Brown Adipose Tissue Thermogenesis and Metabolic Rate Contribute to the Variation in Obesity among Rats Fed a High Fat Diet

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Summary  A high fat (HF) diet is known to induce obesity, but susceptibility to obesity induced by a HF diet differs not only among different strains of rats but also within the same strain. The present study revealed that the Lee index (an index of obesity) positively correlated with insulin, and inversely correlated with both the mitochondrial oxygen consumption in interscapular brown adipose tissue (BAT) and the resting metabolic rate (RMR) in Sprague-Dawley rats. This suggests the contribution of BAT thermogenesis and RMR, in addition to hyperphagia, to the intrasubject variation in susceptibility to HF diet-induced obesity.

Key words: high fat diet, brown adipose tissue, resting metabolic rate.

It is known that different strains of rats differ in their susceptibility to obesity induced by a high fat (HF) diet [1, 2]. Although hyperphagia is the primary cause of HF diet-induced obesity, differences in the sympathetic regulatory mechanism may account, in part, for variations in metabolic efficiency [3].

Brown adipose tissue (BAT) is a main effector organ of diet-induced thermogenesis [4] and non-shivering thermogenesis [5]. It has been shown that defective control of BAT thermogenesis results in obesity [4], and that elevated thermogenesis in BAT is a contributing factor in the leanness and resistance to obesity [6, 7].

There is considerable variability in the propensity towards dietary obesity, even within the same strain of rats [8]. The reason why some rats are prone to obesity when given a HF diet remains unknown. The purpose of this study was to determine whether resting metabolic rate (RMR) and BAT thermogenesis contribute to susceptibility to HF diet-induced obesity, by measuring RMR and oxygen consumption in BAT mitochondria in Sprague-Dawley rats after 6 weeks on a HF diet.

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Forty 8-week-old female (195–210 g) Sprague-Dawley (SD) rats were purchased from Charles River Japan (Osaka, Japan). The animals were housed individually in wire-bottom cages in a temperature-controlled room (22 ± 2°C) with the lights on from 0600 to 1800 h daily. After 1 week of adaptation to the chow diet (Charles River Japan, Osaka) and laboratory conditions, 20 of the animals were fed HF diets ad libitum for up to 6 weeks. The other 20 rats were fed a low fat (LF) diet. The HF diet contained (in g/100 kcal) 8.33 vitamin-free casein, 9.0 corn starch, 0.74 corn oil, and 4.45 vegetable shortening. With this mix, 36% of calories came from carbohydrate, 44% from fat, and 20% from protein. The LF diet contained (in g/100 kcal) 8.33 vitamin-free casein, 19.0 corn starch, and 0.74 corn oil. For the LF diet, 76% of the calories came from carbohydrates, 4% from fat, and 20% from protein. Both diets contained (in g/100 kcal) 1.47 Rogers-Harper mineral mix, 0.36 vitamin mix, 0.55 desiccated liver, 0.05 dl-methionine, and 4.17 alphacel non-nutritive bulk. Body weight and the nasoanal length were measured. The Lee index was calculated as \(\frac{\text{body weight (g)}}{\text{nasoanal length (cm)}} \times 1,000\). Food intake was recorded daily by subtracting the weight of the uneaten food from the weight of the food provided.

Resting metabolic rate and locomotor activity were estimated after 6 weeks on a HF or LF diet using a closed-circuit metabolic system with an actimonitor (Envirionics, ACM-1, USA) from 0900 to 1200 h at room temperature 22°C, as described elsewhere [9].

BAT thermogenesis was assessed by measuring oxygen consumption in the interscapular BAT (IBAT) mitochondria. After 6 weeks on a HF or LF diet, the animals were killed at 0900 h by cervical dislocation. Blood samples were collected for the determination of serum levels of insulin and triiodothyronine (T₃) by radioimmunoassay (insulin RIA kit, Dinabot Radioisotope Lab., Tokyo, Japan; Spac T; RIA kit, Dinabot Radioisotope Lab., Tokyo, Japan). Rapidly removed IBAT samples were weighed and homogenized in an ice-cold medium containing 250 mM sucrose and 5 mM TES. The IBAT mitochondria were isolated by differential centrifugation, and then the mitochondrial protein content was measured. As previously reported [10], oxygen consumption in the IBAT mitochondria was determined polarographically in 2 ml of medium consisting of 100 mM KCl, 20 mM TES (pH 7.2), 4 mM KH₂PO₄, 2 mM MgCl₂, 1 mM EDTA, 4 µM rotenone, and 10% defatted bovine serum albumin at 25°C using Rank oxygen equipment (Rank Brotteus, Cambridge, UK). Mitochondrial protein (850 µg) was added to 2 ml of medium containing 10 mM α-glycerophosphate as a substrate for mitochondrial respiration. Statistical analysis was performed using the Student’s t-test. This study was performed following “Principles for the Care and Use of Animals” approved by the Council of the Japan Physiological Society.

The HF diet produced greater weight gain and variation in obesity over the 6-week period than the LF diet. Total energy intake and serum insulin levels were significantly greater in the HF-fed group than in the LF-fed group. IBAT weight and intra-abdominal (mesenteric and retroperitoneal) white adipose tissue (WAT)
Table 1. Body weight, Lee index, total food intake, serum IRI and T₃, RMR, intra-abdominal WAT weight, BAT weight, mitochondrial (Mt) protein content in BAT, and BAT·Mt O₂ consumption in HF-fed and LF-fed groups.

<table>
<thead>
<tr>
<th></th>
<th>HF</th>
<th>LF</th>
</tr>
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<tbody>
<tr>
<td>No.</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>295.1±7.8**</td>
<td>279.3±3.6</td>
</tr>
<tr>
<td>Lee index</td>
<td>288.5±2.1***</td>
<td>281.1±1.1</td>
</tr>
<tr>
<td>Food intake (kJ)</td>
<td>9,366±352*</td>
<td>8,260±281</td>
</tr>
<tr>
<td>IRI (µU/ml)</td>
<td>68.0±4.0***</td>
<td>33.9±3.1</td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>99.2±3.4</td>
<td>96.1±4.6</td>
</tr>
<tr>
<td>RMR (ml/min W⁰.⁷⁵)</td>
<td>9.0±0.2**</td>
<td>10.1±0.4</td>
</tr>
<tr>
<td>WAT weight (g)</td>
<td>15.4±0.5*</td>
<td>13.3±0.5</td>
</tr>
<tr>
<td>BAT weight (mg)</td>
<td>353.2±11.9***</td>
<td>300.9±6.8</td>
</tr>
<tr>
<td>BAT·Mt protein content (mg)</td>
<td>3.53±0.18</td>
<td>3.63±0.10</td>
</tr>
<tr>
<td>BAT·Mt O₂ consumption</td>
<td></td>
<td></td>
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<tr>
<td>Specific (nmol/(min·Mt protein))</td>
<td>24.8±1.0***</td>
<td>29.3±1.2</td>
</tr>
<tr>
<td>Total (nmol/(min·tissue))</td>
<td>88.0±6.4*</td>
<td>106.4±5.4</td>
</tr>
<tr>
<td>Locomotor activity (U/h)</td>
<td>218.1±35.1</td>
<td>286.3±40.3</td>
</tr>
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Values are mean±SE. Significant difference between HF-fed and LF-fed groups: *p<0.05, **p<0.02, ***p<0.01.

weight in the HF-fed group was greater than in the LF-fed group, but mitochondrial protein content in IBAT did not differ between the two groups. The mean RMR and mitochondrial oxygen consumption (both specific and total) in the IBAT for the HF-fed group were significantly lower than the respective values for the LF-fed group, but there was no difference in serum T₃ levels (Table 1). There were highly significant correlations between the Lee index and both total food intake (Fig. 1) and the serum insulin levels (r=0.63, p<0.01), but not between the Lee index and serum T₃ levels in the HF-fed group. Significant inverse correlations were observed between the Lee index, and both oxygen consumption in the IBAT mitochondria and the RMR in HF-fed rats (Fig. 2), but not in LF-fed rats (Fig. 3).

The development of dietary obesity in animals is usually dependent upon consumption of a high energy dense diet. The greater efficiency with which dietary fat enters the fat stores, compared to dietary carbohydrates, is a partial explanation for the greater obesity in rats eating the HF diet. Rothwell and Stock [2] fed four strains of rats “cafeteria” diets and concluded that the variations in weight gain among the strains were due mainly to differences in metabolic efficiency. Schemmel et al. [1] studied seven strains of rats and found that SS/PI rats exhibited only small weight gains on a HF diet, whereas Osborne-Mendel rats became quite obese. Fiser et al. [3] and Yoshida et al. [7] reported that the resistant SS/PI rats had a higher norepinephrine turnover rate, an indicator of sympathetic nervous system (SNS) activity and thermogenesis in BAT, than the sensitive Osborne-Mendel rats.
when fed a HF diet.

Variations in the susceptibility to dietary obesity are present even within the same strain of rats. Levin and colleagues [8] reported that SD rats showed a variability in their susceptibility to diet-induced obesity (DIO), and found that DIO rats had lower sympathetic activity associated with hyperinsulinemia than diet-resistant (DR) rats [11]. In this study, BAT weight in HF-fed rats was greater than in LF-fed rats, which was consistent with a previous report [12]. Both specific and total mitochondrial oxygen consumption in BAT in HF-fed rats were significantly reduced compared to their respective values in LF-fed rats. Since the protein content in BAT mitochondria of HF-fed rats was similar to that of LF-fed rats, the heavier BAT weight in HF-fed rats may be due to the accumulation of lipids. Our present study demonstrated a significant inverse correlation between the Lee index and oxygen consumption in the BAT mitochondria of HF-fed rats, but not of LF-fed rats. This suggests that differences in SNS activity and BAT thermogenesis, in addition to inter-strain variability, may play a role in intra-strain variations in the propensity towards dietary obesity. Hyperinsulinemia in the model of hypothalamic obesity plays a central role in the development of obesity [13]. In HF diet-induced obesity, the whole body insulin resistance, especially in BAT, is likely to be a significant factor in the reduced thermogenesis and progression to obesity [14], which may explain the positive correlation between the Lee index and the serum insulin level.
Fig. 2. Correlations between the Lee index and both mitochondrial oxygen consumption in BAT (A) and RMR in the HF-fed group (B). A regression line was plotted using linear regression analysis with a correlation coefficient \( r \) of $-0.54 \ (p < 0.02)$ for the mitochondrial oxygen consumption in BAT and $-0.45 \ (p < 0.05)$ for RMR.

An inverse correlation between the Lee index and the RMR was observed in the HF-fed rats. BAT accounts for about 10% of the RMR at steady state, but for over 50% of stimulated heat production (during cold-exposure or adrenergic stimulation) in animals [15]. The decrease of the RMR in HF-fed rats could be attributed to reduced BAT thermogenesis, since locomotor activity and serum $T_3$...
levels did not differ between the HF-fed and LF-fed rats. In addition, we have found a significant positive correlation between RMRs before and after HF diet administration ($r=0.48$, $p<0.05$) [16]. This suggests that reduced BAT thermogenesis and RMR may be a cause rather than a result of HF diet-induced obesity.

Recently, Change and associates [17] studied Wistar rats fed a HF diet (60% of calories from fat) for 4 weeks. They found no significant difference in daily energy expenditure between the obesity-prone and obesity-resistant rats. The
different strains used may explain the discrepancy between their finding and ours, as SD rats are more susceptible to the effects of a HF diet than Wistar rats [1].

In conclusion, the present study demonstrates that BAT thermogenesis and metabolic rate, in addition to hyperphagia, may contribute to variations in obesity induced by a HF diet in SD rats.

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REFERENCES


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