Functional atrophy of brown adipose tissue during lactation in mice

Effects of lactation and weaning on mitochondrial GDP binding and uncoupling protein

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The thermogenic activity and capacity of brown adipose tissue were determined in mice during lactation and after weaning. There was a marked fall during lactation in the mitochondrial content of the tissue, and in GDP binding and the specific mitochondrial concentration of uncoupling protein. The lactation-induced functional atrophy of brown adipose tissue was fully reversible after weaning; mitochondrial content and the mitochondrial concentration of uncoupling protein were both restored, although GDP binding was not normalized.

INTRODUCTION

Alterations in the thermogenic activity of brown adipose tissue are now viewed as being important in the regulation of whole-body energy balance in laboratory rodents (see [1,2]). Conditions in which changes in thermogenesis have been observed include obesity, overfeeding, pregnancy, lactation and starvation (see [2]). A major functional atrophy of brown adipose tissue occurs during starvation and in lactation [3–8]. In lactating rats and mice there is a decrease in mitochondrial mass and a fall in mitochondrial GDP binding, indicative of a decrease in mitochondrial proton conductance [4,7,8].

The capacity for non-shivering thermogenesis in the whole animal is also decreased during lactation [4,9], consistent with the changes that take place in brown adipose tissue. A recovery of non-shivering thermogenesis is, however, evident after weaning of the pups [9]. This suggests that the functional atrophy of brown adipose tissue in lactation may be reversed after weaning. This possibility is strengthened by the observation that sympathetic activity returns to normal after weaning, having been decreased during the period of lactation itself [10].

The primary aim of the present study was to investigate whether the thermogenic activity and capacity of brown adipose tissue are restored after weaning. A failure to reverse the lactation-induced changes in the tissue would have important implications for thermoregulatory heat production and the regulation of energy balance in the dam, post-weaning. A further aim of the study was to determine whether the fall in the activity of the proton-conductance pathway in brown fat of the lactating animal relates to a decrease in the specific mitochondrial concentration of uncoupling protein, or to an 'in-activation' of the protein. Preliminary reports of part of this work have been presented elsewhere [11,12].

EXPERIMENTAL

Animals

The animals used in these experiments were female mice of the 'Aston' variety, bred at the Dunn Nutrition Laboratory. They were housed at $23 \pm 1^\circ\text{C}$, with a 12 h-light/12 h-dark cycle (lights on at 07:00 h). The mice were mated at about 7 weeks of age, the males being removed at mid-late pregnancy. Only mice suckling their first litter were used, and the study was restricted to dams with between seven and ten pups. The litters were weaned at 21 days of age, and the dams were then housed either singly or in pairs. Mice were studied at early lactation (1–3 days *post partum*), mid lactation (9–12 days *post partum*), late lactation (16–18 days *post partum*), weaning, and at 1 and 3 weeks post-weaning. Virgin mice, caged in pairs, were taken as controls.

The mice were given free access to a rodent breeding diet (LAD-1; K and K Greef Chemicals, Croydon, U.K.), containing (w/w) 21.3% protein, 3.4% fat, 41.8% starch and 2.8% sucrose.

Brown adipose tissue

The mice were killed by cervical dislocation, and brown adipose tissue was removed from the interscapular site and cleaned of adhering tissues. The brown adipose tissue was then weighed and homogenized in 10 vol. of a buffer containing 250 mM-sucrose, 1 mM-Hepes and 0.2 mM-EDTA (pH 7.2). Homogenization was performed at 4°C with a motor-driven Teflon/glass Potter-Elvehjem homogenizer (six strokes at 600 rev./min). Samples of the homogenate were removed for assay of cytochrome oxidase activity, by a spectrophotometric method, and for measurement of total tissue protein [13]. Bovine serum albumin (Sigma, Poole, Dorset, U.K.) was used as a standard in the protein assay. Most of the homogenate was used for the preparation of mito-

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Table 1. Weight, protein content and cytochrome oxidase activity of interscapular brown adipose tissue of mice during lactation and after weaning

The results are means $\pm$ S.E.M. for the numbers of animals shown in parentheses. For experimental details see the text. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with virgin control mice.

Mice	Body wt. (g)	Brown adipose tissue wt. (mg)	Protein content (mg)	Cytochrome oxidase activity (μ mol of cytochrome c oxidized/min)
Virgin controls	29.4 $\pm$ 0.6 (34)	68 $\pm$ 3 (34)	9.5 $\pm$ 0.3 (34)	21.8 $\pm$ 1.0 (34)
Lactating:				
Early	40.4 $\pm$ 0.8*** (17)	107 $\pm$ 9*** (17)	10.3 $\pm$ 0.8 (11)	21.8 $\pm$ 2.6 (11)
Mid	43.1 $\pm$ 1.1*** (16)	118 $\pm$ 6*** (16)	7.3 $\pm$ 0.4** (6)	12.0 $\pm$ 1.3*** (6)
Late	46.7 $\pm$ 1.0*** (13)	113 $\pm$ 8*** (13)	6.8 $\pm$ 0.5*** (7)	11.4 $\pm$ 0.6*** (7)
Weaning	42.9 $\pm$ 0.6*** (16)	129 $\pm$ 6*** (16)	8.4 $\pm$ 0.4 (7)	14.4 $\pm$ 1.1*** (7)
Post-weaning:				
1 week (single)	37.1 $\pm$ 0.9*** (17)	127 $\pm$ 10*** (17)	10.7 $\pm$ 0.6* (9)	23.8 $\pm$ 1.8 (9)
1 week (paired)	37.4 $\pm$ 0.7*** (13)	118 $\pm$ 9*** (13)	8.3 $\pm$ 0.5 (7)	14.5 $\pm$ 1.2** (7)
3 weeks (single)	37.2 $\pm$ 0.9*** (14)	104 $\pm$ 9*** (14)	10.4 $\pm$ 0.7 (8)	25.9 $\pm$ 3.4 (8)
3 weeks (paired)	37.7 $\pm$ 0.7*** (20)	103 $\pm$ 4*** (20)	10.0 $\pm$ 0.2 (20)	30.9 $\pm$ 1.4*** (20)

chondria [13]. Mitochondrial protein was determined by the same method as for the tissue homogenates.

GDP binding was measured by incubating freshly prepared mitochondria with 10 μ M-[3 H]GDP (at pH 7.1) for 6 min at room temperature [13]. [3 H]GDP and [U- 14 C]sucrose were obtained from Amersham International (Amersham, Bucks., U.K.). The concentration of uncoupling protein in mitochondria was measured by a solid-phase radioimmunoassay [14], by using a rabbit anti-(rat uncoupling protein) serum. A rat uncoupling-protein standard was used in the radioimmunoassay, and this was prepared from brown adipose tissue of cold-acclimated rats by the method of Lin & Klingenberg [15].

Statistical analysis

The statistical significance of differences between control animals and experimental groups was assessed by Student's t test.

RESULTS AND DISCUSSION

The body weight of the mice was substantially greater throughout lactation than in the virgin controls (Table 1). Although there was a decrease after weaning, body weight remained significantly higher than in the virgin animals. The amount of interscapular brown adipose tissue was also increased during lactation, and even by 3 weeks after weaning it had not returned to the value in control animals (Table 1). In contrast with mice, a decrease in the amount of interscapular brown adipose tissue during lactation is apparent in rats [4,8,16]. The

total protein content of the interscapular pads was significantly lower at mid and late lactation than in the virgin controls, but it returned to normal on weaning (Table 1).

Cytochrome oxidase activity, which was measured as an index of mitochondrial mass, was similar in early lactation to that in control mice (Table 1). This indicates that there was no change in the mitochondrial content of brown adipose tissue during pregnancy, in keeping with previous studies [8,17]. There was, however, a marked fall in cytochrome oxidase activity between early and mid lactation, the lower activity being maintained until weaning. Cytochrome oxidase activity was normalized by 1 week after weaning in dams caged singly. In contrast, in dams caged in pairs there was little change in tissue cytochrome oxidase activity by 1 week after weaning, but by 3 weeks post-weaning the activity had risen to a value significantly greater than in the control mice.

Mitochondrial GDP binding, an index of the activity of the 'thermogenic' proton-conductance pathway in brown adipose tissue, was substantially decreased during lactation (Table 2), in agreement with previous studies in both rats [4,8] and mice [7]. The GDP-binding values were lowest at weaning. Although there was some recovery in the binding after weaning, even by 3 weeks post-weaning GDP binding was significantly lower than in the virgin controls (Table 2). This suggests that the activity of the proton-conductance pathway is not normalized post-weaning. There appeared to be little difference in GDP binding between dams caged singly or in pairs after weaning.

Table 2. Mitochondrial GDP binding and the concentration of uncoupling protein in brown adipose tissue of mice during lactation and after weaning

The results are means $\pm$ S.E.M. for the numbers of animals shown in parentheses. For experimental details see the text. The relative uncoupling-protein content of interscapular brown adipose tissue was calculated from the specific mitochondrial concentration and from the changes in cytochrome oxidase activity in the whole tissue; values for virgin control mice were taken as being equal to 1. * $P < 0.001$ compared with virgin controls

Mice	GDP bound (pmol/mg of mitochondrial protein)	Uncoupling- protein concentration (μ g/mg of mitochondrial protein)	Relative uncoupling- protein content of tissue
Virgin controls	344 $\pm$ 22 (11)	52.4 $\pm$ 5.0 (11)	1.00
Lactating:			
Early	112 $\pm$ 11* (6)	65.0 $\pm$ 9.6 (4)	1.24
Mid	91 $\pm$ 9* (10)	22.7 $\pm$ 2.5* (10)	0.24
Late	99 $\pm$ 18* (6)	8.5 $\pm$ 1.5* (4)	0.08
Weaning	61 $\pm$ 9* (9)	14.6 $\pm$ 3.1* (6)	0.18
Post-weaning:			
1 week (single)	117 $\pm$ 11* (8)	38.8 $\pm$ 4.1 (8)	0.81
1 week (paired)	138 $\pm$ 20* (6)	—	—
3 weeks (single)	176 $\pm$ 13* (6)	62.9 $\pm$ 4.4 (6)	1.43
3 weeks (paired)	214 $\pm$ 20* (7)	48.0 $\pm$ 3.0 (7)	1.29

The mitochondrial concentration of the brown-adipose-tissue specific uncoupling protein was not significantly altered in early lactation, but, as with cytochrome oxidase activity, there was a marked fall in the concentration of the protein between early and mid lactation (Table 2). The lowest concentration was found in late lactation (16 % of the virgin controls). There was, however, a marked increase after weaning, such that by 3 weeks post-weaning the concentration had essentially returned to normal, despite the absence of a full recovery in the extent of GDP binding.

The overall capacity for thermogenesis in brown adipose tissue is determined by the total uncoupling-protein content of the tissue. The proportional changes in the total amount of uncoupling protein in brown fat at different stages of lactation, and after weaning, were calculated from the specific mitochondrial concentration of the protein and the relative cytochrome oxidase activity of the tissue [6,18]. By mid lactation the uncoupling-protein content of the interscapular pad was only one-quarter of that in the control animals, and by late lactation it had fallen to just 8 % of the control value (Table 2). The amount of uncoupling protein recovered after weaning, and by 3 weeks post-weaning it was higher than in the control animals, particularly in the mice caged in pairs.

These results clearly indicate that there is a major functional atrophy of brown adipose tissue during lactation in mice. Although neither mitochondrial mass nor the specific mitochondrial concentration of un-

coupling protein was decreased in early lactation, the GDP-binding results indicate that thermogenesis in brown adipose tissue falls initially through a 'masking' of purine-nucleotide-binding sites on uncoupling protein. The initial decrease in thermogenesis in lactation would therefore seem to be exclusively through an 'inactivation' of existing uncoupling protein. This is followed by a decrease in the mitochondrial content of the tissue and in the concentration of uncoupling protein within the mitochondria, both parameters falling to a minimum in late lactation.

It is also evident from the present results that lactation does not result in a permanent loss of function of brown adipose tissue, the thermogenic capacity of the tissue being readily restored. Thus lactation is unlikely to lead to a long-term decline in the ability to respond to low environmental temperatures, or to impair the capacity for diet-induced thermogenesis. Indeed, since the total amount of uncoupling protein was higher in mice at 3 weeks after weaning than in virgin animals, it appears that the potential for both diet-induced and thermoregulatory thermogenesis may actually be increased after lactation. Weaning does not appear to be associated with an 'unmasking' of GDP-binding sites on uncoupling protein, the amount of GDP bound increasing to a lesser extent than the specific mitochondrial concentration of uncoupling protein.

A surprising aspect of the results is the effects of housing the dams singly or in pairs on weaning. The controls were caged in pairs throughout, although

previous work has indicated that there is no significant effect on indices of thermogenic activity in brown adipose tissue if mice are housed either singly or in groups of two per cage at normal room temperatures [19]. There does, however, seem to be a marked influence of caging conditions during the initial recovery of brown fat after lactation, in that at 1 week post-weaning the cytochrome oxidase activity in the tissue had fully recovered in the mice caged singly, whereas little change was evident in the mice caged in pairs (Table 1). By 3 weeks post-weaning this difference had largely disappeared, although the specific mitochondrial concentration of uncoupling protein was significantly greater ($P < 0.02$) in the animals kept singly (Table 2).

The activity of the sympathetic nervous system in brown adipose tissue is decreased during lactation [10,20], but is rapidly normalized after weaning [10]. Since the sympathetic system plays a central role in the growth and development of brown adipose tissue [21], the decrease in sympathetic activity is likely to be the cause of the functional atrophy of the tissue in lactating animals. Correspondingly, the normalization of sympathetic activity after weaning is the probable mechanism by which thermogenic capacity in brown fat is restored.

We have previously argued that the suppression of thermogenesis in brown adipose tissue during lactation should lead to a saving of up to 40% of the normal non-lactational component of maternal energy expenditure [7,22]. This value is based on mice studied at mid lactation, and housed at a temperature of approx. 22 °C. The results obtained here support the general conclusions on the energetic impact of the lactation-induced changes in brown-adipose-tissue function, since they demonstrate that thermogenesis is largely suppressed throughout lactation. It should be noted that the energy savings associated with the suppression of thermogenesis would be expected to increase at lower environmental temperatures; even at a temperature as low as 13 °C the thermogenic activity of brown fat in lactating mice is minimal (P. Trayhurn & G. Jennings, unpublished work).

Conclusions

The present results indicate that the functional atrophy of brown adipose tissue occurring during lactation in mice results from a loss of mitochondria, together with a fall in the specific mitochondrial concentration of uncoupling protein. The atrophy is reversible after weaning of the pups, both mitochondrial mass and the specific mitochondrial concentration of uncoupling protein being restored within 3 weeks post-weaning. It is

concluded that the lactation-induced changes in brown adipose tissue do not lead to a long-term decrease in the capacity for thermogenesis, although the GDP-binding results suggest that thermogenic activity may be lower. The thermogenic capacity of brown fat may in fact be increased after lactation, since the total amount of uncoupling protein in the tissue at 3 weeks post-weaning was higher than in virgin mice.

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