Acta Veterinaria (Beograd), Vol. 54. No. 2-3, 95-104, 2004.

UDK 619:612.018.2

ULTRASTRUCTURAL ALTERATIONS OF RAT BROWN ADIPOCYTES AFTER SHORT-TERM CORTICOSTERONE TREATMENT

ČAKIĆ-MILOŠEVIĆ MAJA*, KOKO VESNA**, DAVIDOVIĆ VUKOSAVA***
and RADOVANOVIĆ JELENA*

**Institute of Zoology, Faculty of Biology, University of Belgrade, **Institute for Medical Research, Belgrade, ***Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade*

(Received 14. February 2004)

The aim of the present study was to examine ultrastructural alterations of rat brown adipocytes and their mitochondria after short-term corticosterone treatment. Animals were subcutaneously treated with three daily doses of corticosterone, 5 mg/kg each, dissolved in a small amount of ethanol and diluted with saline. Control animals received vehicle injection only (ethanol-saline). The results showed that short-term corticosterone treatment exerted a strong inhibitory effect on BAT thermogenic function, demonstrated by increased body mass gain. It also conspicuously affected the ultrastructural organization of brown adipocytes. Stereological analysis showed significant cell enlargement owing to lipid accumulation, together with decreased volume density of the nucleus and mitochondria. The mitochondrial machinery was not able to utilize imported free fatty acids complitely, which was demonstrated by sporadic occurrence of small lipid droplets in the mitochondrial matrix. Morphological signs of mitochondrial fusion, considered as indications of reduced function, were also noticed. In our opinion, besides its effect on brown adipocytes via glucocorticoid receptors, corticosterone also acts directly on the genome of their mitochondria.

Key words: brown adipocytes, corticosterone, mitochondria, rat.

INTRODUCTION

In many mammalian species, especially in small rodents and human neonates, brown adipose tissue (BAT) plays an important role in the maintenance of body temperature during exposure to cold (non-shivering thermogenesis; Himms-Hagen, 1986) and in the control of energy balance after overfeeding (diet-induced thermogenesis; Rothwell and Stock, 1986a).

The typical brown adipocyte is usually polygonal, contains many small lipid droplets scattered throughout the cytoplasm and is described as multilocular. Numerous mitochondria with well organized cristae, as well as the usual complement of other organelles (nucleus, rough and smooth endoplasmic

reticulum, Golgi complex, ribosomes, peroxisomes and lysosomes) are also present.

Metabolic heat production in BAT is related to the existence of highly specialized uncoupling protein-1 (UCP-1) in the cristae of brown adipocyte mitochondria. UCP-1 is directly responsible for poor coupling of substrate oxidation to ATP synthesis, which leads to heat production/energy dissipation (Nicholls and Locke, 1984; Klingenberg, 1990; Fleury *et al.*, 1997; Nedergaard *et al.*, 2001).

Thermogenesis in BAT is mediated by noradrenaline released from the sympathetic nerve endings in the tissue (Rothwell and Stock, 1986b; Trayhurn and Ashwell, 1987). Noradrenaline acts via ß-adrenergic receptors on brown adipocyte membrane and initiates processes in the cell that lead to raised heat production (Himms-Hagen, 1991). BAT thermogenic activity can be modified by several hormones. Thus, thyroid hormones, insulin and glucagon, for example, exert positive effects, while gonadal hormones are responsible for reduced tissue activity, particularly during pregnancy and lactation (for review see: Himms-Hagen, 1986; Janský, 1995). Glucocorticoids, however, play a particularly interesting role in compounded the regulatory mechanisms of BAT thermogenesis. Namely, they are necessary, in a permissive sense, for cold-induced thermogenesis (Fellenz *et al.*, 1982) but they strongly suppress diet-induced thermogenesis (Galpin *et al.*, 1983a; Fukushima *et al.*, 1985; York, 1989).

As far as we know, fine ultrastructural and stereological analysis of brown adipocytes after glucocorticoid treatment has not yet been carried out. Hence, this study has been performed in order to determine the effects of short-term treatment with corticosterone, the major glucocorticoid hormone in the rat, on brown adipocytes in stock-fed animals.

MATERIALS AND METHODS

Twelve male rats of the Wistar strain, weighing about 205-235 g at the beginning of the experiment, were used. The animals, previously acclimated to 21±1°C, were maintained under a 12 h light/dark cycle and given food and water *ad lib*. The rats were divided into two groups. One group was fed a stock diet, given water *ad lib*. and treated subcutaneously with corticosterone (Sigma Chemical Co., St. Louis, MO, USA) (5 mg/kg for 2 days). Before the injection, corticosterone was dissolved in a small amount of ethanol and diluted with saline. The other group (control) received vehicle injection only (the same amount of ethanol in saline). On day 3 of the experiment all animals were decapitated and the interscapular BAT was removed, dissected and trimmed free of contaminating tissues at 0°C. For electron microscopy the medial part of interscapular BAT was gently diced into very small pieces and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, at pH 7.4 for 4 hours. This was followed by post-fixation in 2% osmium tetroxide in 0.1 M phosphate buffer at pH 7.4 for 4 hours. Samples were dehydrated through a series of cold alcohols and propylene oxide and embedded in Araldite. One micron thick sections were examined by light microscopy for orientation and appropriate areas selected for thin sectioning. These sections

were cut on an LKB III ultramicrotome equipped with a glass knife, stained with 4% uranyl acetate in methanol and standard lead citrate, and examined in a Philips CM 12 electron microscope. For stereological analysis, performed with a transparent lattice point-counting grid, 10 micrographs per animal at a final magnification of 5400x were used to determine cell and nucleus profile area, volume density of the nucleus, mitochondria, lipid droplets and cytoplasm.

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 corticosterone treated animals.

RESULTS

The data presented in Table 1 indicated that corticosterone administration resulted in an increase of body mass gain in comparison to the control animals, while BAT mass remained unaffected.

Table 1. Effect of corticosterone on body and interscapular BAT mass. BAT - brown adipose tissue.

	control	corticosterone	p
initial body weight (g)	227±2.4	224±4.4	
final body weight (g)	238±4.8	257±3.0	<0.01
body mass gain (g)	11±6.0	33±4.4	<0.05
absolute BAT mass (mg)	246±10.5	248±4.5	

Adipocytes of interscapular BAT showed remarkable ultrastructural alterations after corticosterone treatment (Figure 1). They changed shape from mostly polygonal to prevalently roundish. The obvious enlargement of the brown adipocytes was confirmed by stereological data showing a significantly increased cell profile area (Table 2). From multilocular, the cells became almost unilocular, while a large lipid droplet occupied a central position and pushed the nucleus from the center of the cell towards the plasma membrane. In some instances, the shape of the lipid droplet pointed to coalescence of several smaller droplets into one large one. The nucleus acquired deep invaginations or became crescent-shaped and the quantity of heterochromatin was raised. Sometimes the shape of the nucleus was visibly changed on the account of lipid droplet expansion. The nucleus profile area remained unchanged in comparison to the control, but in the enlarged cell its volume density was decreased. The volume density of lipid droplets was noticeably increased (Table 2).

Mitochondria, packed closely to each other and to the nucleus, were spherical and appeared swollen. Their cristae were often short and seemed interrupted. The number of mitochondria per cell was obviously reduced which, together with the enlargement of the cell, diminished the mitochondrial volume density to approximately half of the control value (Table 2). Mitochondria were

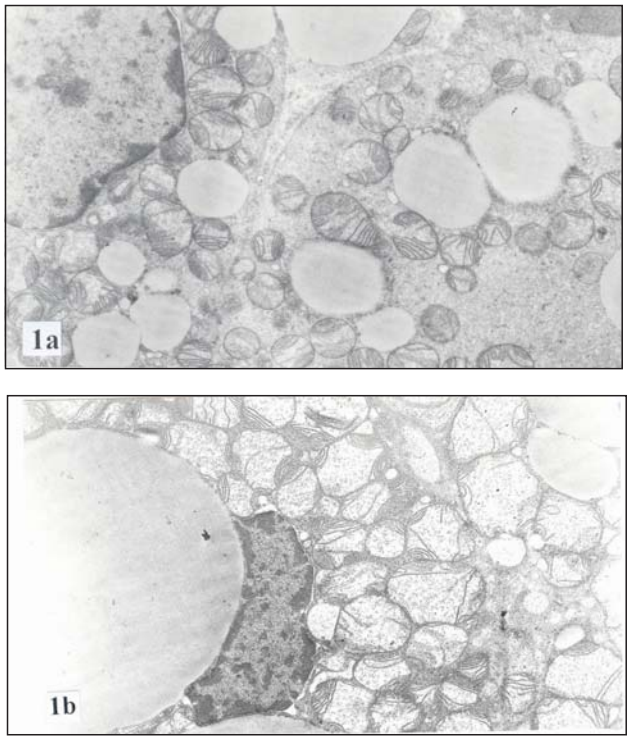

Figure 1. Portions of brown adipocytes in a control (a) and a corticosterone treated rat (b). x 7600

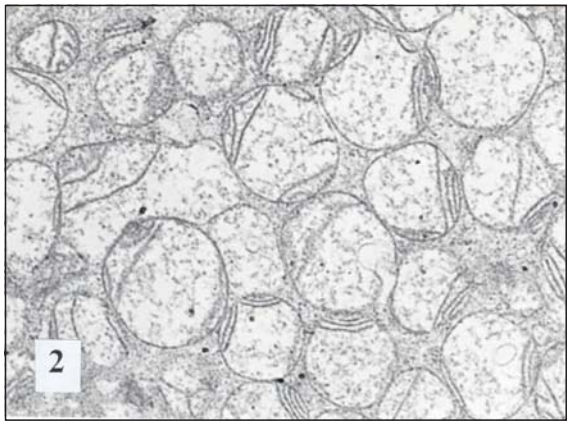

Figure 2. The mitochondrial inner membrane system is poorly developed and mitochondria appeared swollen in corticosterone treated rats. x 16700

evidently enlarged and their inner membrane system was poorly developed (Figure 2). It was also observed that three or four irregularly shaped mitochondria were mosaically placed in a way enabling one part to fit into another with sparse cristae that appeared to be "continuous" (Figure 3a). Along the contact line, membranes of two neighboring mitochondria appeared merged into one in places (Figure 3b). In the matrix of some mitochondria, an individual small lipid droplet was observed (Figure 4).

In the cytoplasmic matrix the other organelles, including polyribosomes were sparse and rarely visible.

Table 2. Effects of corticosterone treatment on some major parameters of brown adipocytes.

	control	corticosterone	p
area (μm^2)			
cell	362 ± 16.5	521 ± 29.4	<0.001
nucleus	18.8 ± 1.4	16.4 ± 1.6	
volume density (μm^2)			
nucleus	5.3 ± 0.3	3.4 ± 0.3	<0.001
mitochondria	33.0 ± 1.6	17.0 ± 0.8	<0.001
lipid droplets	48.0 ± 1.9	65.9 ± 1.7	<0.001
cytoplasm	19.0 ± 0.9	17.1 ± 1.0	

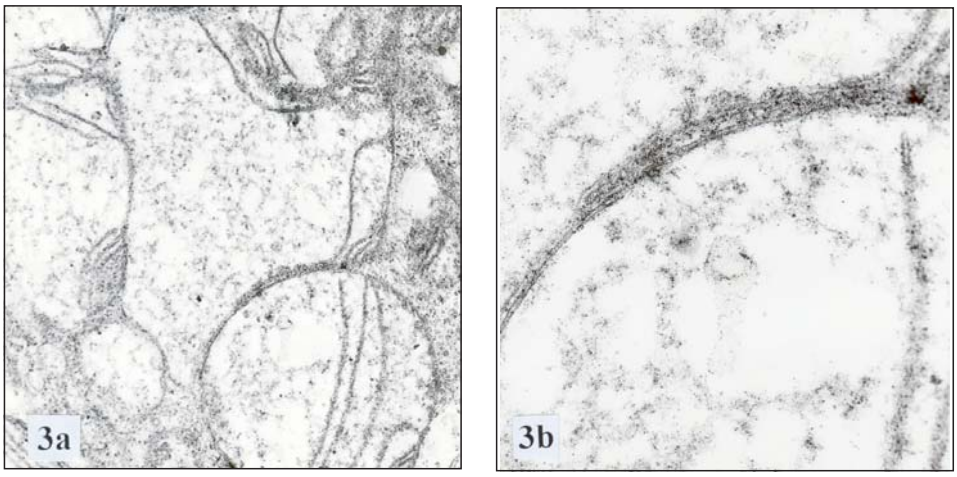

Figure 3. (a) A few more or less irregularly shaped mitochondria are placed in the way to fit one part into another. x 22400. (b) Contact line between two mitochondria where their membranes appear merged. x 63000

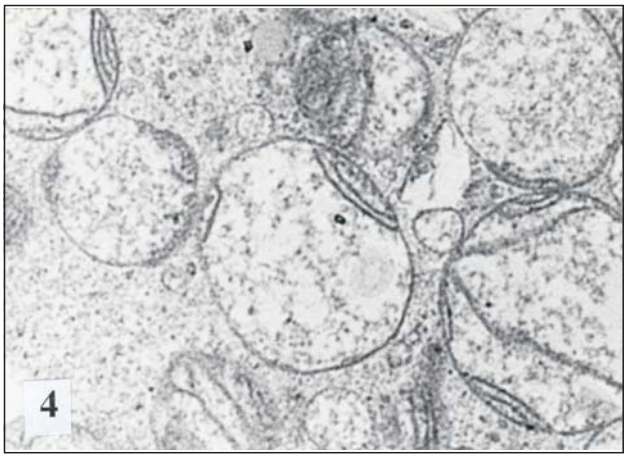

Figure 4. Small individual lipid droplets are visible in the matrix of some mitochondria in the corticosterone treated group of animals. x 22400

DISCUSSION

A number of studies have dealt with the influence of glucocorticoids on the functional status of BAT. Effects of glucocorticoids on BAT activity via alteration in sympathetic activity, modulation of the β-adrenergic pathway at the level of adenylate cyclase and inhibition of corticotropin releasing hormone production (which itself enhances BAT sympathetic activity) are well known (LeFeuvre *et al.*, 1987; Scarpace *et al.*, 1988; Davidović *et al.*, 1992). It has long been recognized that chronic corticosterone administration produces hyperphagia, high metabolic efficiency, hyperinsulinemia and suppression of BAT thermogenesis, which all together lead to obesity (Rastogi and Campbell, 1970; Galpin *et al.*, 1983a; 1983b). To the best of our knowledge, very little is known about the effect of glucocorticoids on BAT morphology. Thus, the intention of this study was to investigate and quantify morphological alterations of brown adipocytes, to establish their relationship with functional alterations and to suggest a possible corticosterone *modus operandi*.

Since the results of body mass measurement showed a significant body mass gain in corticosterone treated rats compared to the controls, it could be accepted that the applied dose of corticosterone (considerably higher than 1 mg/kg b.w. which is enough to completely restore circulating levels of the hormone in adrenalectomized animals; Rothwell and Stock, 1984) and duration of treatment were able to suppress thermogenesis and to produce the expected morphological alterations in the tissue.

Conspicuous remodeling of brown adipocytes provoked by corticosterone treatment is primarily related to the alteration of size and shape (cells become

enlarged and roundish) as well as to the accumulation and redistribution of lipid content. Lipid accumulation is a complex event connected with the reduction of cytoskeletal elements accompanied by changes in cell shape (Spiegelman and Green, 1980; Spiegelman and Farmer, 1982). Coalescence of lipid droplets observed after corticosterone treatment is an energetically more favorable mode of lipid deposition and does not need full cytoskeletal involvement for maintenance as in the multilocular BAT cell. The same applies to the newly acquired roundish ("relaxed") cell shape. This is in agreement with reduced cell engagement in protein synthesis as demonstrated by low numbers of polyribosomes in the cytoplasm.

Brown adipocyte lipid depots are restored with fatty acids derived from blood chylomicrons or very low density lipoproteins; in addition, some fatty acids could be produced by *de novo* synthesis from glucose (Himms-Hagen, 1986; 1991). Lipoprotein lipase present at the surface of the capillary endothelium in BAT enables brown adipocytes to acquire fatty acids (Nilsson-Ehle *et al.*, 1980) and corticosterone positively regulates its activity (for review see Himms-Hagen, 1986). The raised level of circulating corticosterone also provokes the release of insulin, which in turn contributes to lipogenesis with glucose serving as the precursor (Greco-Perotto *et al.*, 1987). At the same time lipolysis and fatty acid oxidation are depressed. The consequent lipid accumulation in brown adipocytes is confirmed by our stereological analysis and morphological observations.

The value for mitochondrial volume density, together with our previously published reports on mitochondrial stereological alterations after corticosterone treatment (Čakić-Milošević *et al.*, 1998) points to a diminished role of mitochondria in cell metabolism and, even more, to the loss of the ability for heat dissipation. These data are understandable if we bear in mind that corticosterone is an "antibrown hormone" which inhibits metabolic activity within BAT (Davidović *et al.*, 1992). On the basis of mitochondrial ultrastructure i. e. reduced number of cristae, we believe that corticosterone inhibits synthesis of UCP in brown adipocytes, as well as intramitochondrial synthesis of some proteins resident in cristae. On the other hand, it is possible that corticosterone could positively regulate BAT mitochondrial protease activity and thus may be involved in the selective loss of UCP-1 from mitochondria (Desautels *et al.*, 1986; Langer and Neupert 1996). We also believe that mosaically placed mitochondria with apparently continuous cristae preceded mitochondrial fusion. This assumption is supported by the fact that glucocorticoids induce fusions of mitochondria in rat liver cells (Kimberg *et al.*, 1968). Mitochondrial fusion in brown adipocytes goes together with morphological alterations of mitochondria (swelling, reduction in number of cristae) described before under the same experimental conditions that reduce BAT thermogenesis (Čakić-Milošević *et al.*, 1998). Hence it seems probable that the impulse for fusion is cessation of mitochondrial function.

The existence of small lipid droplets in the mitochondrial matrix demonstrates the inability of mitochondria to utilize free fatty acids as fuel in oxidative metabolism, but without impediment of their transfer into the matrix. This is in accordance with the findings of Levin *et al.* (1984) on the occurrence of lipid droplets in mitochondria of obese Zucker rats whose thermogenesis is defective.

The data presented in this report demonstrate that the administration of corticosterone produces a variety of morphological changes in brown adipocytes and their mitochondria. The presence of glucocorticoid receptors with a high affinity for corticosterone in brown adipocytes (Feldman, 1978) is reliable evidence that it directly affects these cells. However, it is not still completely clear whether corticosterone could directly affect BAT mitochondria. In the rat liver, Demonakos *et al.* (1993) demonstrated mitochondrial genome sequences with strong homology to nuclear glucocorticoid response elements, which, in our opinion, could point to the possibility that corticosterone achieves its effect on BAT mitochondria directly by regulation of transcription of the mitochondrial genome.

ACKNOWLEDGMENTS
This work was supported by the Ministry of Sciences and Technology, Republic of Serbia, Grant. No. 03E05.

Address for correspondence:
Maja Čakić-Milošević
Institute of Zoology
Faculty of Biology, University of Belgrade
11000 Belgrade, Serbia & Montenegro
e-mail: maja@bf.bio.bg.ac.yu

REFERENCES

1. *Čakić-Milošević M, Koko V, Davidović V, Radovanović J,* 1998, Stereological analysis of rat brown adipose tissue mitochondria after corticosterone treatment, *Folia Anat*, 26, 31-2.
2. *Davidović V, Vasilev I, Stojanović-Šušulić V,* 1992, Dependence of the sympatho-adrenal activity on the nutritional status in corticosterone treated rats, *Comp Biochem Physiol*, 101A, 309-12.
3. *Demonakos C, Đorđević-Marković R, Spandidos DA, Tsawdaroglou NC, Tsiriyiotis C, Sekeris CE,* 1993, The mitochondrial genome as a primary site of action of steroid and thyroid hormones. International Conference on Molecular Endocrinology, Oct 1-3, Athens, Greece. Book of lectures and oral presentations, 12-5.
4. *Desautels M, Dulos R, Mozaffari B,* 1986, Selective loss of uncoupling protein from mitochondria of surgically denervated brown adipose tissue of cold-acclimated mice, *Biochem Cell Biol*, 68, 441-7.
5. *Feldman D,* 1978, Evidence that brown adipose tissue is a glucocorticoid target organ, Endocrinology, 103, 2091-7.
6. *Fellenz M, Trandafillou J, Guilliam C, Himms-Hagen J,* 1982, Growth of interscapular brown adipose tissue in cold-acclimated rats maintained on thyroxin and corticosteron, *Can J Biochem*, 60, 838-42.
7. *Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, et al.* 1997, Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia, *Nature Genetics*, 15, 269-72.
8. *Fukushima M, Lupien J, Bray GA.* 1985, Interaction of light and corticosterone on food intake and brown adipose tissue of the rat, *Am J Physiol*, 249, R753-7.
9. *Galpin KS, Henderson RG, James WPT, Trayhurn P,* 1983a, Effects of corticosterone acetate on energy balance in mice, *Proc Nutr Soc*, 42, 159A.
10. *Galpin KS, Henderson RG, James WPT, Trayhurn P,* 1983b, GDP bonding to brown-adipose-tissue mitochondria of mice treated chronically with corticosterone, *Biochem J*, 214, 265-8.

11. *Greco-Perotto R, Zaninetti D, Assimacopoulos-Jeannet F, Jeanrenaud B,* 1987, Insulin modifies the properties of glucose transporters in rat brown adipose tissue, *Biochem J*, 247, 3-68.
12. *Himms-Hagen J.* 1986, Brown adipose tissue and cold acclimation, In: Trayhurn P, Nicholls DG, editors, *Brown Adipose Tissue*, Edward Arnold, London, 214-68.
13. *Himms-Hagen J,* 1991, Brown adipose tissue metabolism, In: Bjorntorp P, Bradoff NB, editors, *Obesity*, Lippincott Co. Philadelphia, Pennsylvania,15-35.
14. *Janský L.* 1995, Humoral thermogenesis and its role in maintaining energy balance, *Physiol Rev*, 75, 237-59.
15. *Kimberg DV, Loud AV, Wiener J.* 1968, Cortisone-induced alterations in mitochondrial function and structure, *J Cell Biol*, 37, 63-79.
16. *Klingenberg M,* 1990, Mechanisms and evolution of the uncoupling protein of brown adipose tissue, *TIBS,* 15, 108-12.
17. *Langer T, Neupert W.* 1996, Regulated protein degradation in mitochondria, *Experientia*, 52, 1069-76.
18. *LeFeuvre RA, Rothwell NJ, Stock MJ.* 1987, Activation of brown fat thermogenesis in response to central injection of corticotropin releasing hormone in the rat, *Neuropharmacology,* 26,1217-21.
19. *Levin BE, Finnegan MB, Marquet E, Sullivan AC,* 1984, Defective brown adipose oxygen consumption in obese Zucker rats, *Am J Physiol,* 247, E94-100.
20. *Nedergaard J, Golozoubova V, Matthias A, Asadi A, Jacobsson A, Cannon B.* 2001, UCP 1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency, *Biochim Biophys Acta*, 1504, 82-106.
21. *Nicholls DG, Locke RM,* 1984 Thermogenic mechanism in brown fat, *Physiol Rev*, 64, 1-69.
22. *Nilsson-Ehle P, Garfinkel AS, Shotz MC,.* 1980, Lipolytic enzymes and plasma lipoprotein metabolism, *Annu Rev Biochem,* 48, 667-93.
23. *Rastogi KS, Campbell J.* 1970, Effect of growth hormone on corticosterone-induced hyperinsulinemia and reduction in pancreatic insulin in the mouse, *Endocrinology*, 87, 226-32.
24. *Rothwell NJ, Stock MJ,* 1984, Sympathetic and adrenocorticoid influences on diet-induced thermogenesis and brown fat activity in the rat, *Comp Biochem Physiol*, 79A, 575-9.
25. *Rothwell NJ, Stock MJ.* 1986a, Brown adipose tissue and diet-induced thermogenesis, In: Trayhurn P, Nicholls DG, editors, *Brown Adipose Tissue,* Edward Arnold, London, 269-98.
26. *Rothwell NJ, Stock M J,* 1986b, Whither brown fat? *Biosci Rep,* 6, 3-17.
27. *Scarpace PJ, Baresi L, Morley JE.* 1988, Glucocorticoids modulate b-adrenoceptors subtypes and adenylate cyclase in brown fat, *Am J Physiol,* 255, E153-8.
28. *Spiegelman BM, Farmer SR,* 1982, Decreasing in tubulin and actin gene expression prior to morphological differentiation of 3T3 adipocytes, *Cell,* 29, 53-60.
29. *Spiegelman BM, Green H,* 1980, Control of a specific protein biosynthesis during the conversion of 3T3 cells, *J Biol Chem,* 255, 8811-8.
30. *Trayhurn P, Ashwell M,* 1987, Control of white and brown adipose tissues by the autonomic nervous system, *Proc Nutr Soc*, 46, 135-42.
31. *York DA.* 1989, Corticosteroid inhibition of thermogenesis in obese animals, *Proc Nutr Soc,* 48, 231-5.

Acta Veterinaria (Beograd), Vol. 54. No. 2-3, 95-104, 2004.
Čakić-Milošević Maja *et al*. Ultrastructural alterations of rat brown adipocytes after short-term corticosterone treatment

ULTRASTRUKTURNE PROMENE ADIPOCITA MRKOG MASNOG TKIVA PACOVA POSLE KRATKOTRAJNOG TRETMANA KORTIKOSTERONOM

ČAKIĆ-MILOŠEVIĆ MAJA, KOKO VESNA, DAVIDOVIĆ VUKOSAVA
i RADOVANOVIĆ JELENA

SADRŽAJ

Cilj ovog rada bio je ispitivanje ultrastrukturnih promena mrkih adipocita pacova, kao i njihovih mitohondrija, posle kratkotrajnog tretmana kortikosteronom. Kortikosteron je primenjivan tokom tri dana u dozi od 5 mg/kg. Rezultati primenjenih analiza su pokazali da je kortikosteron ispoljio snažan inhibitorni efekat na termogenu funkciju mrkog masnog tkiva, što se manifestovalo povećanim prinosom telesne mase. Kortikosteron je takođe doveo do promene ultrastrukturne organizacije mrkih adipocita. Stereološka analiza je pokazala da je došlo do povećanja ćelija na račun povećane akumulacije lipida, uz smanjenje zapreminske gustine nukleusa i mitohondrija. Mitohondrije nisu bile sposobne da potpuno iskoriste unete slobodne masne kiseline, što se manifestovalo sporadičnim prisustvom malih lipidnih kapi u matriksu. Pojava fuzije mitohondrija tumačena je kao znak njihove smanjene termogene aktivnosti. Po našem mišljenju, pored uticaja na mrke adipocite posredstvom glukokortikoidnih receptora, kortikosteron ispoljava i direktni efekat na genom njihovih mitohondrija.