tissue trophism. The absolute number of vessels in the stroma of brown adipose tissue did not differ significantly ($p>0.05$) among groups 1, 2, and 4, amounting to 6.3±0.65, 6.6±0.42, and 6.5±0.42, respectively. The absolute number of vessels also did not differ signifi-

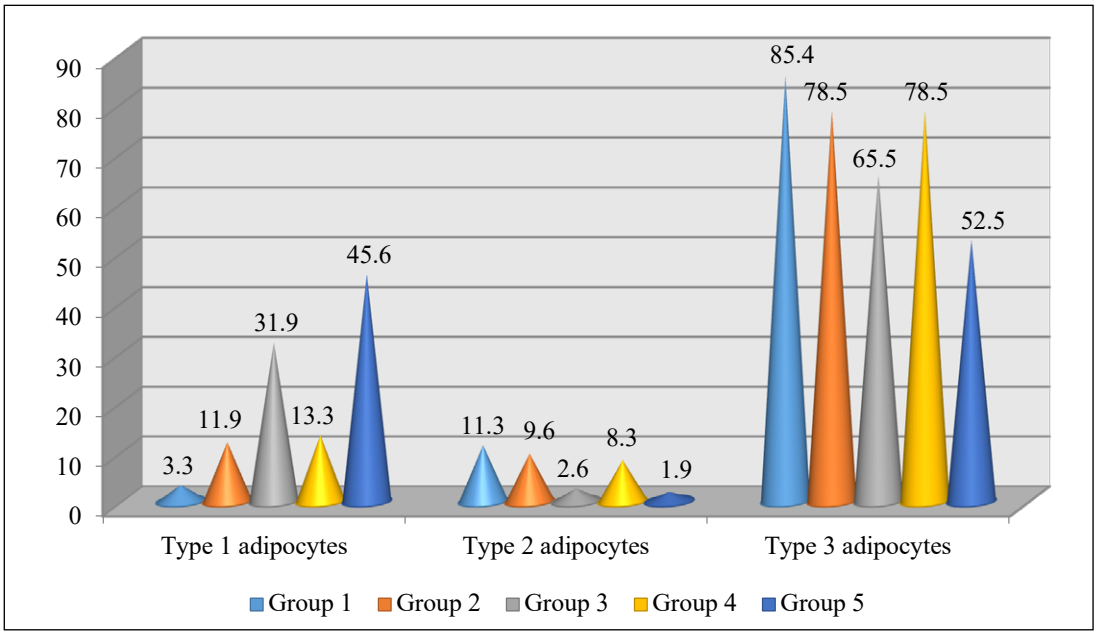

Fig. 2. Results of counting the relative (%) number of adipocytes in brown adipose tissue from the interscapular region of rats in groups 1-5.

cantly ($p>0.05$) between group 3 (13.9±0.64) and group 5 (14.1±0.64). In the latter groups, this morphometric parameter was higher ($p<0.05$) compared with groups 1, 2, and 4. The stimulation of angiogenesis observed in groups 3 and 5 represents a morphological manifestation of brown adipose tissue activation. Pronounced angiogenesis in brown adipose tissue is essential for the rapid distribution of heat throughout the body and for supplying it with nutrients and oxygen.

DISCUSSION

The authors' examination of the experimental material made it possible to identify the morphological features of brown adipose tissue under conditions of intermittent cold exposure, in DHEA-induced PCOS, and during DHEA administration combined with intermittent cold exposure.

Brown adipocytes are known to be characterized by the presence of a large number of mitochondria and high expression of uncoupling protein-1 (UCP1). In the mitochondria of brown adipocytes, the presence of high levels of UCP1 diminishes the proton gradient by uncoupling cellular respiration without producing adenosine triphosphate, and dissipating energy in the form of heat [12]. Another distinctive feature of brown adipocytes is the presence of multiple lipid droplets allows the cell to increase the lipid droplet surface-to-volume ratio, which facilitates the rapid consumption of lipids in cellular respiration reactions, leading to thermogenesis [12].

In the model of intermittent cold exposure, a moderately expressed activation of brown adipose tissue in the interscapular region was observed, morphologically manifested by an increase in the number of type 1 adipocytes and a decrease in the number of type 2 and 3 adipocytes, which was due to a reduction in the number of lipid droplets. These changes in adipocytes led to a decrease in their area. An increase in the nuclear area of adipocytes was also detected, indicating activation of their morphofunctional state.

The mechanism of brown adipose tissue activation under cold exposure has been well studied in vivo and in vitro. Cold stimulates skin thermoreceptors, leading to activation of the sympathetic nervous system and norepinephrine release. Norepinephrine stimulates brown adipocytes via β-adrenergic receptors and initiates intracellular events, including triglyceride hydrolysis, oxidation of the resulting fatty acids, and ultimately UCP1 activation and heat production [9, 13, 14].

Administration of DHEA to the animals resulted in a polycystic ovarian morphology, as previously described by us [11], and a minimally expressed activation of brown adipose tissue was observed. Morphologically, this was manifested by a decrease in the number of type 3 adipocytes and an increase in the number of type 1 adipocytes, a reduction in adipocyte area, and an increase in adipocyte nuclear area. A similar morphological pattern of brown adipose tissue activation was also observed in animals receiving purified and sterilized olive oil, which, in our opinion, may be regarded as a stress response. It has been demonstrated that stress induces sympathetic and neuroendocrine reactions, thereby activating brown adipose tissue [15].

The literature presents conflicting data regarding brown adipose tissue activity in DHEA-induced PCOS.

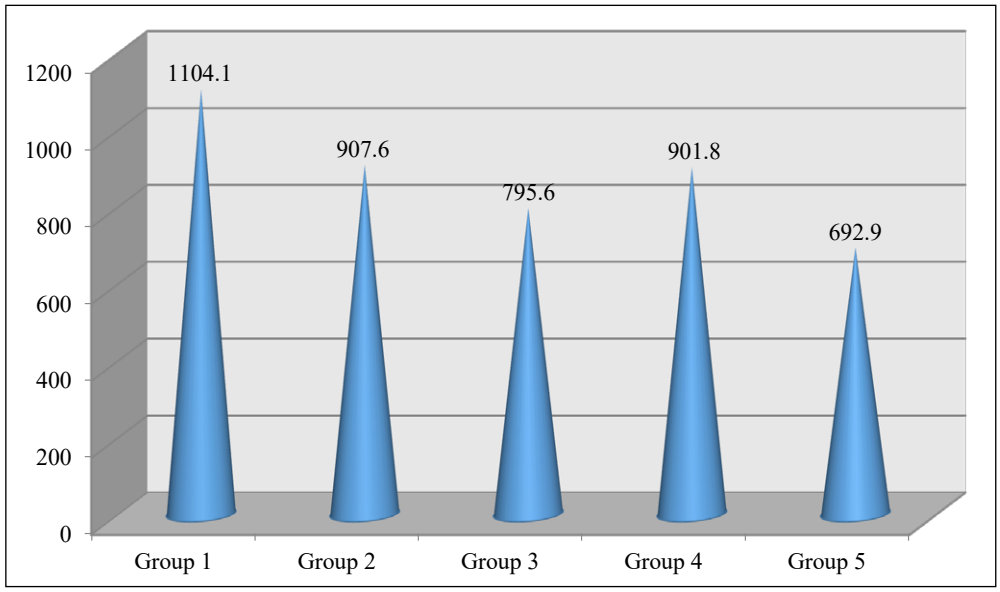

Fig. 3. Results of measuring the area (μm^2) of adipocytes in brown adipose tissue of rats in groups 1-5.

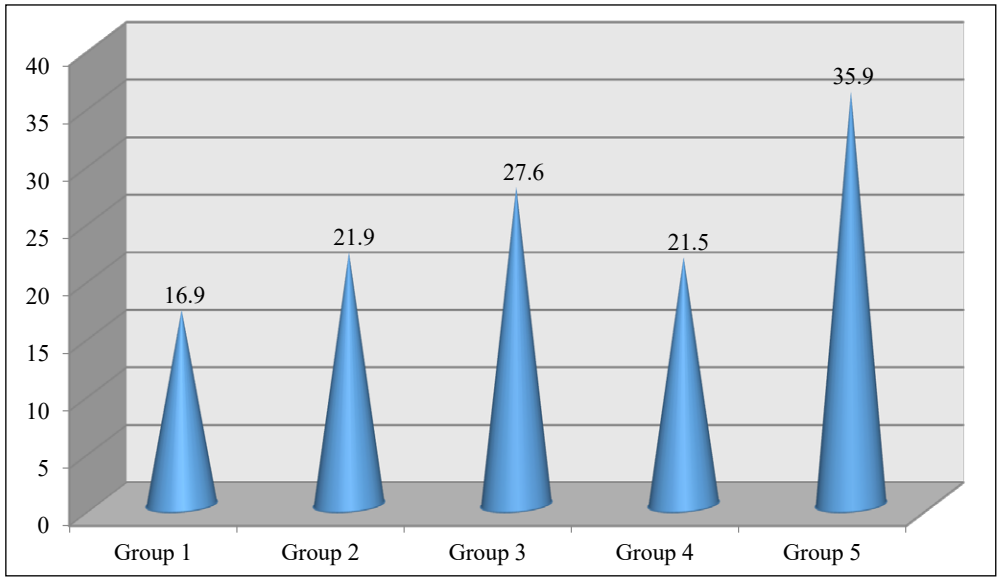

Fig. 4. Results of measuring the nuclear area (μm^2) of adipocytes in brown adipose tissue of rats in groups 1-5.

Some studies report unchanged brown adipose tissue activity, whereas others indicate its reduction [16].

Administration of DHEA to animals under intermittent cold exposure was associated, on morphological examination, with normalization of the morphofunctional state of the ovaries, as determined and previously reported by us [11], and the most pronounced activation of brown adipose tissue. In our opinion, this was due to the synergistic effects of intermittent cold exposure and DHEA administration. Morphologically, brown adipose tissue activation was manifested by a decrease in the number of type 2 and type 3 adipocytes, an increase in the number of type 1 adipocytes, an increase in the number of type 3 adipocytes, a reduction in adipocyte area, and an increase in adipocyte nuclear area.

Examination of the stromal component of brown adipose tissue under intermittent cold exposure and during DHEA administration combined with intermittent cold exposure revealed an increased number of blood vessels, which, in our opinion, indicates activation of angiogenesis. Stimulation of angiogenesis improves the trophic support of brown adipose tissue during its activation under cold exposure.

According to various researchers, the number of adipocytes increases during brown adipose tissue activation [17, 18]. This may occur through active angiogenesis. It is known that the vasculature of adipose tissue serves as the primary niche for multipotent precursor cells, which give rise to new adipocytes [19].

In a limited number of studies, cold exposure has been shown to increase the expression of vascular endothelial growth factor and the number of blood vessels in the stroma of brown adipose tissue [20].

CONCLUSIONS

In DHEA-induced PCOS, under intermittent cold exposure, and during DHEA administration combined with intermittent cold exposure, activation of brown adipose tissue in

the interscapular region is observed, with the degree of activation being minimally, moderately, and maximally expressed, respectively. Activation of brown adipose tissue in DHEA-induced PCOS is characterized by a decrease in the number of type 3 adipocytes and an increase in the number of type 1 adipocytes; a reduction in adipocyte area, and an increase in adipocyte nuclear area. Under intermittent cold exposure and during DHEA administration combined with intermittent cold exposure, brown adipose tissue activation is manifested by a decrease in the number of type 2 and type 3 adipocytes and an increase in the number of type 1 adipocytes; a reduction in adipocyte area; an increase in adipocyte nuclear area; and an increase in the number of blood vessels in the stroma.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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