Brown adipose tissue in the parametrial fat pad of the mouse

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Cold acclimation has been shown to produce a substantial increase in the number of brown adipocytes in the parametrial fat pad of female BALB/c mice - a site normally thought to consist of typical white adipocytes. The brown adipocytes have been identified not only on the basis of their morphology using light and electron microscopy, but also on the basis of the content of the mitochondrial 'uncoupling protein' (M_r = 32000) which is characteristic of the proton conductance pathway of brown adipose tissue.

Brown adipose tissue *Parametrial white adipose tissue* *Mitochondria* *Cold acclimation* *Radioimmunoassay* *Uncoupling protein*

1. INTRODUCTION

The exact relationship between white and brown adipocytes has never been fully understood although it has been the subject of much speculation. One school of thought believes that white and brown adipocytes have different origins [1]; the other view is that they are derived from a common precursor and therefore are possibly interconvertible [2]. Whatever their origin, they have usually been thought to reside in anatomically separate adipose tissue regions, e.g. the perigenital fat pads are thought to be a typical white adipose tissue depots whereas the interscapular fat pad is thought to be a typical brown adipose tissue depot [3]. Traditionally, brown adipocytes have been distinguished from white adipocytes by virtue of their multilocular fat droplets, their centrally placed nucleus and their numerous mitochondria with complex internal structure as viewed by electron microscopy [4]. The mitochondria of brown adipocytes also have the apparently unique feature of being able, via their proton conductance pathway, to generate heat by uncoupling substrate oxidation from the production of ATP [5]. This pathway can be blocked by purine nucleotides, which bind to a specific 'uncoupling protein' (M_r = 32000) situated in the inner membrane of brown adipose tissue mitochondria [6].

During a preliminary survey of the effects of cold acclimation on female BALB/c mice, we noticed some brown areas in the perigenital fat pad. Since this observation was contrary to the popular view of distinct white and brown fat regions, we decided to set up this study so that morphological and biochemical criteria could be used to differentiate between white and brown adipocytes in the parametrial fat pad from BALB/c mice before and after cold acclimation. Our results provide evidence for the existence of some brown fat cells in this traditional white fat cell pad and for a substantial increase in the numbers of these cells after cold acclimation.

2. MATERIALS AND METHODS

Female BALB/c mice (24–26 g) obtained from Charles River UK were housed at either 4°C or 23°C for a period of 4 weeks. During this time they were allowed pelleted stock diet ad libitum. At sacrifice, the interscapular brown adipose tissue depot was removed together with both parametrial fat pads. Brown adipose areas were carefully dissected away from white adipose areas in the parametrial fat pad.

For light microscopy, conventional histology techniques were used. The samples were fixed in 10% (w/v) Formol saline and embedded in wax

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prior to sectioning. The sections were stained with haematoxylin and eosin. For electron microscopy, samples were fixed in 2.5% (w/v) glutaraldehyde followed by 1% (w/v) osmium tetroxide, both buffered with Pipes [piperazine-*N,N'*-bis(2-ethanesulphonic acid)], pH 7.0. The samples were then dehydrated, embedded in resin and sectioned. The sections were stained with uranyl acetate and lead citrate.

The maximal activities of oxoglutarate dehydrogenase and citrate synthase activities were assayed in tissue homogenates [7,8]. Mitochondria were prepared [9] from the interscapular brown fat depot and from brown and white areas of the parametrial fat pad from groups of 3 animals. Proteins were separated by discontinuous slab gel electrophoresis in the presence of sodium dodecyl sulphate [10] using 10% (w/v) acrylamide. Uncoupling protein (M_r = 32000) was located using protein markers (M_r 14000–64000). Only the 32000 protein showed increases in height relative to other mitochondrial proteins bands with cold acclimation. Its height was therefore measured relative to an adjacent band M_r 30000 in each gel. Results in table 2 are expressed in terms of this ratio with the ratio for mitochondria from control mice set at 100%.

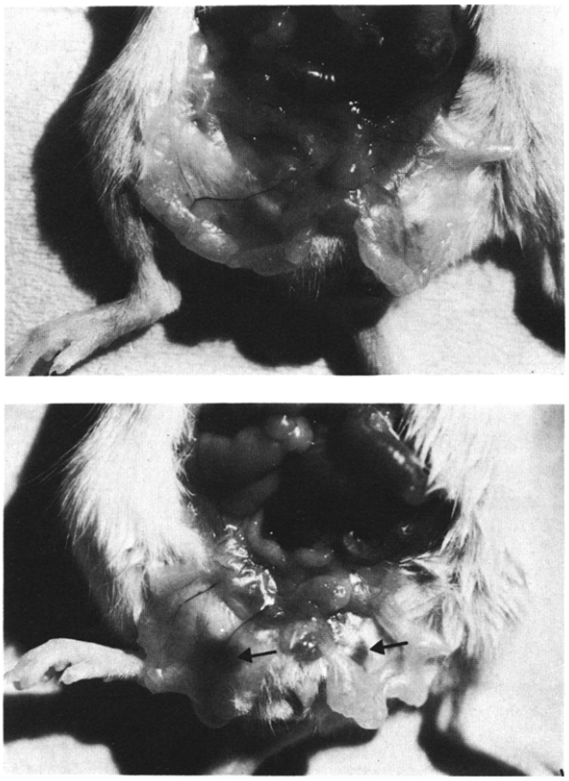
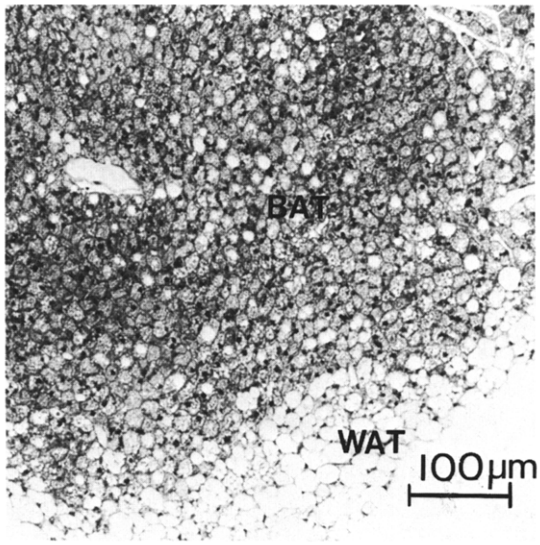


Fig. 1.(a) Parametrial fat pads on a 23°C acclimated mouse. (b) Parametrial fat pads of a cold-acclimated mouse. The arrows show regions of brown tissue.



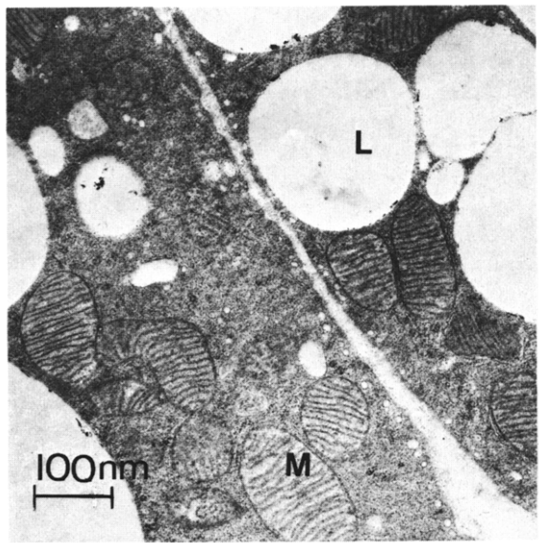


Fig. 2.(a) Light microscopy of the brown adipose tissue in the parametrial fat pad of a cold acclimated mouse. BAT, brown adipose tissue; WAT, white adipose tissue. (b) Electron micrograph of the brown adipose tissue in the parametrial fat pad of a cold acclimated mouse. M, mitochondria; L, lipid droplet.

Table 1

Activities of oxoglutarate dehydrogenase and citrate synthase in brown and white areas of adipose tissue from the parametrial and interscapular fat pads

	Enzyme activity (μmol · min^{-1} · g wet wt^{-1})	
	Oxoglutarate dehydrogenase	Citrate synthase
Parametrial white	<0.1	3.2 ± 0.9
Parametrial brown	3.8 ± 0.8[a]	92.4 ± 17.2[b]
Interscapular brown	11.6 ± 2.3[b]	201.6 ± 40 [b]

[a]p <0.01, [b]p <0.001 compared to values for parametrial white adipose tissue using an unpaired t-test

Tissues were taken from mice that had been cold-acclimated for 4 weeks. Results are the means ± SE of 6 observations

Mitochondria were also lysed using 0.01% Triton X-100 in phosphate-buffered saline (pH 7.5) and diluted to a final concentration of 500 μg protein/ml. The lysates were assayed for uncoupling protein using rabbit anti-rat uncoupling protein serum in a solid phase radioimmunoassay [11]. Uncoupling protein isolated from mice [12] was used to produce a standard curve.

3. RESULTS

Brown areas of adipose tissue could be detected as a diffuse region close to the blood vessels in the parametrial fat pads of adult BALB/c mice (fig.1a) maintained at 23°C. With cold acclimation this region darkened and increased to approximately 15 mg/mouse, corresponding to 6% of the weight of the pad. Also other diffuse brown tissue areas appeared within the pad (fig.1b).

Histological examination showed that cells in the brown adipose tissue area of cold acclimated mice were centrally nucleated and contained multilocular fat droplets (fig.2a), in contrast to the unilocular fat cells with eccentric nuclei observed in the surrounding white area of adipose tissue. Electron microscopy revealed a high concentration of mitochondria within the brown adipose tissue (fig.2b). This observation was supported by biochemical studies that showed high activities of the mitochondrial marker enzymes, oxoglutarate dehydrogenase and citrate synthase, in the brown, but not in the surrounding white areas of the parametrial fat pad (table 1).

In addition, mitochondria prepared from the brown areas of the parametrial fat pads were enriched in uncoupling protein (M_r 32000) (table 2). The total amount, as measured either from scans of polyacrylamide gels or by the more specific radioimmunochemical method increased substantially with cold acclimation. A 2-fold in-

Table 2

Effect of cold acclimation of the uncoupling protein (M_r 32000) content of parametrial and interscapular adipose tissue as measured from polyacrylamide gels and by specific radioimmunoassay

Acclimation temperature (°C)	Adipose tissue type	Proportion of M_r 32000 protein (%)	Uncoupling protein by radioimmunoassay (μg/mg mitochondrial protein)	
23	Parametrial	Brown	100.0 ± 4.7 (6)	16.5 ± 3.6 (6)
4	Parametrial	Brown	153.7 ± 11.5 (6)[a]	73.4 ± 6.0 (6)[b]
23	Parametrial	White	100.0 ± 2.8 (6)	3.3 ± 0.9 (6)
4	Parametrial	White	93.4 ± 5.3 (6)	15.8 ± 2.7 (6)[a]
23	Interscapular	Brown	100.0 ± 4.1 (6)	27.3 ± 2.7 (6)
4	Interscapular	Brown	152.3 ± 8.4 (6)[b]	75.3 ± 8.0 (6)[b]

[a]p <0.01, [b]p <0.001

Results are the means ± SE of the number of observations shown in parentheses. Statistical comparison using an unpaired t-test compares the same tissue at the two temperatures

crease between 22°C and 4°C as measured by radioimmunoassay has previously been reported for the interscapular brown fat site of mice and was confirmed in these experiments [13].

4. DISCUSSION

Our results have indicated that areas of brown fat cells exist amongst the traditional white fat cells which make up the parametrial fat pad of female BALB/c mice and that these brown fat cells are particularly numerous after the mice have been cold acclimated. It is interesting to note that brown areas of parametrial fat have been noticed, but not characterized, in lean mice of the Aston strain [14]. In our preliminary studies, we found that the parametrial fat pads of cold acclimated female CD1 mice and female C57BL/6 mice also contain small areas of brown tissue, but these mice have less brown tissue than female BALB/c mice. However, no brown tissue can be observed in the epididymal fat pad of male BALB/c mice even after cold acclimation.

Brown fat cells could arise in the parametrial fat pad by recruitment from precursor cells or by transformation from existing white fat cells. Our current measurements do not allow us to draw any firm conclusions about the way in which these cells arise. Increases in cell number in epididymal sites in response to cold acclimation have been reported [15,16]. Cell number was determined from DNA estimations and it was assumed that cell number was increased by the addition of white fat cells. However, recruitment of brown fat cells would also result in an increase in total cell number. On the other hand, our own histological observations of the parametrial fat pads of non-cold-acclimated BALB/c mice have shown the existence of areas of white cells which are smaller than average for the fat pad; these areas are those in which brown cells occur on cold acclimation. This observation would suggest that transformation, rather than recruitment, is occurring.

Whatever the ontogeny of the brown cells, these results are the first to show that they can lie dormant in a traditional white adipose tissue depot and that the stimulus of cold can cause considerable hyperplasia of those cells in the same way as it does in traditional BAT depots. Furthermore, cold acclimation causes an enrichment of mitochondria within the BAT cells and a particular enrichment in the concentration of M_r 32 000 uncoupling protein. Since this particular protein is crucial for BAT thermogenesis it follows that the total thermogenic potential of the tissue is increased.

BAT has recently been shown to be quantitatively important in both non-shivering thermogenesis and diet-induced thermogenesis [17]. So far, evidence has been largely restricted to studies of the interscapular BAT and calculations of the total thermogenic potential of an animal have been based on the amount of BAT in traditionally recognised depots. The demonstration that brown fat cells can lie dormant in traditional white adipose tissue depots suggests that adipose tissue with thermogenic properties may be more generally distributed than previously supposed. It may also help to explain the difficulty experienced with the positive identification of brown adipose tissue in man in spite of its postulated role in diet-induced thermogenesis.

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