Responses to cafeteria feeding in mice after the removal of interscapular brown adipose tissue

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Feeding a cafeteria diet to mice resulted in an increased energy intake of approximately 30% and this led to increases in the wet weight, total protein content, and total cytochrome oxidase activity of interscapular and dorso-cervical brown adipose tissue. Surgical removal of interscapular brown adipose tissue, followed by cafeteria feeding, gave rise to an elevation in dorso-cervical brown adipose tissue wet weight, total protein content, and total cytochrome oxidase activity, compared to intact cafeteria-fed mice. Cafeteria feeding with or without the removal of interscapular brown adipose tissue did not lead to significant increases in body weight compared to stock-fed control mice, but both cafeteria-fed groups of mice showed significant elevations in body fat content indicating that the induced hyperphagia led to a relative obesity in the cafeteria-fed groups. The results presented are consistent with an increased thermogenic activity in the brown adipose tissue of cafeteria-fed mice, and the effect of the removal of interscapular brown adipose tissue further indicates the quantitative importance of the tissue in the control of body weight.

Susceptibility to some forms of obesity may be associated with a reduced capacity for dietary-induced thermogenesis (DIT) and it is well established that brown adipose tissue (BAT) is involved in DIT in the rat (Rothwell & Stock, 1979; Himms-Hagen et al., 1981). If rats are presented with an energy-dense and palatable diet, the so-called 'cafeteria diet', they become voluntarily hyperphagic and consequently DIT is elevated, which tends to prevent any increase in body weight in response to the diet (Rothwell & Stock, 1979). BAT has been implicated as the tissue exhibiting DIT since the wet wt., protein content, total cytochrome oxidase activity, and metabolic activity of the tissue increase markedly on cafeteria feeding (Rothwell & Stock, 1979; Himms-Hagen et al., 1981). The extent to which an animal responds to the induced hyperphagia is both age-dependent (Sclafani & Gorman, 1977; Stephens et al., 1981) and strain-dependent (Rothwell & Stock, 1980).

In a previous study (Connolly et al., 1982) we examined the effect of the removal of interscapular BAT (IBAT) on body weight and energy balance in the mouse. Removal of IBAT in normal, stock-fed...
mice led to a rapid increase in body weight compared to intact mice and this increase was attributable to an elevation of body fat content. The removal of IBAT did not lead to any changes in the size or activity of other BAT sites in these mice, and this indicated that the threshold required to produce a response in BAT had not been exceeded (Connolly et al., 1982).

Cafeteria feeding represents another degree of imposed stress on an animal and we felt it would be appropriate to look at the response of mice without IBAT to this dietary regime. These animals would be expected to respond to a greater extent than intact, cafeteria-fed mice, since some 25% of the thermogenic tissue was absent. We decided to look at the wet-weight and metabolic changes in the BAT of these mice since these changes are closely allied to, and indicative of, the thermogenic activity of the tissue (Rothwell & Stock, 1979).

Animals and Methods

The mice used were from our stocks of first-generation crosses between C3H and Manchester Black Strains. Forty-five male mice aged between 8 and 12 weeks were divided into three groups: 18 cafeteria-fed controls (CC); 18 cafeteria-fed with interscapular brown adipose tissue surgically removed (IBATX); and 9 stock-fed controls (CST). The animals were caged in groups of three in metal cages with a layer of sterilized wood shavings. Surgical operations on the IBATX group were carried out exactly as described previously (Connolly et al., 1982), except that neither of the control groups was given a dose of anaesthetic. The animal room was maintained at 22°C on 12-h-light/12-h-dark cycle, the light coming on at 08.00 h.

All the animals were given free access to stock pellets (4IB Rat Cube, Pillsbury's, Birmingham) and water. In addition, however, both cafeteria groups were given access to four different energy-rich and palatable foods each day. The cafeteria foods presented included milk chocolate, fudge, cereals, bread, biscuits, peanuts, ginger cake, madeira cake, and wafer biscuits. The cycle of cafeteria feeding was 4 days such that the selection presented on day 5 was the same as on day 1. After each day the food remaining in each cage was separated and weighed and in this way food consumption was calculated. The energy content of cafeteria foods was estimated from food tables (Paul & Southgate, 1978).

After 22 days the animals were killed with halothane and the IBAT and dorsocervical BAT (DCBAT) deposits were dissected free of adhering muscle and white adipose tissue, weighed, and homogenized in medium (0.25 M sucrose, 0.2 mM EDTA, 1 mM HEPES, pH 7.2). IBAT and DBCAT were homogenized in 2 ml and 1 ml respectively. Cytochrome oxidase activity was assayed polarographically at 37°C as previously described and protein was estimated by the modified Lowry method of Schacterle and Pollack (1973) after precipitation with trichloroacetic acid (100 g/l (Connolly et al., 1982).

Results

During the 21 days of the experiment, feeding a varied and palatable diet led to voluntary hyperphagia in both of the cafeteria groups. Intact, cafeteria-fed mice (CC) and cafeteria-fed mice without IBAT (IBATX) increased their energy intake by 31% and 25%
Table 1. Body weight gain, body fat content, and food consumption of mice on a cafeteria diet

Mean values with their standard errors: no. of observations in parentheses. CST, stock-fed controls; CC, cafeteria-fed controls; IBATX, cafeteria-fed with IBAT surgically removed. Food consumption was calculated for cafeteria animals using food tables (Paul & Southgate, 1978). Animal carcasses were dried to constant weight in an oven, minced with scissors, and placed in a soxhlet apparatus for the extraction of body fat with chloroform:methanol (2:1 v/v).

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body wt.</th>
<th>Final body wt.</th>
<th>Fat</th>
<th>Fat-free mass</th>
<th>Total food consumption in 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0 (g)</td>
<td>day 21 (g)</td>
<td>(%)</td>
<td>(g) body wt.</td>
<td>(g) body wt. (kJ)</td>
</tr>
<tr>
<td>CST</td>
<td>28.55 ± 0.84 (9)</td>
<td>29.58 ± 0.80 (9)</td>
<td>1.96 ± 0.11 (9)</td>
<td>6.67 ± 0.37 (9)</td>
<td>27.61 ± 0.84 (9)</td>
</tr>
<tr>
<td>CC</td>
<td>28.33 ± 0.84 (17)</td>
<td>29.67 ± 1.06 (17)</td>
<td>3.29 ± 0.18 (17)</td>
<td>11.09 ± 0.47 (17)</td>
<td>26.38 ± 0.47 (17)</td>
</tr>
<tr>
<td>IBATX</td>
<td>27.93 ± 0.80 (18)</td>
<td>29.25 ± 0.83 (18)</td>
<td>3.32 ± 0.11 (18)</td>
<td>11.37 ± 0.28 (18)</td>
<td>25.93 ± 0.28 (18)</td>
</tr>
</tbody>
</table>

Significance of results (Student’s t-test) * P < 0.001 compared to CST.

respectively compared to stock-fed controls (CST; Table 1). Despite this elevated energy intake neither of the cafeteria-fed groups showed any significant increase in body weight over stock-fed control animals. The percentage weight gains after the 21 days for CST, CC, and IBATX groups were 3.6%, 4.7%, and 4.7% respectively (Table 1). However, the body fat content of both the cafeteria-fed groups was significantly elevated over that of stock-fed mice (CC 66% and IBATX 70% compared to CST; Table 1). The fat-free mass is similar in both the cafeteria-fed groups but is higher in the stock-fed mice, and this may be explained by the increased body water content in these animals (Table 1). The mean dry-matter content (excluding fat) of the animal carcasses for CST, CC, and IBATX groups was 6.49 g, 6.41 g, and 6.29 g respectively. It seems, therefore, that the increased fat deposition led to a decreased body water content in cafeteria-fed mice, thus allowing the body weight to remain constant. It is clear that body weight, in itself, is a poor index of the energy state of an animal.

Table 2 shows the results of dissection of the animals on day 22 of the experiment. The effect of cafeteria feeding on BAT is indicated by comparison of the CC and CST groups. Cafeteria feeding led to a 38% increase in the wet weight of IBAT and a 23% increase in the wet weight of DCBAT. The specific body content (mg/g body weight) of both sites of BAT was elevated in the cafeteria-fed group although this was only significant in the IBAT (CC compared to CST, Table 2). Cafeteria feeding led to significant increases in the total protein content of both IBAT and DCBAT of 50% and 20% respectively (Table 2). Similarly, the total cytochrome oxidase activity was elevated in the BAT of the CC group compared to CST mice, with both IBAT and DCBAT displaying a significant 38% increase (Table 3). Cafeteria feeding did not significantly change the specific activity of cytochrome oxidase (Table 3).

The animals lacking IBAT (IBATX) showed an elevation of the DCBAT site over that seen in the intact cafeteria-fed mice, indicating a further compensation for the lack of IBAT. The DCBAT of the IBATX group showed significant increases in wet weight and total
Table 2. Wet weight and total protein contents of IBAT and DCBAT deposits in cafeteria-fed and control mice

Mean values with their standard errors; no. of observations in parentheses. CST, stock-fed controls; CC, cafeteria-fed controls; IBATX, cafeteria-fed with IBAT surgically removed. Protein was measured by the modified Lowry method of Schacterle and Pollack (1973).

<table>
<thead>
<tr>
<th>Group</th>
<th>IBAT Specific body weight (mg)</th>
<th>Total wet wt. content (mg)</th>
<th>DCBAT Specific body weight (mg)</th>
<th>Total wet wt. content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CST</td>
<td>128 ± 4.37 ± 7.31 ±</td>
<td>17.76 ± 0.609 ± 1.32 ±</td>
<td>CST</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 (9) 0.38 (9) 0.46 (9)</td>
<td>1.13 (9) 0.032 (9) 0.10 (9)</td>
<td>CST</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>177 ± 5.99 ± 10.94 ±</td>
<td>21.81 ± 0.746 ± 1.59 ±</td>
<td>CC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 (17) 0.34 (17) 0.29 (17)</td>
<td>0.91 (17) 0.038 (17) 0.07 (17)</td>
<td>CC</td>
<td></td>
</tr>
<tr>
<td>IBATX</td>
<td>-</td>
<td>-</td>
<td>IBATX</td>
<td></td>
</tr>
</tbody>
</table>

Significance of results (Student's t-test):
+ P < 0.05 compared to stock-fed animals.
* P < 0.02 compared to stock-fed animals.
++ P < 0.01 compared to stock-fed animals.
+++ P < 0.005 compared to stock-fed animals.
+++ P < 0.001 compared to stock-fed animals.
† P < 0.02 compared to cafeteria-fed controls.

Protein content of 31% and 45% respectively compared to CST. The total protein content was also significantly higher (21%) than the intact cafeteria-fed group (Table 2). Total cytochrome oxidase activity in the DCBAT of the IBATX group was significantly higher than both CST and CC groups. There was a 60% increase above CST mice and a 15% increase above CC mice and these changes were associated with no significant change in the specific activity of the enzyme (Table 3). It therefore seems that the BAT of the IBATX group has proliferated to a greater extent on the cafeteria diet than that of the intact (CC) group.

Table 3. Cytochrome oxidase activity of IBAT and DCBAT of stock- and cafeteria-fed mice

Values are means with their standard errors, with the number of observations in parentheses.

<table>
<thead>
<tr>
<th>Group</th>
<th>IBAT</th>
<th>Total activity (μmol O2/min)</th>
<th>Sp. activity (μmol O2/min per mg protein)</th>
<th>DCBAT</th>
<th>Total activity (μmol O2/min)</th>
<th>Sp. activity (μmol O2/min per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CST</td>
<td>12.62 ± 0.95 (9)</td>
<td>1.74 ± 0.10 (9)</td>
<td>2.70 ± 0.18 (9)</td>
<td>CST</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.38 ± 0.78 (17)</td>
<td>1.59 ± 0.06 (17)</td>
<td>3.72 ± 0.09 (17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBATX</td>
<td>-</td>
<td>-</td>
<td>4.29 ± 0.14 (18)</td>
<td>IBATX</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significance of results (Student's t-test):
+ P < 0.005 compared to CST
* P < 0.005 " " CC
++ P < 0.001 " " CST
Discussion

The importance of BAT as a mediator of thermogenesis has been demonstrated in cafeteria-fed rats (Rothwell & Stock, 1979) and in cold-adapted rats (Foster & Frydman, 1978), although the extent to which this applies to man is uncertain (Himms-Hagen, 1979; James & Trayhurn, 1981). In view of this, much effort has been directed towards discovering the exact mechanism controlling the dissipation of excess energy by BAT in the hope that such investigations may lead to a better understanding of the human obese state.

Cafeteria feeding induces rats to consume more energy than stock-fed controls and consequently leads to an increased thermogenesis in these animals. This thermogenesis is associated with BAT as indicated both by in vivo blood-flow studies (Rothwell & Stock, 1981) and by the proliferation of the tissue sites in cafeteria-fed animals. The increased thermogenic function is characteristically accompanied by increases in the wet weight, total protein content, and total cytochrome oxidase activity of the tissue as well as an elevated amount of GDP binding to the BAT mitochondrial proteins (Brooks et al., 1980; Himms-Hagen et al., 1981).

The results presented here indicate that cafeteria feeding in mice leads, as expected, to an increased BAT content in the body and this is accompanied by the increases in protein and mitochondrial (as measured by cytochrome oxidase) contents in the tissue. The excess energy intake of the CC group compared to the CST group was 492 kJ. If thermogenesis were proceeding at the same rate in both CC and CST mice, then all this excess energy intake would be converted to body fat. Assuming the cost of energy gain for 1 g of body fat is 53 kJ (Pular & Webster, 1977), then the CC group should have gained 9.3 g of body fat over the 21-day experiment, whereas in fact they only gained 1.3 g. Moreover the energy cost of depositing fat of 53 kJ/g body fat is probably an overestimate for a diet as high in fat as the cafeteria regimen (Stephens et al., 1981), and therefore a fat gain of 9.3 g may be an underestimation. Consequently, it is reasonable to assume that DIT is elevated in both the cafeteria-fed groups to maintain a lean body weight, and that the changes observed in BAT reflect the changes in thermogenesis.

We have previously shown that surgical removal of IBAT leads to an increased retention of ingested energy in stock-fed mice (Connolly et al., 1982). Removal of IBAT represents a reduction of some 25-30% of the thermogenic tissue in the body, but this did not induce any compensatory changes in the DCBAT site, indicating that the threshold required for activation of the remaining 70% of BAT was not exceeded on the stock diet and consequently the IBATX animals gradually gained weight as fat was laid down (Connolly et al., 1982). Where cafeteria feeding is further imposed upon the pressure of IBAT removal, the other BAT sites (as indicated by DCBAT) show an enhanced compensation to that seen in intact cafeteria-fed animals. In this case, clearly the threshold of energy load has been passed and the importance of the lack of IBAT then becomes significant. Since the BAT of the two cafeteria groups has been activated and proliferation has occurred, the body weight of these mice is maintained at or near that of stock-fed mice. The fat gained represents the
excess energy intake of both cafeteria groups that could not be dissipated by the increased BAT function in these animals.

The effect of cafeteria feeding on BAT in mice agrees with similar findings by Trayhurn et al. (1982), and the enhanced response of the BAT of IBATX mice reinforces the view that BAT is involved with DIT in the mouse and agrees with similar findings for the rat (Stephens et al., 1981). The question still remains, however, as to the nature of the threshold which must be overcome to cause BAT proliferation and how this compensation is initiated.

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References