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ULTRASTRUCTURAL AND MORPHOMETRIC ANALYSIS OF RAT BROWN ADIPOCYTES AFTER SHORT-TERM SUCROSE CONSUMPTION

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The detailed ultrastructural and stereological study of rat brown adipocytes was carried out after short-term sucrose consumption since it is known that sucrose overfeeding stimulates brown adipose tissue metabolic activity. Electron microscopy morphometric analysis was performed using the point-counting procedure. The results obtained showed that the cell and nucleus profile areas as well as nucleus volume density were increased in sucrose-treated rats. Lipid droplet volume density remained unchanged, whereas cytoplasm volume density was increased. The number of mitochondria was significantly increased, their cristae being more numerous, and the volume density of mitochondrial matrix reduced as compared with the control. Ultrastructural examination revealed that the fine internal structure of the cell, particularly that of mitochondria, was also affected by sucrose treatment: the nucleus was euchromatic and organelles involved in synthetic processes were more prominent; mitochondrial cristae, packed closely together, were straight and usually transversed the whole width of mitochondria; small mitochondria, with a well developed system of cristae, were numerous.

The results of this analysis demonstrate the mode of altering the ultrastructure of rat brown adipocytes accompanying a sucrose-rich diet. They indicate that there are no significant differences, at the level of cell morphology, between diet-induced and thermoregulatory thermogenesis, previously widely explored by other authors.

Key words: brown adipose tissue, rat, sucrose, morphometry.

INTRODUCTION

Brown adipose tissue (BAT) is the main site of heat production during thermoregulatory (or non-shivering) and diet-induced thermogenesis in many mammalian species (Suter, 1969 a; Rothwell and Stock, 1979). BAT thermogenic activity directly depends on stimulation of noradrenergic tissue (Arch, 1989; Trayhurn, 1989). Brown adipocyte mitochondria, that contain uncoupling protein (thermogenin) in the membranes of their cristae, play the crucial role in the process of heat release (Nicholls and Rial, 1984).

Exposure to cold induces BAT growth associated with increased sympathetic activity, blood flow and vascularity, as well as the number of brown adipocytes (Foster and Frydman, 1978; Bukowiecki et al., 1982; Himms-Hagen 1986; Lončar et al., 1986). In brown adipocytes, the intensive thermogenic activity is manifested by an increase in mitochondrial number as well as their morphological and biochemical ability for thermogenesis (Skala et al., 1970; Himms-Hagen, 1990; Ricquier et al., 1991; Desautels and Dulos, 1994) and by the rearrangement of lipid depots, i. e. numerous small lipid droplets appear instead of a few large ones.

Although BAT has been known as the principal effector of diet-induced thermogenesis for nearly 20 years, ultrastructural analyses of brown adipocytes were mostly concerned with morphological changes related to cold-induced thermogenesis or with tissue morphogenesis during the perinatal period (Suter, 1969 b, c; Lončar, 1991). Bearing in mind the lack of evidence in the literature about alterations of brown adipocytes during diet-induced thermogenesis, the purpose of the present study was to perform detailed ultrastructural and stereological analysis of rat brown adipocytes during short-term diet-induced thermogenesis and to compare our findings with existing data obtained under different experimental conditions.

MATERIALS AND METHODS

Twelve male albino rats of the Wistar strain, weighing about 220-235 g at the beginning of the experiment, were used. The animals, previously acclimatised to 21 ± 1^0C, were maintained under intermittent 12 hr periods of light and dark and given food and tap water ad lib. The rats were housed in individual cages and divided into two equal groups: one group was allowed to drink a 10% sucrose solution instead of water in addition to regular rat chow, over two days; the other group was an untreated control.

On day 3 of the experiment, all animals were weighed and sacrificed. The interscapular BAT was removed, transferred on ice, trimmed free of contaminating tissues and weighed. For electron microscopy the medial part of each 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 hr. Fixation was followed by postfixation in 2% osmium tetroxide in 0.1 M phosphate buffer, at pH 7.4 for 4 hr. Samples were dehydrated through a series of cold alcohol 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 ultrotome equipped with a glass knife, stained with 4% uranyl acetate in methanol, and standard lead citrate and examined with a Philips CM 12 electron microscope.

Stereological analysis was performed with a transparent lattice point counting grid with lattice ratio r = 3, using the method of Weibel et al. and Weibel and Bolender, as descibed by Aherne and Dunnill (1982). Micrographs at a final magnification of (a) 5400x were analyzed to determine cell and nucleus profile area, volume density of the nucleus, mitochondria, lipid droplets and cytoplasm and number of mitochondria per cell; (b) 11400x for mitochondrion profile area and number of cristae per mitochondrion; (c) 36000x for volume density of mitochondrial matrix.

The results are presented as means ± SE. All data were subjected to statistical analysis using Student's t-test for differences between the control and sucrose-treated animals.

RESULTS

The obtained results show that in sucrose overfed animals body weight gain as well as absolute and relative BAT masses were not changed significantly (Table 1).

Table 1. Body and tissue weights in control and sucrose overfed rats. There are no significant differences between the groups. BAT - brown adipose tissue.

	control	sucrose
initial body weight (g)	227 ± 2.4	228 ± 11.4
final body weight (g)	238 ± 4.8	232 ± 9.9
change in body weight (g)	11.0 ± 6.0	4.2 ± 2.0
absolute BATmass (mg)	246 ± 10.5	255 ± 22.8
relatve BAT mass (mg)	104 ± 5.2	109 ± 6.7

Adipocytes of the rat interscapular BAT showed remarkable ultrastructural and stereological alterations after sucrose consumption. Thus, a typical brown adipocyte in the control group (Figure 1) was roundish or polygonal in shape. It had a slightly irregularly formed nucleus with some shallow indentions and more abundant heterochromatin along the inner membrane of the nuclear envelope. Mitochondria were closely packed and some of them were in close proximity to the nucleus, girdling its whole circumference. Mitochondria appeared swollen with a pale matrix and the cristae formed two or more often slightly curved parallel and separated systems. The other organelles were usually not prominent. Lipids were distributed within lipid droplets of varying size which did not coalesce.

After sucrose treatment, the typical brown adipocyte was elongated or polygonal with a spherical, euchromatic nucleus located in the center of the cell (Figure 2). Around the nucleus there was a seemingly empty, thin cytoplasmic rim.

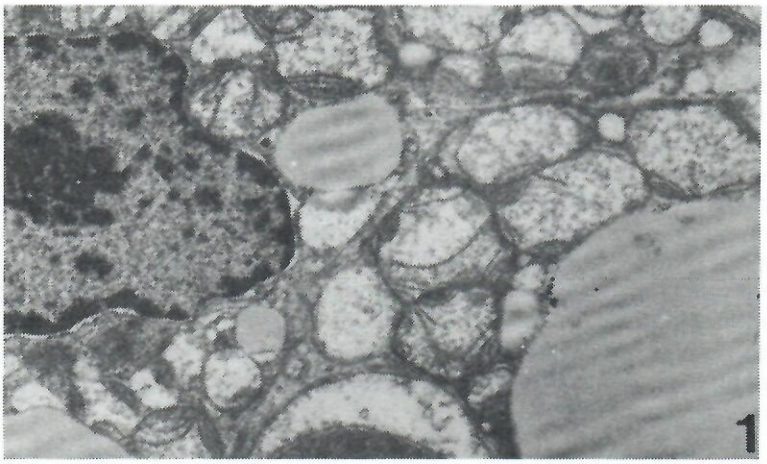

Figure 1. Portions of two brown adipocytes in a control rat. The shape of the nucleus is irregular and mitochondria are positioned nearby. Magnification x 13300.

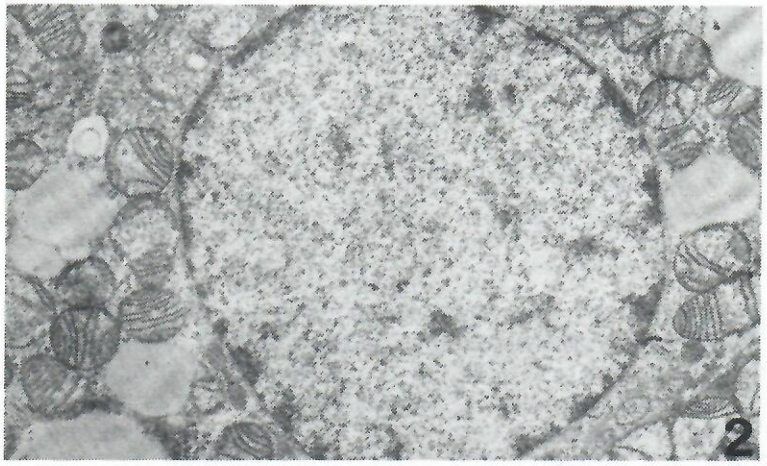

Figure 2. Portion of a brown adipocyte from a sucrose-treated rat. The nucleus is large, pale and spherical. Golgi apparatus, peroxisomes, rough endoplasmic reticulum and free ribosomes are visible among the mitochondria. Magnification x 13300.

Both cell and nucleus areas as well as nucleus volume density were significantly increased (Table 2). The number of mitochondria was increased but

mitochondrial volume density remained unchanged (Table 2). In the majority of cells mitochondria were separated from each other by the cytoplasmic matrix where other organelles were noticed. Most mitochondria were rounded, their matrix often being more dense than in the control group and the volume density markedly decreased (Table 2). Mitochondrial cristae were more numerous, straight and transversed the whole width of the mitochondria. The average size of the mitochondria was unchanged (Table 2) despite great variations existing between different brown adipocytes from the same animal. In this group there was a considerable increase in the number of small mitochondria with well-developed cristae. Cytoplasm volume density was increased (Table 2) and the cytoplasmic matrix was granulated due to the presence of numerous ribosomes. There was a large number of evenly distributed, mostly small and medium sized lipid droplets. Their volume density was unchanged in comparison to the control animals (Table 2).

Table 2. Effects of sucrose overfeeding on some major parameters of brown adipocytes and their mitochondria.

	control	sucrose	p
area (μm^2)			
cell	362 ± 16.5	466 ± 23.8	< 0.001
nucleus	18.8 ± 1.4	25.0 ± 1.6	< 0.001
volume density (μm^0)			
nucleus	5.3 ± 0.3	6.4 ± 0.3	< 0.05
mitochondria	33.0 ± 1.6	27.0 ± 1.7	n.s.
lipid droplets	48.0 ± 1.9	47.8 ± 2.9	n.s.
cytoplasm	19.0 ± 0.9	25.3 ± 2.0	< 0.001
number of mitochondria per cell (N)	92 ± 3.7	114 ± 7.3	< 0.05
mitochondrion profile area (μm^2)	0.99 ± 0.06	0.86 ± 0.07	n.s.
number of cristae per mitochondrion (N)	7.9 ± 0.28	8.8 ± 0.12	< 0.001
volume density of mitochondrial matrix (μm^0)	70.8 ± 1.8	52.6 ± 1.8	< 0.001

Generally, multilocularity was very conspicuous in sucrose-treated rats and lipid droplets were of more uniform size than in the control rats. Organelles were often absent immediately around the lipid droplets. The mainly well developed Golgi apparatus was usually positioned in the periphery of the cell. Components of the lysosomal system seemed numerous, especially residual bodies, and the presence of numerous peroxisomes was conspicuous (Figure 3).

a constant supply of fatty acids as substrate for oxidation and heat production (McKee and Andrews, 1989; Himms-Hagen 1991), as well as for uncoupling protein activation (Nicholls and Rial, 1984), Glucose, as the sucrose metabolite, provokes insulin release (Ohno and Kuroshima,1983). Insulin has beneficial effects on thermogenesis (Rothwell, 1989) and in turn, contributes to lipogenesis with glucose serving as the precursor (McCormack, 1982).

The enhanced lipid metabolism was accompanied by increased multilocularity. This leads to an increase in the total lipid droplet surface accessible for hormone-sensitive lipase which releases fatty acids from lipid stores. The "empty" space around the lipid droplets on our micrographs probably corresponds to the microfibrillar basket-like supporting structure previously described by Lončar and Afzelius (1989) which prevents lipid droplet coalescence. Moreover, well developed cytoskeletal elements are necessary for maintenance of the highly energized irregular shape of brown adipocytes and for keeping the nucleus centrally positioned.

The well developed Golgi apparatus is involved in the formation of lysosomes whose number, in the form of residual bodies, increases in metabolically active brown adipocytes. The components of the lysosomal system are related to morphological transformations of brown adipocytes from a less to a more active form whereby the cell machinery is partly changed due to the lysosomal degradation of preexisting cell components (Nedergaard et al., 1986).

Our ultrastructural and morphometric examinations, for the first time performed on the BAT of sucrose-treated animals, have shown that there is a correlation between morpho-stereological aspects of brown adipocytes during diet induced and non-shivering thermogenesis. Although these two types of thermogenesis are controlled by different central mechanisms, the response of the effector tissue (or, more precisely, effector cells) is generally the same. Some specificities are mainly linked to the presence of mitochondria that are small but structurally seem capable of thermogenesis. Finally, most results are strictly in accordance with data from functional and biochemical examinations previously obtained by other authors.

A c k n o w l e d g m e n t

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ULTRASTRUKTURNA I MORFOMETRIJSKA ANALIZA ADIPOCITA MRKOG MASNOG TKIVA PACOVA POSLE KRATKOTRAJNOG UNOSA SAHAROZE

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SADRŽAJ

Imajući u vidu činjenicu da povećan unos ugljenih hidrata dovodi do metaboličke aktivacije mrkog masnog tkiva kod mnogih vrsta sisara, sprovedena je detaljna ultrastrukturna i stereološka analiza adipocita mrkog masnog tkiva pacova posle kratkotrajnog konzumiranja saharoze. Elektronske mikrografije celih adipocita mrkog masnog tkiva i njihovih delova analizirane su metodom brojanja testnih tačaka koje padaju na odgovarajuće strukture u ćeliji. Utvrđeno je da su, kod pacova koji su pili rastvor saharoze umesto vode, povećane površine obrisa ćelija i nukleusa, kao i zapreminska gustina nukleusa. Zapreminska gustina lipidnih kapi nije promenjena, a citoplazma je povećana. Značajno je povećan i broj mitohondrija, njihove kriste su brojnije, dok je zapreminska gustina matriksa redukovana u odnosu na kontrolu. Ultrastrukturna ispitivanja su pokazala da je primenjeni tretman uticao na finu strukturu adipocita mrkog masnog tkiva i njihovih mitohondrija: nukleusi su euhromatski, a u ćelijama je istaknuto prisustvo organela uključenih u sintetske procese; mitohondrijalne kriste su međusobno paralelne, bliske i često se pružaju kroz čitavu mitohondriju; matriks mitohondrija je tamniji nego u kontrolnoj grupi. Brojne su male mitohondrije sa dobro razvijenim sistemom kristi.

Prikazani rezultati demonstriraju način na koji dijeta obogaćena ugljenim hidratima utiče na izmenu ultrastrukture adipocita mrkog masnog tkiva i ukazuju da na nivou ćelijske morfologije nema bitne razlike između dijetom indukovane i, ranije morfološki detaljno opisane, termoregulatorne termogeneze.