Effects of Exercise Training on Brown Adipose Tissue Thermogenesis in Ovariectomized Obese Rats

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Abstract

The effect of exercise training on brown adipose tissue (BAT) thermogenesis was studied by measuring cytochrome oxidase activity, as a marker of mitochondrial abundance, mitochondrial guanosine-5'-diphosphate (GDP) binding, as an indicator of thermogenic activity and oxygen consumption in BAT in ovariectomized (OVX) obese rats and sham-operated rats. Six-week exercise training significantly suppressed body weight gain in OVX rats to the level of sedentary control rats, although food intake in exercise trained OVX rats increased more than in the sedentary OVX rats. Exercise training increased cytochrome oxidase activity, mitochondrial GDP binding and oxygen consumption in BAT in OVX rats, which were reduced in a sedentary condition, as well as in the control rats. These results suggest that exercise training potentiates BAT thermogenesis, which may contribute to the reduction of body weight in OVX obese rats.

It is now recognized that brown adipose tissue (BAT) is a common effector of body temperature and energy balance in rodents (Trayhurn, 1986), and that a defect in or the absence of BAT thermogenesis would predispose to obesity (Rothwell and Stock, 1979; Himms-Hagen, 1985). Exercise training is known to retard body weight gain by decreasing fat deposition. This response has been attributed to the increased energy expenditure of exercise training per se. To date, however, data on the effect of exercise training on BAT thermogenesis is less substantial and is controversial. Some reports (Hirata et al., 1981; Hirata, 1982) showed that exercise training increased norepinephrine-induced blood flow to BAT, whereas others (LeBlane et al., 1982; Wickler et al., 1987; Richard et al., 1986, 1987) failed to find any effect of exercise on BAT function.

The present study is therefore designed to investigate whether exercise training potentiates BAT thermogenesis by measuring cytochrome oxidase activity, mitochondrial GDP binding and oxygen consumption, which are thermogenic indicators, in BAT.

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mitochondria in OVX obese rats which have a reduced BAT function and in sham operated rats.

Materials and Methods

Forty female Sprague-Dawley rats (approximately 200 g, 8 weeks old) were purchased from Charles River Japan (Osaka, Japan). The rats were housed individually in wire-mesh cages at a constant temperature of 22±2°C and artificial light from 0600h to 1800h each day, and they were allowed free access to commercial powdered chow (Charles River Japan) and tap water. Under anesthesia with intraperitoneal administration of pentobarbital (45 mg/kg), ovariectomy (OVX) was performed on 20 rats and the remaining 20 rats were sham-operated (control). One week after the surgery both 12 OVX and control rats were submitted to an exercise training program for 6 weeks, whereas the other rats in both OVX and control groups were kept in a sedentary condition.

The exercise training program consisted of running (23 m/min, 0° incline, for 60 min, 5 days/week) on a motor-driven treadmill. Initial running time and speed (days 1–3 of experiment) were 20 min and 10 m/min. Second running time and speed (days 4–6 of experiment) were 40 min and 15 m/min. After the 7th day of the experiment, the rats ran continuously for 60 min at a speed of 23 m/min for a further 5 weeks. The rats that finished the exercise program were 8 rats each in both OVX and control groups, and they were used in the following experiments. Food intake was assessed by weighing the food administered and subtracting the amount remaining at the end of a 24 hour period, every day, in each group for 7 days before the experiments.

Brown adipose tissue thermogenesis

BAT thermogenesis was estimated 24 hours after the last period of exercise by measuring cytochrome oxidase activity, mitochondrial GDP binding and oxygen consumption in interscapular BAT (IBAT) in either sedentary or exercise trained OVX and control rats. IBAT was rapidly removed and dissected from the connective tissue. IBAT samples were collected from 2 rats in either sedentary or exercise trained OVX and control rats for one measurement of the parameters described below. IBAT samples were weighed and homogenized in an ice-cold medium (pH 7.2) containing 250 mM sucrose and 5 mM TES. Portions of the homogenates were used for the measurement of cytochrome oxidase activity. The mitochondria were isolated by differential centrifugation according to the procedure by Cannon and Lindberg (1973). The tissue protein and mitochondrial protein were estimated by the method of Lowry et al. (1951). Mitochondrial GDP binding was determined by incubation and filtration according to a minor modification (Yoshioka et al., 1988) of the method of Nicholls (1976). The mitochondria were incubated at 20°C in 0.5 ml of medium containing [3H]-GDP (1.30 μCi), [14C]-sucrose (0.123 μCi), 100 μM sucrose, 100 μM potassium atractyloside, 20 mM TES (pH 7.1), 10 mM choline chloride and 5 μM rotenone. After 7 minutes of incubation, 0.4 ml aliquots containing 0.26 mg of mitochondrial protein were withdrawn and filtered through a nitrocellulose membrane filter (Sartorius, Göttingen, W. Germany, pore size 0.45 μm). The filters were counted for [3H] and [14C] in a scintillation counter (Packard, Downers Grove, IL, USA). Cytochrome oxidase activity was measured spectrophotometrically with a Double-Beam Spectrophotometer (UV-140-02, Shimadzu, Kyoto) at 25°C in 1 ml of medium consisting of 100 mM KH₂PO₄, 1 mM EDTA and 30 μM reduced cytochrome c after treating the homogenates with 1% Lubrol by the method of Yonetani and Ray (1965). Mitochondrial respiration 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 BSA at 25°C with Rank oxygen equipment (Rank Brotteus, Cambridge, UK). Mitochondrial protein (850 μg) was added to the medium and 10 mM α-glycerophosphate was added as a substrate for mitochondrial respiration. All data are presented as the mean±S.E. Statistical analysis was done by Student’s t-test.

Results

Table 1 shows that OVX led to a significant increase in body weight and food intake. In the sedentary condition, cytochrome oxidase activity and mitochondrial
Table 1. Body weight, Food intake, Interscapular BAT weight, Total tissue protein and Mitochondrial (Mt) protein content in IBAT, Cytochrome oxidase activity, GDP binding and Oxygen consumption in IBAT of OVX and control rats in sedentary and exercised groups.

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th></th>
<th>Exercised</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>OVX</td>
<td>Control</td>
<td>OVX</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>(n=8)</td>
<td>293±4</td>
<td>347±8**</td>
<td>283±5</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>(n=8)</td>
<td>19.3±1.0</td>
<td>23.2±1.3*</td>
<td>20.3±1.2</td>
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<tr>
<td>IBAT weight (mg)</td>
<td>(N=4)</td>
<td>199±17</td>
<td>191±10</td>
<td>324±28#</td>
</tr>
<tr>
<td>Total tissue protein content in IBAT (mg)</td>
<td>(N=4)</td>
<td>13.9±1.2</td>
<td>11.1±1.3</td>
<td>29.2±0.7##</td>
</tr>
<tr>
<td>Mt. protein content in IBAT (mg)</td>
<td>(N=4)</td>
<td>1.82±0.17</td>
<td>1.50±0.12</td>
<td>3.04±0.42#</td>
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<td>Cytochrome oxidase activity in IBAT</td>
<td>(N=4)</td>
<td>4.15±0.07</td>
<td>4.05±0.07</td>
<td>5.07±0.43##</td>
</tr>
<tr>
<td>specific (µmol/min·mg)</td>
<td></td>
<td>58.4±2.3</td>
<td>44.0±4.0*</td>
<td>147.5±13.7##</td>
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<td>total (µmol·tissue)</td>
<td></td>
<td>204.0±8.9</td>
<td>174.2±3.5*</td>
<td>326.7±14.1##</td>
</tr>
<tr>
<td>GDP binding in IBAT</td>
<td>(N=4)</td>
<td>376.8±30.1</td>
<td>265.2±14.1</td>
<td>970.8±131.4##</td>
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<tr>
<td>specific (µmol/mg)</td>
<td></td>
<td>35.2±2.3</td>
<td>29.7±1.8</td>
<td>49.4±2.0##</td>
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<tr>
<td>total (µmol·min·tissue)</td>
<td></td>
<td>66.3±7.2</td>
<td>41.0±6.9</td>
<td>176.9±17.5##</td>
</tr>
</tbody>
</table>

Values are shown as the mean±S.E.  n: Number of animals in each group  N: Number of measurements in each group  Control vs OVX:  * p<0.05,  ** p<0.01  Sedentary vs Exercised:  # p<0.05,  ## p<0.01

Oxygen consumption per tissue in IBAT in OVX rats were significantly reduced as compared with those in the control rats. Mitochondrial GDP binding both per mitochondrial and per tissue were also decreased in OVX rats as compared with those in the control rats. Six-week exercise training markedly suppressed body weight gain in OVX rats to the level of sedentary control rats. Food intake in exercise trained OVX rats increased more than in the sedentary group, but not in the control rats. IBAT weight, tissue and mitochondrial protein content in IBAT were significantly increased both in the exercise trained control and OVX groups as compared with those in the sedentary groups. Exercise training significantly increased the cytochrome oxidase activity, mitochondrial GDP binding and oxygen consumption in IBAT in OVX rats as well as in the control rats.

Discussion

Brown adipose tissue is now recognized as being a main effector of cold-induced thermogenesis (Foster and Frydman, 1979) and diet-induced thermogenesis (Rothwell and Stock, 1979). A defect in or the absence of BAT thermogenesis would predispose to obesity (Rothwell and Stock, 1986; Himms-Hagen, 1985; Yoshida et al., 1984a, 1987a, 1987b; Yoshioka et al., 1988) and a stimulation of BAT function would result in a reduction in body weight (Yoshida et al., 1983, 1988; Arch et al., 1984). The OVX rats in a sedentary condition had reduced BAT thermogenesis, as evidenced by
the decreased levels of cytochrome oxidase activity, mitochondrial GDP binding and oxygen consumption in IBAT, which is completely in accordance with our previous studies (Yoshida et al., 1987; Yoshioka et al., 1988).

Six-week exercise training significantly potentiated reduced BAT thermogenesis in OVX rats, and it markedly retarded body weight gain in OVX rats to the level of the sedentary control group, although food intake in exercise trained OVX rats increased more than in the sedentary OVX group. The present results showing that exercise training stimulated BAT thermogenesis is in accordance with the reports of Hirata et al. (1981, 1982) who reported an increased whole body thermogenesis response to norepinephrine and an increased norepinephrine-induced blood flow to BAT in swim-trained rats.

In contrast, several reports showed that exercise training did not affect BAT thermogenesis. LeBlanc et al. (1982) failed to find any increase in non-shivering thermogenesis in swim-trained rats. Wickler et al. (1987) has shown that treadmill running did not affect resting oxygen consumption, norepinephrine-induced oxygen consumption or brown fat blood flow. In their studies, the exercise program differed from ours; their average running speed is 17 m/min, whereas ours is 23 m/min. In addition, they measured blood flow to BAT after a 12-hour fast. Fasting suppresses sympathetic nervous system (SNS) activity (Young and Landsberg, 1980; Yoshida et al., 1983), and that may account for the lack of changes in blood flow to BAT in exercised rats. Richard et al. (1986, 1987) also have demonstrated that 33-day exercise training (25 m/min, 9° incline, 7 days/week) did not potentiate BAT thermogenesis as assessed through mitochondrial GDP binding both in the control and OVX rats, although the intensity of their exercise program was greater than that of our study. They used Wistar rats, while we used Sprague-Dawley rats. In their studies, the level of GDP binding in OVX rats did not decrease more than in the control rats, whereas it decreased significantly in our study. Moreover, the level of GDP binding both in the control and OVX rats in the sedentary condition of their experiments is one and a half times to twice as high as that in our experiments, in which it was approximately at the level of those in the cold-exposed rats in our previous study (Yoshioka et al., 1988). Because the average environmental temperature is lower in Canada than in Japan, a low environmental temperature might influence (Himms-Hagen, 1986) the levels of GDP binding in their rats in a sedentary condition, which could account for the discrepancy between their study and ours.

Exercise is known to increase SNS activity (LeBlanc et al., 1964; Ostman-Smith, 1979) and the plasma catecholamine concentration. The increase in plasma epinephrine is thought to be derived from the adrenal medulla (Galbo et al., 1986) and that in norepinephrine (NE) from leakage of neurotransmitters from noradrenergic fibers (Goldstein et al., 1983; Keller et al., 1984) or from muscle vascular beds (Petronnet et al., 1988). BAT thermogenesis is regulated primarily by the SNS (Landsberg and Young, 1984) and NE stimulates BAT thermogenesis. Therefore, the activation of BAT thermogenesis by exercise training in OVX rats, which have reduced SNS activity in BAT (Yoshida et al., 1987), might result from the stimulation of SNS activity or from the direct action of circulating catecholamines derived from the adrenal medulla. Further examination is required to evaluate the mechanism of exercise on BAT thermogenesis, by measuring NE turnover in BAT, which is a reliable indicator of SNS activity (Young and Landsberg, 1977; Yoshida and Bray, 1984b), or by investigating BAT thermogenesis in adrenalectomized animals.
In conclusion, exercise training potentiated reduced BAT thermogenesis in OVX obese rats, which might contribute to the retardation of body weight gain.

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References


