Brown adipose tissue: structure and function

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Within the context of the biology of brown adipose tissue (BAT), a clear understanding of physiological and biochemical mechanisms requires a sound knowledge of the histology and ultrastructure of this fascinating tissue. Recent research interest in BAT has been concentrated on two aspects of its function in the body, namely non-shivering thermogenesis and diet-induced thermogenesis. Both these functions involve the important fact that, whereas all cells of the body produce heat as a by-product of their metabolic processes, the main function of the cells of BAT is heat production. Thus metabolic procedures such as maintenance of deep body temperature, rewarming after hibernation and food intake–energy balance are intimately related to the function of BAT, and hence to its structure. In the adult rat at thermoneutral temperature (28–30°) BAT comprises about 1% of total body-weight (Hammar, 1895) and contributes 1% to the overall metabolic rate. When stimulated (by cold exposure) this contribution can rise to 50% of total metabolic rate (Foster, 1986) and the BAT weight rises to 3–5% of total body-weight.

Anatomically, BAT differs from white adipose tissue (WAT). Whereas the latter is distributed throughout the body in a diffuse state (i.e. in the subdermis), BAT occurs as discrete lobes invaginated by thin strands of connective tissue. BAT is of a brown–red colour, due to high vascularization and the presence of mitochondrial cytochromes, as opposed to the yellow appearance of WAT. BAT is more vascular than WAT, being supplied with a rich capillary plexus. BAT is also highly innervated by postganglionic sympathetic fibres. At light microscope level, the BAT cell can be seen to be considerably smaller (25–40 μm) (Girardier, 1983) than the cell of WAT (60 μm). Its nucleus is characteristically round, compared with the flattened or 'crescent shaped' nucleus of the WAT cell. The cytoplasm stains acidophilically and is an obvious feature of the BAT cell on light microscopy. However, the predominant difference between the cells of the two types of adipose tissue is the state of division of the lipid which they carry. Under conditions of even slight cold stress, the BAT cell can be seen to contain multiple lipid droplets. The white adipocyte always presents a unilocular appearance. The size of the lipid droplets of BAT and their numbers vary considerably, dependent on the metabolic status of the animal. At thermoneutrality, the BAT cell may present an unilocular appearance; hence the locularity of the cell cannot be used alone to distinguish the two cell types. The electron microscope must be employed to discern further differences between the white and brown adipocyte. Although in both cells there are relatively few cell organelles, a major difference lies in their mitochondria. Those of the BAT cell are characteristically more numerous (Nedergaard & Lindberg, 1982) and larger (>0.5 μm) than those of the WAT cell (<0.3 μm) (Afzelius, 1970). The cristae typically cross the whole width of the organelle, and are tightly packed. The surface area of the inner membrane is considerably greater than that of the outer membrane (Lindgren & Barnard, 1972) and appears to be directly related to the role of energy production of the mitochondrion. The organization of the cristae of mitochondria of the WAT cell is less obvious. In addition to the much increased surface area of the inner membrane, its structure differs from that of the mitochondrion of the WAT cell and cells

of other tissues, in that it lacks elementary particles (Lindberg et al. 1967). Related to these structural differences of the inner mitochondrial membrane is the presence of an uncoupling component (uncoupling protein) which is specific to the thermogenic capacity of BAT (for review, see Ricquier & Bouilland, 1986). This protein alters the proton conductance sequence, enabling protons to re-enter the mitochondrion without ATP generation. The amount of uncoupling protein (and hence the surface area of the inner membrane) is directly related to the heat-producing capacity of the tissue (Nicholls et al. 1986). These facts indicate the essential role played by the mitochondrion of the brown adipocyte in the performance of heat generation by the cell.

Considerable efforts have been made to relate the ultrastructure of the brown adipocyte to changes of metabolic status of the animal. Two major structural changes occur during the chronic stimulation of BAT: increase in multilocularity of the lipid content, and increase in the surface area of the inner membrane of the mitochondrion (e.g. Suter, 1969; Hull & Vintner, 1984; Nechad, 1986). However, since the area available for viewing with the electron microscope is necessarily very limited in comparison with the area of a longitudinal section through an entire lobe of BAT, great care must be taken in sampling procedures.

In our work we have noted that the lipid droplets appear to be consistently larger at the cranial end of the interscapular lobe of laca mice, and smaller at the caudal end. A distinct boundary between these two populations is not evident. Also, at the edge of the interscapular BAT (IBAT) pad, the lipid distribution tends to be unilocular. It is possible in adult mice that, although the BAT in this region remains for the most part active and capable of heat production if required, the outer parts of the lobe have undergone involution and replacement with WAT. In addition to these observations an inhomogeneity of the BAT structure has been observed in proximity to large blood vessels, where the lipid droplet size is consistently small. Thus, in any experiment where sampling for histology and, particularly, electron microscopy is involved, care should be taken to reduce apparent structural changes which may in fact be due to anatomical variations in the tissue. In this laboratory, we have consistently discarded the outermost parts of the IBAT pad, whether or not these regions could be described as 'white' on visual examination. We have taken samples from the central region of the upper half of the pad. In making quantitative measurements using either light or electron microscopy, we have avoided regions containing large blood vessels.

Dimensions of lipid droplets are frequently measured using the light microscope. The upper limits of the size range can be accurately assessed, but the lower size ranges are approaching the limits of resolution of the light microscope. This rarely presents a problem when 'resting' BAT (i.e. from an animal at thermoneutrality) is examined, since in our work we found the normal range of the area of lipid droplets in mouse IBAT to be 365 (SD 240) μm^2. However, in cold-exposed animals, where the BAT is functional in heat production, the lipid droplet areas are consistently much smaller and the variation relatively greater. The light microscope alone cannot be used for the entire range of droplet sizes. The electron microscope, even at low magnifications, cannot accommodate the larger droplets, but can give values for the bottom end of the range. Hence, particularly in experiments where the effects of varying times of cold exposure are being monitored, a combination of light and electron microscopy is essential.

The remaining part of the present paper will be devoted to a description of our studies of four examples of the inter-relation, in animal models, of structure and function in BAT. The examples used will be: (1) cold exposure in the lean mouse; (2) cold exposure in the Aston, genetically obese (ob/ob) mouse; (3) arousal after hibernation in the hamster; (4) nutritional status in the mouse.

Cold exposure in the lean mouse

A major role of BAT is that of heat production. It has been estimated that, in rats placed in a cold environment, 60% of the extra oxygen utilized by the animal supplies the brown fat (Foster & Frydman, 1979). Several studies of the changes in brown adipocyte ultrastructure as a result of cold exposure have been carried out (Cameron & Smith, 1964; Suter, 1969). In our studies (Simba *et al.* 1984) we have used adult male laca mice, warm-acclimated at about thermoneutral temperature (28°). The animals were exposed to cold stress (10°) for times varying between 30 min and 14 d, then killed by cervical dislocation, and the IBAT was studied by light and electron microscopy. Warm-housed animals acted as controls. The immediate response of the adult homeotherm to sudden temperature decrease is thermogenesis by shivering. This is energy consumptive and is progressively replaced by non-shivering thermogenesis; it is accepted that BAT plays a major part in this adaptation (Bruck, 1970; Nicholls & Locke, 1984). Such adaptation would be expected to be reflected in ultrastructural changes. The brown adipocytes from the warm-acclimated animals, while not unilocular, contain predominantly large lipid droplets. After 3 h at 10° three changes are apparent: (1) the size of the lipid droplets progressively decreases with the time of cold exposure, (2) the vascularity of the tissue increases (one of the causes of the dark red–brown appearance of BAT on dissection), (3) the number and internal organization of the mitochondria increase.

The response of the tissue is extremely rapid, the initial changes in lipid droplet size being detectable within 3 h. The mitochondrial changes are in direct relation to the decreasing lipid droplet size, indicating an increase in the thermogenic capacity of the BAT. Mitochondrial involvement in the cold adaptation of rats has been established by biochemical techniques (Bukowieki *et al.* 1978), but to date no parallel study has been done on mice. In our histological analysis, changes in both lipid droplet size and mitochondrial structure are well established between 12 and 48 h of cold exposure, indicating a breakdown from large to small lipid droplets, and hence provision of a larger surface area for lipolysis. Mitochondrial changes lag slightly (24 h) behind those of lipid droplet sizes, possibly due to synthesis of internal membrane material.

Cold exposure of genetically obese mice

Genetically obese (*ob/ob*) mice are known to have poor thermoregulatory ability (Davis & Mayer, 1954). This is suggested to be due to a defect in the ability of their BAT to produce heat (Hogan & Himms-Hagen, 1980). For this reason, in our laboratory, we have compared the response of cold stress in the Aston strain of mouse using both the obese mice and their lean littermates, in which the gene was not expressed, to the laca strain described previously. All animals (male adults) were warm acclimated at 28°. The obese mice could not survive temperatures of 10°, and were cold stressed at a temperature above that at which they entered torpor (17°) for periods of time from 12 h to 14 d. In warm-acclimated animals, as for the warm-acclimated laca mice, the droplet size was large. The mitochondria, similar to those of the warm-acclimated laca mice, showed few looped, peripherally-located cristae. After 24 h in the cold (17°) the droplet size of adipocytes of the lean heterozygotes decreased, analogous to that of the laca mice. Following these changes, a progressive increase in the surface area of mitochondrial inner membrane, as indicated by increased numbers of cristae, is evident. The *ob/ob* mice showed no rapid decrease in lipid droplet size, the cells remaining unilocular in appearance during the cold exposure period. However, the mitochondrial order increased, after a lag phase of 3 d (Mehigan *et al.* 1985). Thus, although the mitochondrial inner membrane area increases, no lipid breakdown is occurring. It is

possible that a defect in the proton conductance chain plays an important part in the lack of thermogenic response of the *ob/ob* mice; lowered sympathetic activity may also be involved. Fluorescent microscopy studies on lean cold-acclimated mice show the presence of catecholamine-dependent fluorescence around both the blood vessels and the adipocytes (Ashwell, 1985). In warm-acclimated animals BAT lobes showed fluorescence only around the blood vessel walls. At all temperatures the obese mice resembled the warm-acclimated lean animals, indicating a reduced sympathetic input to BAT in these mice at all times, and hence an inability to increase non-shivering thermogenesis on cold stress. This would then lead to reduced lipolysis and hence the retention of large lipid droplets as observed by Mehigan *et al.* (1985).

The role of BAT in the hibernation sequence

BAT has long been associated with the process of hibernation (Rasmussen, 1923) and, indeed, was referred to as the 'hibernating gland'. In this laboratory, we have followed the morphological changes in BAT during arousal of the hibernating hamster (Andrews *et al.* 1984). The hamster is induced to enter hibernation by temperature reduction (to 5°) and, hence, is in a cold-adapted state. Thus, the lipid droplet size is small. As arousal proceeds the droplet size remains low, and the tissue vascularity noticeably increases. The BAT of the hibernating animal contains elongated mitochondria with well orientated cristae (Umahara, 1968). As arousal progresses, the overall size and length of the mitochondria decreases. The inner membrane surface area remains high at 60 min into the arousal process. These results agree with the studies of lipid utilization during arousal by Nedergaard & Cannon (1984) and definitively implicate BAT in the phenomenon of hibernation and arousal. Again, initial changes are rapid indicating the plasticity of the tissue.

Effects of nutritional status on BAT in the mouse

It is known that BAT increases in weight as thermal response of the animal is required, for example, in cold exposure, or in diet-induced thermogenesis, and these mechanisms seem identical. Considerable information has accumulated on energy balance and diet in model systems (Rothwell & Stock, 1986). However, the use of the 'cafeteria diet' introduces the problem of choice of food item by the animal, and hence lack of control of the energy intake. In our laboratory, we have compared the IBAT structure from mice housed at 28° and fed *ad lib.* on a standard laboratory diet, with mice fed with half their normal intake of laboratory stock diet, and with mice that in addition to the laboratory diet have been offered chocolate (known to stimulate energy intake (Younger & Trayhurn, 1984)). After 3 weeks, the animals were killed and the IBAT pads were examined using light and electron microscopy. In comparison with the controls (droplet area 374·5 μm^2) the average size of the underfed animals was significantly decreased (83·9 μm^2). This would indicate a mobilization of the lipid for use as an energy source at the other sites in the body. In both *ad lib.*-fed and half-fed animals, the mitochondria present an inactive profile, with few, curved cristae, indicating little thermogenic activity. In the case of the hyperphagic animals, the lipid droplet size is again significantly reduced (35·3 μm^2), but in this case the mitochondria appear well organized with many straight cristae, indicating metabolic activity of the BAT in heat production (C. Murphy, J. F. Andrews and E. Arbuthnott, unpublished results). This work might have been added to significantly by an assay of the total lipid content of the animals.

These examples of variations in structure of the brown adipocyte with changes in metabolic and nutritional status serve to emphasize the specialized functional significance of this tissue. Because of its rich sympathetic innervation and its highly vascular

nature the activity of BAT can be increased very rapidly in response to metabolic requirements of the animal, and the heat so produced can be quickly and effectively dispersed.

Ultrastructural observations implicate, in all the examples given, the role of the mitochondrion in the increased metabolic activity of the adipocyte. Both the cold-exposure experiments and the nutrition studies indicate that the same pathway is being followed; namely an increase in surface area of the inner mitochondrial membrane, allied to a decrease in lipid droplet size. The increase in inner membrane surface area and, hence, an increase in available uncoupling protein is involved in the production of heat by the lipid oxidation sequence. This is specific to the brown adipocyte mitochondrion. Thus, the BAT cell provides an insight into the metabolic control mechanisms of the small rodent.

It is known that BAT is present in the human neonate and that it persists in the adult, the number of sites decreasing with the age of the subject (Heaton, 1972). Its role in diet-induced thermogenesis in the human being is debatable (Rothwell & Stock, 1983; Lean & James, 1986); however, in cases of acute cold stress, ageing or starvation, a depletion of the lipid content of BAT in human subjects has been recorded (Aherne & Hull, 1966; Heaton, 1973). This finding may suggest that under conditions of varying metabolic status 'resting' brown fat may become reactivated, as is known to be the case for the small mammal.

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Printed in Great Britain