Presence of the brown fat-specific mitochondrial uncoupling protein and iodothyronine 5'-deiodinase activity in subcutaneous adipose tissue of neonatal lambs

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Subcutaneous adipose tissue of neonatal lambs has been examined for the presence of markers diagnostic of thermogenic brown fat. Uncoupling protein, uncoupling protein mRNA, and iodothyronine 5'-deiodinase activity were each detected in subcutaneous adipose tissue, as well as in the major internal fat depot (perirenal), of newborn lambs. These brown fat markers were not present, however, in adipose tissue of adult sheep. It is concluded that subcutaneous fat in newborn lambs is functionally 'brown', and similar to the internal fat; subcutaneous and internal adipose tissues follow a similar developmental path — from 'brown' to 'white'.

Brown adipose tissue; White adipose tissue; Uncoupling protein; Iodothyronine 5'-deiodinase; Western blotting; Northern blotting

1. INTRODUCTION

Brown adipose tissue is characterized by the presence of a tissue-specific mitochondrial uncoupling protein (UCP), M_r 32,000 [1–4]. The tissue, which generates heat by a regulated uncoupling of oxidative phosphorylation [1], has been identified in a number of mammals on the basis of the immunological detection of UCP [4,5], including newborn ruminants such as lambs and reindeer [5–7]. Although UCP is present at birth in the internal fat depots (perirenal, pericardial) of these precocial animals, it disappears over the first days or weeks of postnatal life, indicating that there is a rapid transition from brown to white adipose tissue [6,7]. In contrast to the internal depots, UCP and its mRNA have not been detected in the subcutaneous fat of newborn lambs [6,8], suggesting that there may be fundamental differences in the nature and development of the subcutaneous and internal adipose tissues.

During studies on adipose tissue development in neonates, we have been able to further examine and redefine the nature of the subcutaneous fat in the newborn lamb. UCP, the critical marker of brown fat, and its mRNA, were detected in subcutaneous adipose tissue, indicating that the tissue is functionally 'brown' at birth. High iodothyronine 5'-deiodinase activity, a further indicator of brown adipose tissue, was also evident. UCP and iodothyronine 5'-deiodinase activity were not found, however, in subcutaneous adipose tissue of adult

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sheep. These results suggest that the development of subcutaneous adipose tissue is similar to that of the internal fat depots.

2. MATERIALS AND METHODS

2.1. Animals and tissues

Seven lambs [Suffolk × (Border Leicester × Scottish Blackface)] were obtained from a flock at the Rowett Research Institute, and killed within 3 h of birth by an intravenous overdose of sodium pentobarbitone. Adipose tissue was rapidly removed from the subcutaneous (rear, near the hindlimbs; and from the shoulder area), perirenal, and pericardial regions, and frozen in liquid N_2. Adipose tissues were also obtained from a group of adult sheep, treated similarly to the newborn. All tissues were stored at −80°C, until analysis. Contaminating tissue was removed, and separate samples taken for each assay.

2.2. Uncoupling protein

Tissues were homogenized in 250 mM sucrose, 1 mM HEPES, 0.2 mM EDTA (pH 7.2), and mitochondria prepared [9]. Mitochondrial proteins were measured with bovine serum albumin as a standard, and separated according to molecular weight by SDS-PAGE [7]. The proteins were blotted onto nitrocellulose (Hybond-C extra; Amersham International), and probed with a rabbit anti-(ground squirrel UCP)serum, followed by a goat anti-(rabbit IgG)serum conjugated with horseradish peroxidase [7]. Antigen/antibody complexes were detected using a sensitive chemiluminescence procedure (ECL; Amersham International). UCP purified from the axillary brown fat of Richardson's ground squirrel (*Spermophilus richardsonii*), was used as a reference on Western blots [7].

2.3. Uncoupling protein mRNA

Total RNA was extracted by a guanidinium isothiocyanate–phenol method [10], and subjected to agarose gel electrophoresis [11]. The RNA was then transferred to a charged nylon membrane (Boehringer Mannheim) by capillary blotting, and fixed with UV light. Pre-hybridization was performed at 42°C for 3 h in 50% formamide, 5 × SSC, 2% blocking reagent (Boehringer Mannheim), 0.1% *N*-laurylsarcosine, and 0.02% SDS. A 27-mer oligonucleotide (3′-TGGAAGG-

GCGACCTGTGGCGGTTTCAG-5′) was used to probe for UCP mRNA [11]. The oligonucleotide was synthesized (British Bio-technology) with digoxigenin (Boehringer Mannheim) conjugated at the 5′-end. Hybridization was at 42°C overnight in 50% formamide, 5 × SSC, 2% blocking reagent, 0.1% N-lauroylsarcosine, 0.02% SDS, together with the oligonucleotide (50 pM). Washes were performed at 48°C [11].

The membranes were incubated with an anti-(digoxigenin)serum/ alkaline phosphatase conjugate (Boehringer Mannheim). Hybridization was detected with the chemiluminescence substrate AMPPD, followed by exposure to film for up to 4 h.

2.4. Iodothyronine 5′-deiodinase activity

Tissues were homogenized in 125 mM potassium phosphate, 1 mM EDTA (pH 7.4) and centrifuged at $80 \times g$ (4°C) for 10 min. The infranantant was taken and iodothyronine 5′-deiodinase activity measured with 2 nM [^{125}I]rT3 (Amersham International) as substrate [12]. Dithiothreitol (1 and 30 mM) and propylthiouracil (1 mM), an inhibitor of iodothyronine 5′-deiodinase activity, or thyroxine (100 nM), were added to assess whether the Type I or Type II form of the enzyme predominated.

3. RESULTS

In the initial investigation, mitochondria from adipose tissues of newborn lambs were examined for the presence of UCP by Western blotting, using an anti-(UCP)serum. Immunoreactivity was present in each adipose tissue examined – perirenal, pericardial and subcutaneous – at a M_r of 32,000, consistent with the presence of UCP (Fig. 1). UCP was detected in two separate subcutaneous sites, one from near the hindlimbs and the other close to the shoulder region. In contrast to newborn lambs, UCP was not evident in either internal (perirenal) or subcutaneous adipose tissue (rear) of adult sheep (Fig. 1).

Adipose tissues of the newborn were then examined for the presence of the mRNA for UCP, by Northern blotting, using a 27-mer oligonucleotide complementary

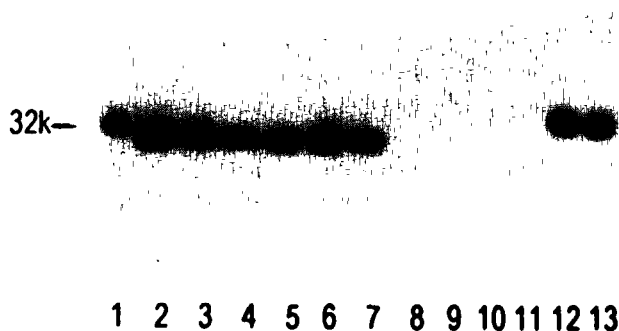**1 2 3 4 5 6 7 8 9 10 11 12 13**

Fig. 1. Western blot of uncoupling protein in mitochondria from subcutaneous and internal adipose tissue of newborn lambs. Tissues were obtained, mitochondria prepared, and Western blotting performed as described in section 2. Lanes 1 and 13, uncoupling protein standard (20 ng); lanes 2 and 3, newborn perirenal; lanes 4 and 5, newborn subcutaneous (rear); lane 6, subcutaneous (shoulder); lane 7, newborn pericardial; lanes 8 and 9, adult perirenal; lanes 10 and 11, adult subcutaneous (rear); lane 12, mouse interscapular brown adipose tissue. 1–5 μg of mitochondrial protein were applied to each lane.
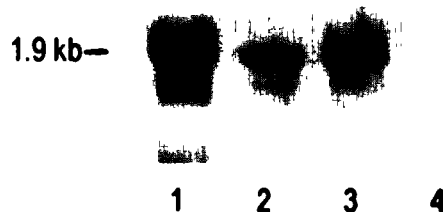
Fig. 2. Northern blot of mRNA for uncoupling protein in adipose tissues of newborn lambs. Adipose tissues were taken within 3 h of birth, and processed as described in Materials and Methods. The blots were probed with a 27-mer oligonucleotide. Lane 1, perirenal; lane 2, pericardial; lane 3, subcutaneous (shoulder); lane 4, subcutaneous (rear). 20 μg of total RNA were applied to each lane.

to a highly conserved sequence of the UCP gene [11]. A 1.9 kb band, characteristic of UCP mRNA in lambs [11], was detected in perirenal, pericardial, and shoulder subcutaneous adipose tissue (Fig. 2). It was not detected, however, in the rear subcutaneous fat.

Perirenal and subcutaneous (rear) adipose tissues were also examined for the presence of iodothyronine 5′-deiodinase activity. High deiodinase activity was found in both these fat depots in newborn lambs (Table I). Inhibition studies with propylthiouracil and thyroxine indicated that the Type I form of iodothyronine 5′-deiodinase predominates in the subcutaneous and perirenal adipose tissues, although some Type II may also be present (results not shown). In contrast to newborn lambs, no iodothyronine 5′-deiodinase activity was detectable in either the subcutaneous or perirenal fat of adult sheep (Table I).

4. DISCUSSION

The present study has detected UCP, and UCP mRNA, in both the internal (perirenal and pericardial) and subcutaneous adipose tissues of newborn lambs. Since UCP is the critical marker of brown adipose tissue, enabling it to be differentiated from white fat [1–5], the results indicate that in the newborn lamb subcutaneous, as well as internal, adipose tissue is functionally

Table I

Iodothyronine 5′-deiodinase activity in subcutaneous and perirenal adipose tissue of newborn and adult lambs

	Perirenal	Subcutaneous
	(pmol I released/h/mg of protein)	
Newborn	21.8 ± 0.4	6.2 ± 0.5
Adult	N.D.	N.D.

Results are given as mean values ± S.E. ($n = 3$), with rT3 as substrate in the presence of 1 mM dithiothreitol. N.D., not detectable (< 0.015 pmol I released/h/mg protein).

'brown'. This view is reinforced by the presence of high iodothyronine 5'-deiodinase activity, an additional marker of brown fat, in the subcutaneous adipose tissue. Previous studies have failed to detect either UCP, or its mRNA, in subcutaneous fat of lambs, although both have been identified in the internal adipose tissues [6,8].

Despite the presence of UCP itself in the two subcutaneous sites examined, UCP mRNA was detected only in the subcutaneous depot taken from near the shoulder. This suggests that there are regional differences in the subcutaneous adipose tissue in the timing at which the gene coding for UCP is suppressed. UCP was not found in either the perirenal or subcutaneous adipose tissue of adult sheep, nor was there any detectable iodothyronine 5'-deiodinase activity. Thus, in agreement with previous work, brown adipose tissue depots in newborn lambs undergo a major postnatal transition to white fat [6].

Iodothyronine 5'-deiodinase catalyses the conversion of thyroxine to the biologically active thyroid hormone, 3,3',5:triiodothyronine, and this deiodination occurs in brown fat [13]. While high iodothyronine 5'-deiodinase activity is evident in subcutaneous adipose tissue of newborn lambs, the activity was lower than in the perirenal fat. Iodothyronine 5'-deiodinase activity in the perirenal depot of newborn lambs was twice that in lamb liver (unpublished results), and some 500–1000 times that in rat brown fat [12,13]. The predominance in lamb adipose tissues of the Type I form of the enzyme is consistent with previous work on the perirenal depot in this species [14].

In conclusion, it is evident from the present study that the internal and subcutaneous adipose tissues of newborn lambs do not simply represent 'brown' and 'white' fat, respectively. On the contrary, the subcutaneous and internal fat depots would seem to follow a common route during development, from the brown (at birth) to the white form. Overall, there is increasing evidence for the interconvertibility of the adipose tissues [5,15–17].

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