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BROWN ADIPOCYTES OF SUCROSE-OVERFED RATS TREATED WITH CORTICOSTERONE: A STEREOLOGICAL AND ULTRASTRUCTURAL STUDY

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Abstract — The aim of this study was to examine the effects of short-term corticosterone treatment on brown adipocytes of rats overfed with sucrose. Ultrastructural and stereological analysis showed that brown adipocyte components responded to the applied treatment in conformity with their own dynamics and affinity. Although brown adipocytes generally corresponded to thermogenically active cells, some signs of supression of that function, such as mitochondrial degradation and a pattern of lipid accumulation, were noticeable. Taken together, the presented results indicate that a high carbohydrate diet delays the expected inhibitory influence of corticosterone on brown adipose tissue thermogenesis. For the full expression of corticosterone effects, longer treatment is needed.

Key words: Brown adipocytes, sucrose, corticosterone, ultrastructure, stereology

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INTRODUCTION

Brown adipose tissue (BAT) is the site of non-shivering thermogenesis in many small mammals (Himms - Hagen, 1986, 1990). The main structural and functional unit of BAT is the brown adipocyte, a large multilocular cell exceptionally rich in structurally and biochemically specialized mitochondria. Uncoupling protein 1 (UCP1), located in the inner mitochondrial membrane, is directly responsible for uncoupling of substrate oxidation from ATP synthesis, which leads to heat production/energy dissipation (Nicholls and Rial, 1984). The surface area of the inner mitochondrial membrane usually reflects the amount of UCP1 and serves as a criterion for assessment of BAT thermogenic capacity.

BAT thermogenesis is stimulated by noradrenaline released from the sympathetic nerve endings in the tissue (Rothwell and Stock, 1986; Trayhurn and Ashwell, 1987). Acting via β-adrenergic receptors on the brown adipocyte membrane, noradrenaline initiates a cascade of intracellular processes related to heat production (Himms - Hagen, 1991). It also strongly stimulates the synthesis of UCP1 (Ricquier and Cassard-Doulcier, 1993).

In addition to its role in cold-induced thermogenesis, BAT also takes part in so-called diet-induced thermogenesis (Rothwell and Stock, 1979). A diet supplemented with palatable food (such as sucrose) increases the activity of BAT, as demonstrated by enhanced noradrenaline turnover in BAT. Furthermore, this kind of diet positively affects BAT mass and UCP1 content (Rothwell et al., 1983; Landsberg and Young, 1983; Bell et al., 2002).

Many hormones exert positive or negative effects on BAT function. Thus, corticosterone suppresses BAT thermogenesis, as demonstrated by decrease in noradrenaline turnover and UCP1 expression in the tissue (Davidović et al., 1992; Moriscot et al., 1993; Strack et al., 1995).

Interestingly, despite its strong negative effect on BAT thermogenesis, corticosterone fails to inhibit sympathetic activity and reduce noradrenaline turnover in BAT of sucrose-overfed rats (Davidović et al. 1992). This finding shows that the action of

corticosterone depends on the nutritional status of the animal and suggests its complex interaction with insulin at the level of BAT.

The results of our previous experiments (Čakić - Milošević, et al. 1997; Čakić - Milošević et al. 2004) showed that the effects of sucrose overfeeding on brown adipocyte ultrastructure were clearly opposite to those obtained after short-term corticosterone treatment. Both sets of ultrastructural changes were in accordance with alterations of BAT function. To be specific, brown adipocytes in sucrose-overfed rats were enlarged, with prominent organelles involved in synthetic processes, and particularly numerous mitochondria with high thermogenic potential, as judged by the number of cristae. On the other hand, corticosterone treatment changed the ultrastructural organization of brown adipocyte, especially their lipid depots and mitochondria, leading to diminished thermogenic capacity.

In view of the great morphofunctional plasticity of brown adipocytes and opposite effects of sucrose and corticosterone on their structure and function, the object of this study was to discern the effects of corticosterone on brown adipocyte ultrastructure in sucrose-overfed rats. We were interested in possible preferences of these two factors for a certain subcellular compartment. We also sought to establish correlation between the experimental treatment and some unusual ultrastructural phenomenon observed in brown adipocytes.

MATERIAL AND METHODS

Ten male Wistar rats weighing 215-230 g at the beginning of the experiment were used. The animals, kept at 21±1°C and under conditions of a 12:12 light-dark cycle, were divided into two equal groups: one was treated subcutaneously with corticosterone (Sigma Chemical Co., St. Louis, MO, USA) in a dose of 5 mg/kg of body weight within two days and allowed to drink a 10% sucrose solution instead of tap water (S+C group). Before the injection, corticosterone was dissolved in a small amount of ethanol and diluted with saline. According to the data of Rothwell and Stock (1984), this dose was supraphysiological. The animals in the other group received only the vehicle injection only (ethanol-saline) and drank tap water (control group).

On day 3 of the experiment, the animals were sacrificed; interscapular BAT was routinely prepared for electron microscopy as previously described (Čakić – Milošević et al. 1997) and examined with a Philips CM12 electron microscope.

Stereological analysis was performed using a transparent lattice point-counting grid according to Weibel (1979). From each group, 10 cells per animal were analyzed at final magnification of 5400x to determine cell profile area; volume density of the nucleus, mitochondria, lipid droplets, and cytoplasm; and the number of mitochondria per cell. The same number of micrographs per animal were analyzed at final magnification of 11400x to determine mitochondrion profile area and the number of cristae per mitochondrion; and at 36000x to determine volume density of the mitochondrial matrix.

The results are presented as means ±S.E. All data were subjected to statistical analysis using Student's *t*-test for differences between the control and S+C groups.

RESULTS

Quantitative analysis

Treatment of sucrose-overfed rats with corticosterone did not affect body weight gain and interscapular BAT mass, either absolute or relative, in comparison with the control animals (Table 1).

Table 1. Effects of corticosterone on body weight gain and absolute and relative interscapular BAT mass of sucrose-overfed rats. There were no significant differences between the groups.

	C	S+C	p
body mass gain (g)	11±6.0	3±1.9	n. s.
absolute BAT mass (mg)	246±10.5	252±30.1	n. s.
relative BAT mass (g)	104±5.2	116±8.8	n. s.

Stereological analysis of brown adipocytes revealed remarkable alterations in some major parameters of these cells (Table 2). Cell profile area was significantly increased, mitochondrial volume density was decreased, while lipid droplets occupied

a larger portion of the cell than in the control group of animals. Nuclear and cytoplasm volume density remained unchanged.

Table 2. Effects of corticosterone on some major parameters of brown adipocytes in sucrose-overfed rats.

	C	S+C	p
cell profile area (μm^2)	362±16.5	481±23.9	<0.001
volume density (%)			
nucleus	5.3±0.31	4.5±0.42	n. s.
mitochondria	33±1.6	23±2.6	<0.01
lipid droplets	48±1.9	60±4.2	<0.05
cytoplasm	19.0±0.9	16.6±1.9	n. s.

A summary of quantitative analysis of brown adipocyte mitochondria is given in Table 3. There were no significant differences between the results for control and S+C animals with respect to the number and size of mitochondria, although the number was slightly increased. However, cristae were more numerous, while volume density of the matrix was decreased.

Table 3. Quantitative characteristics of brown adipocyte mitochondria after corticosterone treatment of sucrose-overfed rats.

	C	S+C	p
number of mitochondria per cell (N)	92±3.7	108±10.1	n. s.
mitochondrion profile area (μm^2)	0.99±0.059	1.20±0.137	n. s.
number of cristae per mitochondrion (N)	7.9±0.28	8.7±0.12	<0.05
mitochondrial matrix volume density (%)	70.8±1.76	63.6±1.91	<0.05

Ultrastructural analysis

The ultrastructural features of brown adipocytes from the control group of animals were generally in accordance with those usually described in the literature (Fig. 1A). They were roundish or polygonal cells with a slightly irregular nucleus and rarely visible nucleolus. Chromatin along the inner nuclear membrane was densely packed. Lipids were distributed in several lipid droplets of varying size. Mitochondria were close to each other and had a pale matrix. Their cristae were well preserved, arranged in two or three separated and often slightly curved systems. The other organelles were usually not prominent.

In respect to their major morphological characteristics, brown adipocytes of animals from the S+C group corresponded to thermogenically active cells (Fig. 1B). They were polygonal and posessed a roundish euchromatic nucleus with a prominent reticular nucleolus. In some instances, however, nucleolar components were segregated (Fig. 2A).

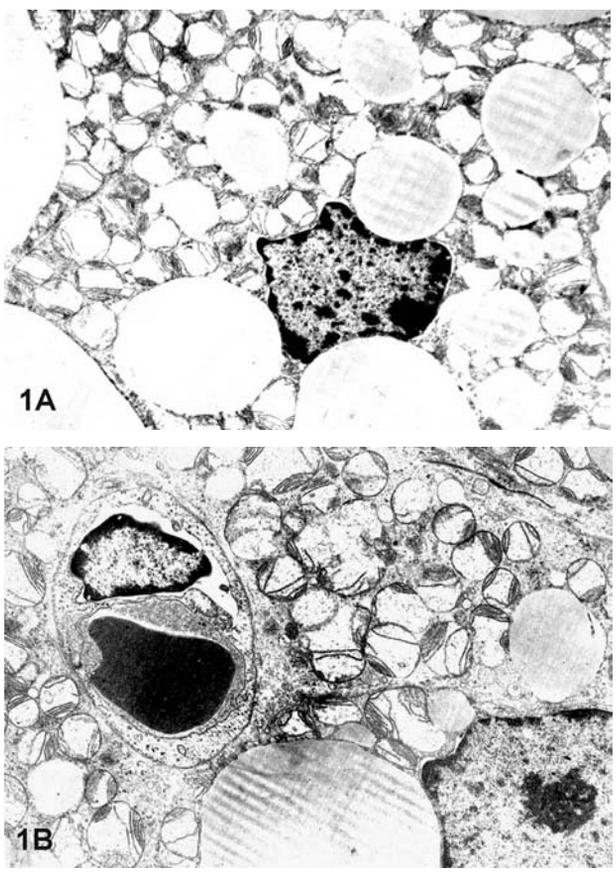

Fig. 1. Portions of brown adipocytes from control (A) and S+C (B) groups of animals. Note bizarre forms of mitochondria in the center of the field in B. Magnification 7000x.

Mitochondria were uniformly distributed throughout the cell. They were mostly circular, but some acquired bizarre forms that suggests fusion and/or rearrangement of the cristae (Fig. 1B). Electron-density of the mitochondrial matrix was low to moderate, and cristae were more numerous than in the control group. In some brown adipocytes, two or three mitochondria were located close to each other, with cristae that appeared to be continuous (Fig. 2B).

Lipid depots were present in the form of a few

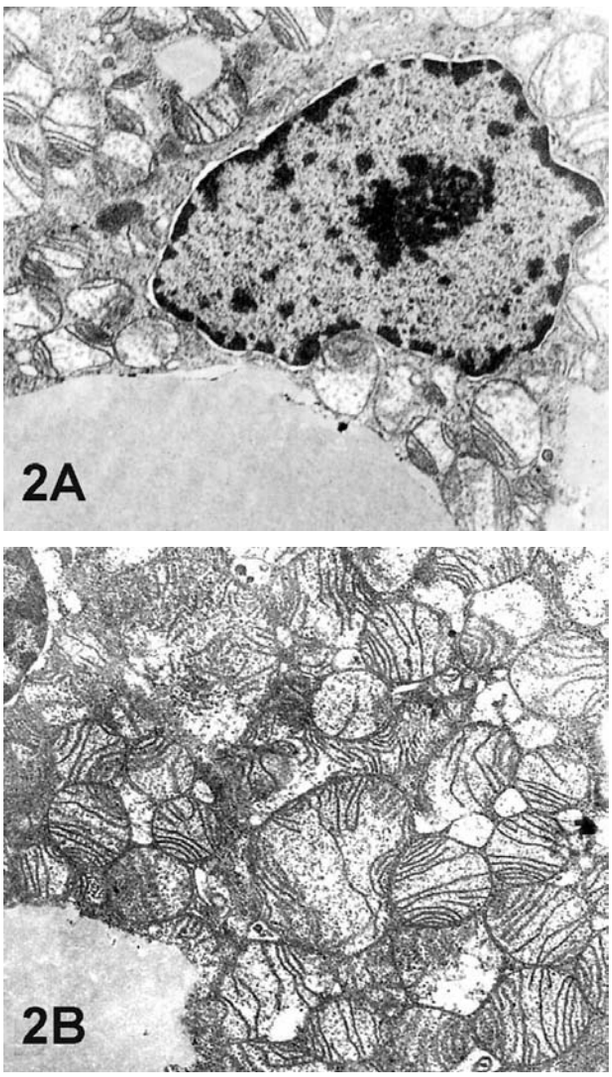

Fig. 2. Some features of S+C brown adipocytes: segregated nucleolar components (A) and mosaically arranged mitochondria (B). Magnification 7000x (A) and 9000x (B).

large lipid droplets that coalesced in some instances.

Dense bodies, recognized as components of the lysosome-peroxisomal compartment and a Golgi apparatus occurred more frequently than in the control.

Vacuoles different in size and content were seen in brown adipocytes from the S+C group. Some of these vacuoles were approximately of mitochondrial size, bounded by a double membrane (Fig. 3A, open arrow), and with finely granular contents (Fig. 3B, asterisk). Cristae remnants were visible in some of them (Fig. 3A, arrows). These vacuoles apparently represent mitochondria in the process of destruction.

Vacuoles of another type were somewhat smaller than mitochondria and filled with very sparse granular material (Fig. 3C). These vacuoles were usually oval or roughly biconvex and enveloped by membrane(s) sometimes divided into layers. Suitable planes of sections revealed that the vacuolar lumen was connected with the interior of well-preserved mitochondria (Fig. 3D).

DISCUSSION

Its remarkable functional plasticity enables BAT to serve as a metabolic buffer in all situations where energy balance is disturbed. Functional adaptability is accompanied by appropriate cytological and histological reorganization.

The main goal of the present study was to investigate ultrastructural alterations of brown adipocytes in sucrose-overfed rats treated with supraphysiological doses of corticosterone.

Our first aim was to estimate the effect of treatment on overall body energetics, i. e., whether it influenced animal body mass during such a short experimental period (48 hours). Data indicating an increased caloric intake in rodents kept for prolonged periods (3-4 weeks) on a diet enriched with palatable food were published previously (Murphy et al., 1989; Younger and Trayhurn, 1989; Kuroshima et al. 1995). However, no significant changes of body mass were observed, since such a diet stimulated BAT activity and energy dissipation (Kuroshima et al., 1995). On the other hand, increased food intake provoked by exogenous administration of corticosterone was correlated with a rather high body mass gain (Galpin et al., 1983; Strack et al. 1995; Čakić–Milošević et al., 2004). Adrenalectomized rats treated with supraphysiological doses of corticosterone and offered sucrose solution to drink ingested more energy and became obese because the supressive effects of corticosterone on BAT thermogenesis inhibited metabolic removal of excess calories (Bell et al., 2000).

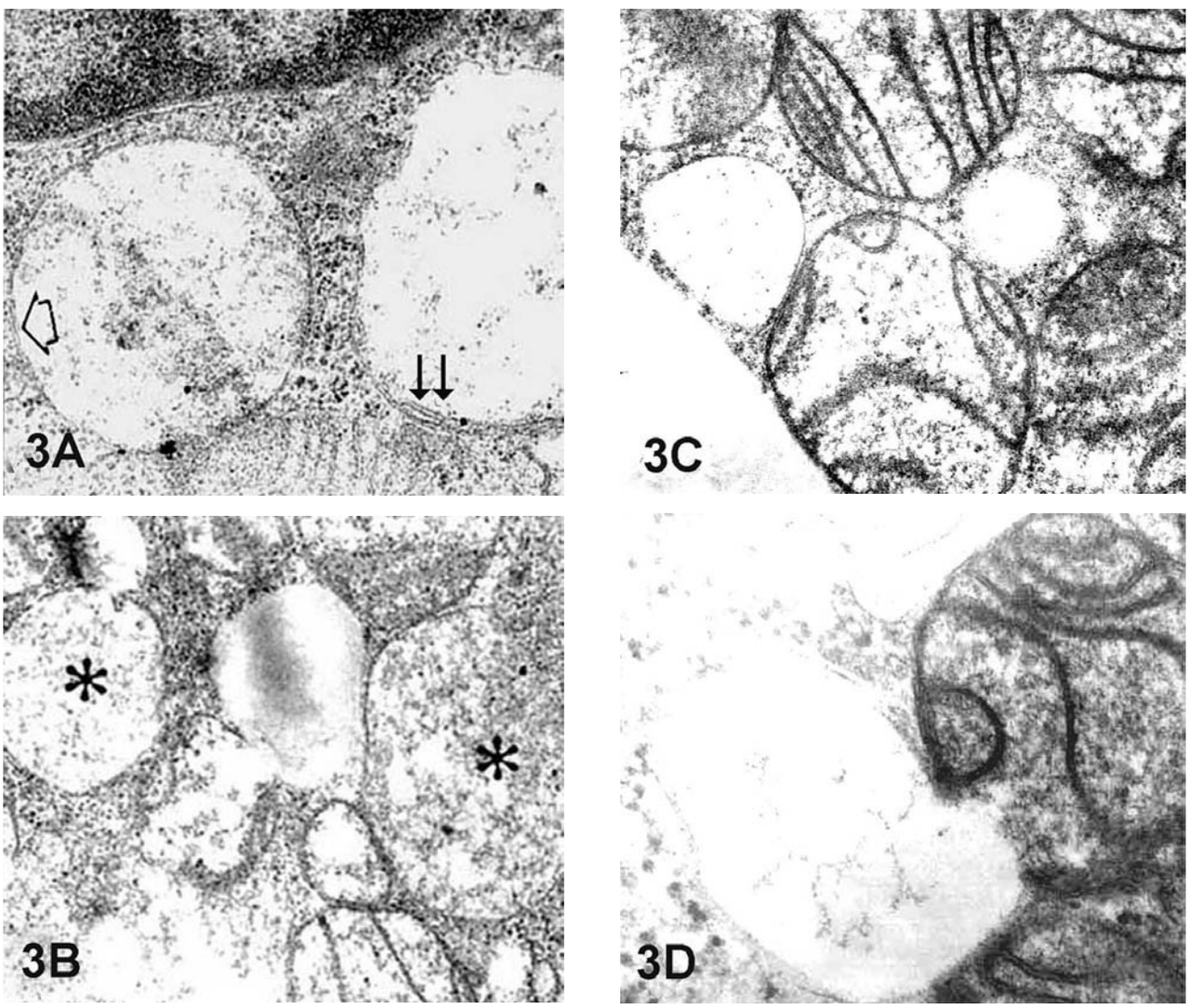

Fig. 3. Different types of vacuoles in brown adipocytes from S+C-treated rats whose appearance suggests mitochondrial origin. A: double-membrane-bounded vacuoles of approximately mitochondrial size (open arrow on double membrane), some with cristae remnants (arrows); B: vacuoles containing finely granular material (asterisk); C, D: vacuoles somewhat smaller than mitochondria, filled with very sparse granular material, in continuity with mitochondria. Magnification 34000x (A, C), 21000x (B), 50000x (D).

We believe that short duration of the experiment was the main reason for the absence of marked body mass change in the S+C group in this study. A similar conclusion might be deduced from the results pertaining to absolute and relative BAT mass, which both remained unchanged.

Brown adipocytes were the main units noticeably affected by the experimental treatment, as demonstrated by stereological and ultrastructural analyses. We expected that when simultaneously administered, sucrose and corticosterone might exert antagonistic, synergistic or independent effects on the thermogenic capacity of brown adipocytes. In turn, brown adipocytes and their subcellular components could be differentially sensitive to inhibitory or stimulatory influences.

Morphological findings, supported by stereological analysis, revealed that brown adipocytes in the S+C group were enlarged due to markedly increased lipid depots. Both insulin, whose secretion was

triggered by sucrose ingestion and corticosterone administration, and corticosterone had an anabolic effect on BAT lipid storage (G r a n n e m a n and C a m p b e l l, 1984; T o k u y a m a and H i m m s – H a g e n, 1987; S t r a c k et al., 1995). Accumulation of lipids in brown adipocytes should be favorable for thermogenesis. However, their disposition in a few large lipid droplets reduced free surface available for mitochondrial approach and fatty-acid oxidation. Our results indicated cooperative effects of sucrose and corticosterone on lipid accumulation in brown adipocytes. The pattern of accumulation was a result of thermogenesis-unfavorable influence of corticosterone alone.

The nucleus of typical brown adipocytes from the S+C group was euchromatic and enlarged in proportion to enlargement of the whole cell. Its appearance for the most part corresponded to the nucleus of a thermogenically active cell. This finding was in keeping with the stimulatory effect of a sucrose-rich diet on brown adipocyte metabolic activity. However, the observed segregation of nucleolar components in nuclei of some cells pointed to transcriptional shut down and inhibition of protein synthesis (S h a v – T a l et al., 2005).

Ultrastructural analysis of mitochondria - organelles directly involved in thermogenesis - gave us reliable information about the thermogenic status of brown adipocytes. Mitochondria were quite numerous, often with a well-preserved inner membrane system, and mosaically arranged in some sort of intracellular energetic units. We believe that this kind of mitochondrial association facilitates thermogenesis in situations when it is impeded and is thereby of functional significance. In addition, aggregation of the mitochondria might precede their fusion, leading to probable decrease of BAT functional capacity later on (G r o d u m s, 1977).

A remarkable characteristic of brown adipocytes in the S+C group was the increased number of intracytoplasmic vacuoles, whose appearance suggested mitochondrial origin. The vacuoles in Figs. 2A and 2B are in different stages of mitochondrial destruction according to the previously proposed model of inner membrane mitoptosis (T i n a r i et al., 2007). In this type of mitochondrial elimination, only the internal matrix and cristae are lost, while the external mitochondrial envelope remains unaltered. Morphological variations among vacuoles as well as cristae remnants reflected different levels of destruction. Another type of vacuoles (shown in Figs. 2C and 2D) could represent cytological manifestation of matrical protein turnover in still functional mitochondria.

To judge from their appearance, the metabolic status of mitochondria varied even in the same cell: some mitochondria appeared to be thermogenically active, while the others were in different stages of degeneration. Corticosterone exerts a strong inhibitory effect on UCP1 gene expression in BAT (M o r i s c o t et al., 1993). Since it inhibits transcription rather than accelerating the degradation of UCP1 mRNA, its effect on mitochondrial ultrastructure is delayed and gradual. Segregation of nucleolar components points to UCP1 mRNA transcriptional arrest. Hence there was no renewal of mitochondrial UCP1 content and "old" mitochondria were subject to degradation.

In our opinion, mitochondria responded faster to the applied diet than to corticosterone, with the result that their number in the S+C group was not reduced in spite of obvious destructive processes. Moreover, the number of mitochondria, as a very dynamic variable, might even be increased at the very outset of the experiment, declining to the value as a result of destruction and fusion.

To summarize, results of the present experiment showed that nutritional status affected the response of brown adipocytes to supraphysiological doses of corticosterone in a short-term experiment. The BAT of sucrose-overfed rats retained the ability to protect the body from excessive fat accumulation for a longer period than in conventionally fed animals. Ultrastructurally, subcellular compartments were able to respond to treatment according to their own affinity in a time-dependent manner. There was clear dissociation between the non-thermogenic pattern of lipid accumulation and the prevalence of what were – judging from morphological criteria – still thermogenically active mitochondria in the

same brown adipocyte.

Despite success in the short-term struggle against body fat accumulation, the observed ultrastructural changes, especially the presence of numerous vacuoles as signs of mitochondrial destruction, suggest gradual fading of the thermogenic function of brown adipocytes. The short duration of the experiment was a limiting factor that prevented us from reaching more reliable conclusions.

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МРКИ АДИПОЦИТИ ПАЦОВА ПРЕХРАЊИВАНИХ САХАРОЗОМ И КРАТКОТРАЈНО ТРЕТИРАНИХ КОРТИКОСТЕРОНОМ: СТЕРЕОЛОШКА И УЛТРАСТРУКТУРНА СТУДИЈА

МАЈА ЧАКИЋ-МИЛОШЕВИЋ, МИРЕЛА УКРОПИНА и АЛЕКСАНДРА КОРАЋ

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Циљ овога рада био је да се испита утицај краткотрајног третмана кортикостероном на фину структуру мрких адипоцита пацова чија је исхрана била обогаћена сахарозом. Резултати морфолошке анализе, подржани стереолошким подацима, показују да различити ултраструктурне компоненте мрких адипоцита одговарају на примењени третман засебно, сопственом динамиком. Иако неки аспекти морфолошке организације мрких адипоцита одговарају термогено активним ћелијама, уочљиви су знаци слабљења те функције. Подаци приказани у овом раду показују да дијета са високим садржајем угљених хидрата одлаже иначе очекивани инхибиторни ефекат кортикостерона на термогену активност мрког масног ткива.