Adaptive changes in the concentration of the mitochondrial 'uncoupling' protein in brown adipose tissue of hamsters acclimated at different temperatures

Paul TRAYHURN, Denis RICHARD, Graham JENNINGS, and Margaret ASHWELL

MRC Dunn Nutrition Laboratory, Downham's Lane, Milton Road, Cambridge, CB4 1XJ, U.K.

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The effect of acclimation at different temperatures on the activity of interscapular brown adipose tissue has been investigated in the hamster, a hibernator. Between 31° and 4°C the cytochrome oxidase activity of the tissue increased 4- to 5-fold, mitochondrial GDP binding per mg of mitochondrial protein doubled, and the amount of uncoupling protein rose from 1.7% to 5.4% of total mitochondrial protein. It is concluded that there are clear adaptive changes induced by temperature in brown adipose tissue of the hamster, but the changes are limited in comparison with those in the mouse.

It is now generally agreed that brown adipose tissue is the principal site of non-shivering thermogenesis in small rodents, both during the neonatal period and in adult life (Smith & Horwitz, 1969; Foster & Frydman, 1978, 1979; Thurlby & Trayhurn, 1980). The central mechanism for thermogenesis in the tissue is considered to be a proton-conductance pathway across the mitochondrial inner membrane (see Nicholls, 1979). This pathway is associated with a specific membrane protein of mol.wt. 32 000 (Heaton et al., 1978), which is now increasingly referred to as 'uncoupling protein'. The proton-conductance pathway is inhibited by purine nucleotides, such as GDP, which bind to the uncoupling protein (Nicholls, 1976). During adaptation of non-hibernating species to cold environments there are considerable changes in the effective proton conductance of brown-adipose-tissue mitochondria, and this is associated with substantial increases in GDP binding (Desautels et al., 1978; Desautels & Himms-Hagen, 1979; Sundin & Cannon, 1980; Ashwell et al., 1983) and in the concentration of uncoupling protein (Ricquier & Kader, 1976; Heaton et al., 1978; Ricquier et al., 1979; Ashwell et al., 1983).

In contrast, studies primarily on the hamster have led to the view that in hibernating species adaptive changes in the proton-conductance pathway do not occur, and that in hibernators brown adipose tissue is always primed for thermogenesis, even in the warm-adapted state (see Nicholls, 1979). Thus the amount of protein in the 32 000-mol.wt.
region, following separation of mitochondrial proteins by poly-
acrylamide-gel electrophoresis in the presence of sodium dodecyl 
sulphate, is reported to be similar in brown adipose tissue from warm-
and cold-acclimated hamsters (Ricquier et al., 1979; Himms-Hagen & 
Gwilliam, 1980). However, some increase in mitochondrial GDP 
binding has recently been observed in cold-acclimated hamsters 
(Himms-Hagen & Gwilliam, 1980; Sundin, 1981), as well as changes in 
respiration and in chloride-induced mitochondrial swelling (Sundin, 
1981). In addition, a study on mitochondrial Ca$^{2+}$ transport in brown 
adipose tissue showed large differences between cold- and warm-
acclimated hamsters (Trayhurn & Fraser, 1983), and this study used a 
thermoneutral temperature for the warm-acclimated group.

In view of these recent observations it seems likely that there is 
some adaptive capacity in the proton-conductance pathway of brown-
adipose-tissue mitochondria of the hamster. In the present study we 
have investigated the effect of acclimating hamsters at different 
temperatures on the mitochondrial concentration of uncoupling protein, 
measured by a sensitive and specific radioimmunoassay (Lean et al., 
1983). Parallel measurements of GDP binding and total tissue 
cytochrome oxidase activity have also been made. The acclimation 
temperatures used ranged from thermoneutrality (30°C) down to 4°C.

**Materials and Methods**

Male golden hamsters (*Mesocricetus auratus*) of the outbred MB 
strain were obtained at 6 weeks of age from Intersimian Ltd. 
(Abingdon, Oxon, U.K.). At 2 months of age they were divided into 
four groups of similar mean body weight, and caged singly in plastic 
cages with the minimum quantity of bedding material. The hamsters 
were then placed at either 30°, 22°, 13°, or 4°, for three weeks; 
30°C is a thermoneutral temperature for the hamster (Pospisilova & 
Jansky, 1976). Temperatures other than 22°C were achieved by the 
use of temperature-controlled cabinets, which were maintained with 
the same 12-h-light/12-h-dark cycle (light period from 0700 h) as the 
main animal house. Each hamster was given free access to water and 
a low-fat/high-carbohydrate diet (Spillers-Spratts Rodent Breeding Diet 

The hamsters were killed by cervical dislocation following light 
anaesthesia with diethyl ether. Interscapular brown adipose tissue was 
removed, dissected free of other tissues, and weighed. The inters-
capular pad was then homogenized in a buffer of 250 mM sucrose/ 
1 mM Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/0.2 
mM EDTA, pH 7.2. Samples of the homogenate were removed for the 
measurement of total tissue protein (Schacterle & Pollack, 1975) and 
for the spectrophotometric assay of cytochrome oxidase activity 
(Yonetani & Ray, 1965). The bulk of the homogenate was used for 
the preparation of mitochondria (Cannon & Lindberg, 1979).

Mitochondrial purine nucleotide binding was measured by incubating 
the freshly prepared mitochondria with 10 μM [3H]GDP, in a buffer at 
 pH 7.1, for 7 min at room temperature, essentially as described 
previously (Nicholls, 1976; Goodbody & Trayhurn, 1981). [3H]GDP and 
[14C]sucrose were obtained from Amersham International (Amersham, 
Bucks, U.K.).
The concentration of uncoupling protein was measured by solid-phase radioimmunoassay in Triton X-100 lysates of mitochondrial suspensions previously stored at -20°C (Lean et al., 1983). Hamster uncoupling protein was used as the standard in the radioimmunoassay, and this was isolated from cold-acclimated (4°C for three weeks) hamsters by the method of Lin and Klingenberg (1980, 1982).

The statistical significance of differences between groups was assessed by Student's unpaired t-test.

Results and Discussion

Tissue protein content and cytochrome oxidase activity

The hamsters housed individually at 4°C lost weight during the three-week acclimation period, but weight was gained at each of the other temperatures (Table 1). The amount of interscapular brown adipose tissue was lowest in the hamsters at 30°C and highest in those at 13°C. The total protein content of the tissue and the cytochrome oxidase activity rose progressively with decreasing temperature from 30° down to 13°C, but between 13° and 4°C the protein content did not change and there was a small, though statistically insignificant (P > 0.05), fall in cytochrome oxidase activity (Table 1). Thus in terms of protein content and cytochrome oxidase activity in brown adipose tissue, the hamsters were fully acclimated at 13°C; the cytochrome oxidase activity of the interscapular pad at 13°C was more than 6 times that at thermoneutrality.

The increase in cytochrome oxidase activity in hamster brown adipose tissue at low temperatures is consistent with the results of previous studies (Pospisilova & Jansky, 1976; Ricquier et al., 1979), although Himms-Hagen and Gwilliam (1980) found no difference in activity between hamsters acclimated at 4° and 26°C. There was no evidence in the present study for the 'heat activation' of brown adipose tissue (i.e. increases in protein content and cytochrome oxidase activity) reported for hamsters acclimated at temperatures above 25°C (Pospisilova & Jansky, 1976).

GDP binding to isolated mitochondria

The binding of GDP to brown-adipose-tissue mitochondria is the most widely used method for assessing the activity of the proton conductance pathway. Table 2 shows the results of a GDP binding study on the hamsters acclimated at each temperature. Binding was lowest at 30°C, and it approximately doubled between 30° and 22°C. There was some further increase in binding between 22° and 13°C, and between 13° and 4°C, but both these increases were small. Thus GDP binding only showed substantial changes with acclimation temperature between 30° and 22°C, but the increase observed is broadly similar to that reported previously for hamsters between 4° and 21° or 26°C (Himms-Hagen & Gwilliam, 1980; Sundin, 1981).

The total increase in GDP binding in hamsters between 30° and 4°C (121%) is much smaller than that recently observed for mice (728%) over a similar temperature range (Ashwell et al., 1983), or for rats over a narrower range (Desautels et al., 1978; Brooks et al.,
Table 1. Weight, protein content, and cytochrome oxidase activity of interscapular brown adipose tissue from hamsters acclimated at different environmental temperatures

Hamsters were acclimated at each temperature for 3 weeks; for full experimental details see the text. The values are means ± S.E.M. with the number of animals shown in parentheses.

<table>
<thead>
<tr>
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<th>Acclimation temperature (°C)</th>
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<tbody>
<tr>
<td></td>
<td>30°</td>
</tr>
<tr>
<td>Initial body wt. (g)</td>
<td>87.2 ± 3.0 (7)</td>
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<tr>
<td>Final body wt. (g)</td>
<td>100.2 ± 4.2 (7)</td>
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<tr>
<td>Body wt. gain (g)</td>
<td>13.0 ± 3.7 (7)</td>
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<tr>
<td>Interscapular brown adipose tissue wt. (mg)</td>
<td>147 ± 16 (7)</td>
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<tr>
<td>Protein content of interscapular brown adipose tissue (mg)</td>
<td>24.7 ± 4.1 (6)</td>
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<tr>
<td>Cytochrome oxidase activity of interscapular brown adipose tissue (μmol cytochrome c oxidized/min)</td>
<td>16.0 ± 3.7 (7)</td>
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<td>22°</td>
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<tr>
<td>Initial body wt. (g)</td>
<td>89.9 ± 2.3 (7)</td>
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<tr>
<td>Final body wt. (g)</td>
<td>107.5 ± 3.1 (7)</td>
</tr>
<tr>
<td>Body wt. gain (g)</td>
<td>17.6 ± 2.2 (7)</td>
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<tr>
<td>Interscapular brown adipose tissue wt. (mg)</td>
<td>168 ± 13 (7)</td>
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<tr>
<td>Protein content of interscapular brown adipose tissue (mg)</td>
<td>49.9 ± 3.9 (6)*</td>
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<td>Cytochrome oxidase activity of interscapular brown adipose tissue (μmol cytochrome c oxidized/min)</td>
<td>45.2 ± 5.4 (7)**</td>
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<td>Initial body wt. (g)</td>
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<td>Final body wt. (g)</td>
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<td>Body wt. gain (g)</td>
<td>10.6 ± 3.0 (7)</td>
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<td>Interscapular brown adipose tissue wt. (mg)</td>
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<td>Protein content of interscapular brown adipose tissue (mg)</td>
<td>109.0 ± 11.6 (6)**</td>
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<td>Cytochrome oxidase activity of interscapular brown adipose tissue (μmol cytochrome c oxidized/min)</td>
<td>99.1 ± 8.7 (7)**</td>
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<td>Initial body wt. (g)</td>
<td>90.0 ± 2.9 (8)</td>
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<td>Final body wt. (g)</td>
<td>82.8 ± 2.6 (8)*</td>
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<td>Body wt. gain (g)</td>
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<td>Interscapular brown adipose tissue wt. (mg)</td>
<td>256 ± 12 (8)**</td>
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<tr>
<td>Protein content of interscapular brown adipose tissue (mg)</td>
<td>107.0 ± 9.7 (6)**</td>
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<td>Cytochrome oxidase activity of interscapular brown adipose tissue (μmol cytochrome c oxidized/min)</td>
<td>84.3 ± 8.9 (8)**</td>
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*P < 0.01, **P < 0.001 compared with hamsters at 30°C.
Table 2. GDP binding and the concentration of 'uncoupling' protein in brown-adipose-tissue mitochondria from hamsters acclimated at different environmental temperatures

Hamsters were acclimated at each temperature for 3 weeks; for full experimental details see the text. The values are means ± S.E.M. with the number of animals shown in parentheses.

<table>
<thead>
<tr>
<th>Acclimation temperature (°C)</th>
<th>30°</th>
<th>22°</th>
<th>13°</th>
<th>4°</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDP bound (pmol/mg of mitochondrial protein)</td>
<td>356.2 ± 55.9 (7)</td>
<td>685.0 ± 40.0 (7)**</td>
<td>755.3 ± 59.6 (7)**</td>
<td>788.3 ± 58.8 (8)**</td>
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<tr>
<td>Uncoupling protein (μg/mg of mitochondrial protein)</td>
<td>17.0 ± 2.6 (7)</td>
<td>30.2 ± 2.0 (7)*</td>
<td>36.3 ± 4.6 (6)*</td>
<td>53.8 ± 4.9 (8)**</td>
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<tr>
<td>Mol GDP bound/mol of uncoupling protein</td>
<td>0.68 ± 0.05 (7)</td>
<td>0.74 ± 0.06 (7)</td>
<td>0.72 ± 0.06 (6)</td>
<td>0.48 ± 0.03 (8)*</td>
</tr>
</tbody>
</table>

*P < 0.01, **P < 0.001 compared with hamsters at 30°C.
This suggests that although there is some capacity for adaptation in the activity of the proton conductance pathway in the hamster, it is rather less than in non-hibernating species.

The GDP binding values for the hamsters acclimated at 30°C are similar to those obtained for mice at 13°C, rather than for mice at thermoneutrality (Ashwell et al., 1983). This lends support to the view that hamsters are primed for thermogenesis even in the warm-adapted state (see Nicholls, 1979).

Mitochondrial uncoupling protein

The results obtained for the measurement of uncoupling protein by radioimmunoassay are also shown in Table 2. At 30°C the protein amounted to 1.7% of total mitochondrial protein, and this increased to 3.0% at 22°C. There was little change in the concentration of the protein between 22°C and 13°C, but at 4°C it had risen to 5.4% of mitochondrial protein. Thus the amount of uncoupling protein per mg of mitochondrial protein at 4°C was just over 3 times that at 30°C. This compares with an approximately 9-fold difference in mice over a similar temperature range (Ashwell et al., 1983).

These results demonstrate that a selective increase in the mitochondrial concentration of uncoupling protein takes place in brown adipose tissue of the hamster on cold-acclimation, which is consistent with recent observations of adaptive changes in respiration (Sundin, 1981) and in Ca²⁺ transport (Trayhurn & Fraser, 1983). The failure of previous studies (Ricquier et al., 1979; Himms-Hagen & Gwilliam, 1980) to observe any increase in the protein can be attributed primarily to the much greater sensitivity and specificity of the radioimmunoassay procedure, compared with quantitation of the 32 000-mol.wt. band following separation of mitochondrial proteins by electrophoresis on polyacrylamide gels. In addition, it is clearly important to use a thermoneutral temperature as the main reference point, rather than room temperature, in view of the changes in the amount of the protein that occurred between 30°C and 22°C.

Although the differences between temperature extremes in the amount of uncoupling protein per mg of mitochondrial protein are limited in the hamster, the changes in the amount of protein in the whole tissue are more extensive, because of alterations in mitochondrial mass. When the differences in cytochrome oxidase activity at each temperature are used to indicate differences in mitochondrial mass (Ashwell et al., 1983), the following ratio is obtained for the total amount of uncoupling protein in interscapular brown adipose tissue at 30°C, 22°C, 13°C, and 4°C, respectively - 1:5:13:17. These changes are again limited compared with those observed in the mouse (Ashwell et al., 1983), and demonstrate the greater importance of increases in mitochondrial mass in the total adaptive response to cold in the hamster.

From the amount of uncoupling protein and the GDP binding results, the GDP bound per mole of uncoupling protein has been calculated for the hamsters at each temperature. Since the uncoupling protein is reported to form a dimer, with 1 mol of GDP being bound per mole of dimer (Lin & Klingenberg, 1980, 1982), a ratio of 0.5 mol of GDP per mole of monomer would be expected at maximum specific binding. However, the results in Table 2 show values in excess of this
at acclimation temperatures of 30\degree, 22\degree, and 13\degree C. Only with the hamsters at 4\degree C is a molar binding ratio close to 0.5 obtained. This may indicate, therefore, that at the higher temperatures there is some non-specific binding of GDP to brown-adipose-tissue mitochondria of the hamster, although this does not occur when using the same assay procedure with mice.

The molar binding ratios obtained with the hamsters at all temperatures are higher than those observed in mice (Ashwell et al., 1983). In this species, binding ratios below 0.5 were consistently obtained at all acclimation temperatures between 33\degree and -2\degree C, and it was consequently suggested that in the fully adapted mouse there is always 'spare' GDP-binding capacity.

The value of 53.8 \mu g/mg mitochondrial protein obtained for the concentration of uncoupling protein in the hamsters at 4\degree C (Table 2) is almost identical to that estimated by Cannon et al. (1982) for cold-acclimated hamsters. Interestingly, their value (which was within the range they obtained by immunoassay) was derived from GDP binding data and the binding ratio of 0.5 mol of GDP/mole of dimer. The present results indicate that the use of the binding ratio to obtain the amount of uncoupling protein is valid for hamsters at 4\degree C, but that at higher temperatures this approach would lead to an overestimate of the amount of uncoupling protein. In contrast, with mice the same approach would result in an underestimate in the concentration of the protein (Ashwell et al., 1983). Thus GDP-binding values, even when Scatchard analysis has been performed, cannot be reliably used to predict the absolute amount of uncoupling protein, although they give an indication of relative changes with acclimation temperature.

Conclusions

The present study demonstrates that the hamster exhibits adaptive changes with environmental temperature in the concentration of uncoupling protein in brown-adipose-tissue mitochondria, consistent with adaptations in the capacity of the proton conductance pathway (Sundin, 1981; Trayhurn & Fraser, 1983). In addition, the study emphasizes the importance of using a thermoneutral temperature as the main comparison point in work on temperature adaptation, and underlines the value of a sensitive radioimmunoassay for the quantitation of the uncoupling protein. The extent of the changes in uncoupling protein in hamsters is limited in comparison with mice, and this is partly due to hamster mitochondria containing a higher concentration of the protein at thermoneutrality than mouse mitochondria (Ashwell et al., 1983), and partly due to the mice achieving a higher concentration of the protein when cold acclimated. Whether or not these differences reflect a general distinction between hibernating and non-hibernating species or relate to the difference in body size between mice and hamsters, will require more extensive comparative studies.

Acknowledgement

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References