Endurance training changes in lipolytic responsiveness of obese adipose tissue

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1Laboratoire des Adaptations de l’Organisme à l’Exercice Musculaire, Hôpital Purpan, 31059 Toulouse Cedex, France; 2Institut National de la Santé et de la Recherche Médicale Unité 317, Université Paul Sabatier, 31062 Toulouse Cedex 4, France; and 3Department of Sport Medicine, Third Faculty of Medicine, Charles University, 10 000 Prague 10, Czech Republic

De Glisezinski, I., F. Crampes, I. Harant, M. Berlan, J. Hejnova, D. Langin, D. Rivière, and V. Stich. Endurance training changes in lipolytic responsiveness of obese adipose tissue. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E951–E956, 1998.—The aim of this study was to investigate the effect of aerobic exercise training on the lipolytic response of adipose tissue in obese subjects. Thirteen men (body mass index = 36.9 ± 1.3 kg/m²) were submitted to aerobic physical training on a cycloergometer (30–45 min, 4 days a wk) for 3 mo. Adipocyte sensitivity to the action of catecholamines and insulin was studied in vitro before and after training. Training induced a decrease in the percentage of fat mass (P < 0.05) without changing the body weight. Basal lipolysis and hormone-sensitive lipase activity were significantly decreased after training (P < 0.05). The lipolytic effects of epinephrine, isoprenaline (β-adrenoceptor agonist), and dobutamine (β1-adrenoceptor agonist) were significantly increased (P < 0.05). Lipolysis stimulation by agents acting at the postreceptor level was unchanged after training. In conclusion, aerobic physical training in obese male subjects modifies adipose tissue lipolysis through an enhancement of β-adrenergic response and a concomitant blunting of adipocyte antilipolytic activity.

Materials and Methods

Subjects

Thirteen obese nondiabetic males [age: 41.2 ± 1.5 yr (range: 27–49 yr) and body mass index 36.9 ± 1.3 kg/m² (range: 29.4–47.1 kg/m²)] were recruited for the study. The characteristics of the subjects are presented in Table 1. Two patients were treated for mild hypertension with calcium channel antagonists, and one patient was treated with small doses of L-thyroxine (75 µg/day) for hypothyroidism. All the subjects were sedentary before the study. Their weight had been stable for 6 mo before the study. Their nutrition had not changed during that period. The subjects were instructed not to change their nutritional habits during the course of the study. The stable nutritional pattern was verified with a 4-day dietary record obtained at the beginning, at the 6th wk, and at the end of the study period. The experiment was approved by the Ethics Committee of the 3rd Medical Faculty of Charles University, Prague. All subjects gave their informed consent to participate in the study.

Design of the Study

Subjects took part in a 12-wk aerobic training program, which is described in Exercise training protocol. Before and during aerobic exercise training, subjects were encouraged to maintain their usual diet and activity patterns. The exercise program consisted of aerobic training (30–45 min, 4 days a wk) performed on a cycloergometer. The subjects were instructed to avoid any changes in their diet during the study period. All subjects were instructed to perform the same exercise protocol daily, and their adherence was monitored by weekly phone calls. The exercise protocol was individualized to each subject, with a target heart rate of 60–75% of the maximum heart rate. At the end of the study, the subjects were asked to complete a questionnaire to evaluate their adherence to the exercise program.

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Table 1. Clinical characteristics of 13 obese subjects submitted to 12 wk of aerobic training

<table>
<thead>
<tr>
<th></th>
<th>Before Training</th>
<th>After Training</th>
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<tbody>
<tr>
<td>Weight, kg</td>
<td>112.9 ± 4.9</td>
<td>111.4 ± 5.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>36.9 ± 1.3</td>
<td>36.3 ± 1.5</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>38.6 ± 1.8</td>
<td>37.2 ± 2.0*</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>68.6 ± 2.2</td>
<td>69.1 ± 2.1</td>
</tr>
<tr>
<td>VO₂max, l/min</td>
<td>3.12 ± 0.14</td>
<td>3.30 ± 0.11*</td>
</tr>
<tr>
<td>VO₂max/FM, ml·kg⁻¹·min⁻¹</td>
<td>46.00 ± 1.69</td>
<td>49.15 ± 1.61</td>
</tr>
<tr>
<td>Maximal heart rate, beats/min</td>
<td>171.85 ± 3.49</td>
<td>169.31 ± 2.87</td>
</tr>
<tr>
<td>Maximal aerobic power, W</td>
<td>228.85 ± 12.23</td>
<td>248.08 ± 9.86*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; FFM, fat-free mass; VO₂max, maximal oxygen output. *P < 0.05 after vs. before training.

after the training program, the following measurements and tests were carried out: body composition measurements, maximal oxygen output (VO₂max) measurements, laboratory evaluations of blood glucose and lipids, and test of lipolysis response to catecholamines and insulin in vitro on isolated adipocytes. The tests were performed 48 h after the last exercise session to exclude its effects on the results.

Maximum aerobic capacity. VO₂max was assessed with a graded test conducted on an electromagnetically braked bicycle ergometer (ergometrics 800s Ergoline). The initial workload was 50 W, and it was increased by 0.2 W/kg of lean body mass every minute until exhaustion. VO₂ was measured with a VO₂max apparatus (Sensor Medics) during the test, and the highest VO₂ value was considered as VO₂max.

Exercise training protocol. The training program consisted of exercise on a bicycle ergometer 4 days a wk; each session lasted between 30 and 45 min, after individual prescription. The subjects cycled at a target heart rate calculated from the Karvonen equation (14); the heart rate corresponded to 50% of the heart rate reserve (maximal heart rate minus resting heart rate) during the first 2 wk and progressed to 60–65% of the heart rate reserve during the subsequent weeks.

Anthropometric assessment. During the study, body weight was measured regularly. The percentage of body fat was determined before and after training by hydrostatic weighing with the method of Siri (27).

Methods

Fat cell isolation and measurement of lipolysis. Fat cells were isolated, and lipolysis was measured as described previously (8). Briefly, a subcutaneous abdominal fat biopsy (200–300 mg) was performed under local anesthesia 10–15 cm from the umbilicus, and adipocytes were isolated with collagenase.

Digestion was performed for 60 min at 37°C in a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 90 mg glucose/100 ml and 4% bovine serum albumin with 0.5 mg/ml collagenase (KRBA) (25). At the end of digestion, the fat cell suspension was filtered and washed three times. Fat cell volume was determined from ~200 adipocytes for each subject before and after the training period.

As an indicator of adipocyte lipolysis, the quantity of glycerol released into the medium was measured. Aliquots (100 µl) of the continuously stirred cell suspension were placed in 1.5-ml conical tubes. The cell concentrations were 14.86 ± 1.77 cell/µl before and 17.18 ± 1.68 cell/µl after training, P = 0.393. Four of these aliquots contained only 20 µl KRBA for determination of spontaneous lipolysis, and the others contained 20 µl of drugs at various concentrations. Another four tubes with 20 µl KRBA were immediately placed on ice to provide an evaluation of the initial quantity of glycerol contained in the medium. The remaining tubes were incubated in a shaking water bath for 4 h.

The quantity of glycerol released into the medium was measured with an ultrasensitive bioluminoscence technique to estimate adipocyte lipolysis (15). The bioluminometer was an LKB 1251 (LKB Wallac, Stockholm, Sweden), coupled with a distributor (LKB 1291) for adding luciferase. All measurements were performed in triplicate.

Glycerol release after addition of various agents was expressed as an increase of glycerol concentration above the level of basal lipolytic rate in micromoles of glycerol released per 100 mg of lipids per 4 h. The results were not modified when expressed in micromoles of glycerol released per 10⁶ cells, adipocyte volume being unchanged by training. The lipolytic responsiveness was assessed with epinephrine, isoproterenol (nonselective β-AR agonist), dobutamine (β₁-AR agonist), and procaterol (β₂-AR agonist). The antilipolytic α₂-AR responsiveness of adipocytes was assessed by the mean of the inhibitory concentration-dependent action of epinephrine in the presence of a β₂-AR antagonist (propranolol). For a better evaluation of the inhibitory action of the α₂-AR and of insulin, the basal levels of glycerol were increased by addition of 2 μg/ml of adenosine deaminase to remove adenosine released into the incubation medium by isolated fat cells.

The effects of agents acting at the postreceptor level were evaluated with forskolin (direct activator of adenylate cyclase), cAMP (stimulator of the protein kinase hormone-sensitive lipase complex), 3-isobutyl-1-methylxanthine (IBMX), and theophylline (inhibitor of phosphodiesterase).

Determination of hormone-sensitive lipase activity. The assay was performed as described by Frayn et al. (13). Packed fat cells (300 µl) that had been stored in liquid nitrogen were homogenized at 4°C in 2.0 ml of a buffer containing 0.25 mol/l sucrose, 1 mmol/l EDTA, 1 mmol/l dithiothreitol, and 20 µg/ml each of the protease inhibitors antipain and leupeptin. The homogenate was then centrifuged at 100,000 g at 4°C for 45 min. The fat cake was removed, and the fat-free infranatant was recovered for analysis of hormone-sensitive lipase (HSL) activity with 1(3)⁻[³H]oleoyl-2-O-oleylglycerol as substrate. A diacylglycerol analog was used as substrate assay activity, because HSL has 10-fold higher activity toward diacylglycerol than triacylglycerol. The diacylglycerol lipase activity is not dependent on the phosphorylation state of the enzyme. Moreover, because this substrate has only one hydrolyzable ester bond at the 1(3)-position, neither the diacylglycerol analog nor its hydrolysis products are substrates for monoacylglycerol lipase, which is abundant in adipose tissue. One unit of enzyme activity equals 1 µmol of fatty acid produced per minute at 37°C. The protein concentration was determined with Lowry’s method (18).

Biochemical determination. Glycerol in plasma (20 µl) was analyzed with an ultrasensitive radiometric method (5); the intra-assay and interassay variabilities were 5.0% and 9.2%, respectively. Blood glucose and plasma NEFA were determined with a glucose-oxidase technique (Biotrol, Paris, France) and an enzymatic procedure (Wako, Unipath, Dardilly, France), respectively. Plasma insulin concentrations were measured with RIA kits (Sandif Diagnostics Pasteur, Marnes la Coquette, France). Plasma triglyceride and cholesterol concentrations were assayed with commercial kits.

Reagents. The following reagents were used during fat cell isolation and measurement of lipolysis: bovine serum albumin; fraction V, fatty acid free; collagenase from Clostridium histolyticum; adenosine deaminase; dibutyryl-cAMP; incubation enzymes; luciferase from Photobacterium fisheri (Boehringer-Mannheim, Meylan, France); epinephrine bitartrate;
The aerobic training program increased \( \dot{V}_{2\text{max}} \) by 5.7%, the increase being significant for the total \( \dot{V}_{2\text{max}} \) \((P < 0.05)\) and not significant when related to either body weight or fat-free mass \( (\text{Table } 1) \). No significant change of body weight \((\text{decrease by } 1.3\%)\) or fat-free mass was observed, whereas the decrease of fat mass by 3.6\% was significant \((P < 0.05); \text{Table } 1)\).

**RESULTS**

The aerobic training program increased \( \dot{V}_{2\text{max}} \) by 5.7%, the increase being significant for the total \( \dot{V}_{2\text{max}} \) \((P < 0.05)\) and not significant when related to either body weight or fat-free mass \( (\text{Table } 1) \). No significant change of body weight \((\text{decrease by } 1.3\%)\) or fat-free mass was observed, whereas the decrease of fat mass by 3.6\% was significant \((P < 0.05); \text{Table } 1)\).

Plasma results. No significant change was observed in plasma glycerol and NEFA concentrations \( (\text{Table } 2) \). Fasting blood glucose and plasma insulin levels were unchanged by training. The blood lipids changed partially during training: total cholesterol decreased significantly \((P < 0.01)\), whereas the decrease of triglycerides was not significant. High-density lipoprotein \((\text{HDL})\) concentrations did not change after training \( (\text{Table } 2) \).

In vitro lipolytic response. The mean adipocyte volume did not change after training \( (0.876 \pm 0.063 \text{ nl after vs. } 0.878 \pm 0.045 \text{ nl before})\), whereas basal lipolysis decreased significantly \((\text{Table } 3)\). HSL activity showed a significant decrease from 225.1 \( \pm 8.0 \text{ mU/mg protein before to } 100.5 \pm 2.3 \text{ mU/mg protein after training} \((P < 0.05)\).

The dose-response curves of epinephrine and the \( \beta\)-AR agonist isoproterenol were significantly different, showing that the \( \beta\)-adrenergic lipolytic effect was higher after than before training \( (\text{Fig. } 1 \text{, A and B})\). The dose-response curves of the \( \beta\)-AR agonist \( (\text{dobutamine})\), but not those of the \( \beta\)-AR agonist \( (\text{propranolol})\) \((\text{not shown})\), were significantly different after training \( (\text{Fig. } 1 \text{C})\).

The dose-response curves of epinephrine in the presence of propranolol \((\text{expressed as a percentage of maximal inhibitory effect in the presence of adenosine deaminase})\) were significantly different, indicating that the antilipolytic \( \alpha_2\)-adrenergic effect was decreased by training \((\text{Fig. } 2 \text{A})\). The antilipolytic action of insulin was also decreased after training \((\text{Fig. } 2 \text{B})\).

To study the mechanism of the observed training-induced increase of \( \beta\)-adrenergic-mediated lipolysis stimulation in fat cells, the effect of the agents acting at the postreceptor level of lipolysis stimulation was evaluated. After subtraction of basal lipolysis values, the lipolytic effects of forskolin, cAMP, IBMX, and theophylline were not modified by training \( (\text{Table } 3)\).

To investigate whether the changes in lipolytic activity are mediated by changes in body fat mass, the correlation \((\text{Pearson’s product-moment correlation coefficient using linear regression})\) between the training-induced change of maximal response to isoprenaline stimulation and the training-induced change of body fat mass was calculated. No correlation was found.

**DISCUSSION**

Previous cross-sectional studies \((8, 9)\) from our group showed a clear training-induced enhancement of the lipolytic responsiveness of adipose tissue in nonobese subjects. The main interest of this study was to investigate whether the changes in regulation of lipolysis that were previously reported in these and other studies \((10)\) in nonobese subjects also occur in obese subjects submitted to a training program. To take into account the gender-specific effect of training on in vitro lipolysis \((9, 11)\), we limited our investigation to obese male subjects.

The main finding of the present longitudinal study is that aerobic training \((\text{corresponding in frequency and intensity to that routinely prescribed for obese subjects in clinical practice})\) promotes an increase in catecholamine-induced lipolytic activity of subcutaneous adipose tissue.

In fact, in our subjects, this kind of aerobic training produced a minor, although significant, change in physical fitness \((\text{assessed by maximum aerobic capacity})\). It did not produce a change in body weight, whereas there

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**Table 3. In vitro lipolysis before and after training**

<table>
<thead>
<tr>
<th>Glyceral Concentrations</th>
<th>Glycerol Concentrations</th>
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<tbody>
<tr>
<td>Before training</td>
<td>After training</td>
</tr>
<tr>
<td>Incl. Basal Lipolysis</td>
<td>Minus Basal Lipolysis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Before training</th>
<th>After training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal lipolysis</td>
<td>2.5 ( \pm 0.5 )</td>
<td>1.3 ( \pm 0.2^* )</td>
</tr>
<tr>
<td>Isoproterenol ((10^{-8} \text{ M}))</td>
<td>3.8 ( \pm 0.5 )</td>
<td>3.4 ( \pm 0.4 )</td>
</tr>
<tr>
<td>Dobutamine ((10^{-5} \text{ M}))</td>
<td>3.6 ( \pm 0.6 )</td>
<td>3.1 ( \pm 0.4 )</td>
</tr>
<tr>
<td>ADA</td>
<td>3.7 ( \pm 0.7 )</td>
<td>2.5 ( \pm 0.3 )</td>
</tr>
<tr>
<td>Forskolin</td>
<td>3.9 ( \pm 0.9 )</td>
<td>2.4 ( \pm 0.3 )</td>
</tr>
<tr>
<td>cAMP</td>
<td>5.0 ( \pm 1.0 )</td>
<td>3.9 ( \pm 0.5 )</td>
</tr>
<tr>
<td>IBMX</td>
<td>3.9 ( \pm 0.9 )</td>
<td>2.4 ( \pm 0.3 )</td>
</tr>
<tr>
<td>Theophylline</td>
<td>4.0 ( \pm 0.9 )</td>
<td>3.1 ( \pm 0.4 )</td>
</tr>
</tbody>
</table>

Values are means \( \pm \text{SE}; n = 13 \). Glyceral concentrations were expressed in \( \text{µmol} \cdot 100 \text{mg lipid}^{-1} \cdot 4 \text{ h}^{-1} \), ADA, adenosine deaminase. * \( P < 0.05; tP < 0.01 \) after vs. before training.
was a slight decrease of fat mass and a small but not significant increase in fat-free mass. The modest effects of this type of training are in agreement with other reports on the effects of moderate training in obese subjects (1, 16). The metabolic changes detected in plasma were mild in accordance with other studies in nonobese and obese subjects with comparable training programs (1, 6, 30).

The training did not induce a change in fat cell volume, which is in agreement with the small change in total body fatness. Other longitudinal studies have not

found a decrease in fat cell weight; this was the case in nonobese male subjects (10) and in obese women (21). However, in the latter group, the training was coupled with hypocaloric diet. Moreover, most cross-sectional studies found a significantly lower fat cell volume in trained subjects, but in most of these studies, groups with very different training status and body fatness were compared (8, 9, 20).

In this study, we found a decrease in basal lipolysis after training. However, basal lipolysis changes by training remain controversial. In fact, our results are in
agreement with previous data (24, 29) in cross-sectional studies but in disagreement with others (12, 20). In a longitudinal study, Després et al. (10) did not find any difference in basal lipolysis after training. Similarly, discrepancies in the effects of training on lipolysis at rest occurred in studies examining lipolysis in vivo with labeled NEFA and glycerol; higher as well as lower lipolytic rates were reported in trained compared with untrained subjects (7, 26). These differences probably came from training volume, duration, mode of exercise, and the moment when the investigations were made. In the present study, the decrease of basal lipolysis was associated with a decrease of HSL expression. HSL catalyzes the rate-limiting step in adipose tissue lipolysis; therefore, the decrease of basal lipolysis during training may be caused by the decrease of HSL expression. This is supported by observations during very-low-calorie diet in obese subjects, which report a concomitant increase (28) or decrease (23) of HSL and basal lipolysis during very-low-calorie diet or after a weight-maintenance period, respectively.

The most important result of the study is the finding that training enhances the lipolytic response of adipocytes to catecholamine stimulation. This confirms that the training-induced sensitization of adipose tissue found previously in vitro in nonobese subjects (10) also occurs in obese subjects and that this sensitization can be achieved with training volumes feasible in routine practice. The present study shows, in agreement with previous studies (8, 9), that the β-adrenergic pathway is responsible for this sensitization phenomenon. The obese subjects have been shown to present decreased β-adrenergic stimulation of lipolysis (4, 19, 22), and the data reported here suggest that the decrease in the β-adrenergic responsiveness of lipolysis seen in obese subjects could be partially improved by exercise training. Furthermore, in an attempt to understand which populations of β-AR mediate the training-induced effect, we explored the stimulatory effects of selective β1- and β2-AR agonists (dobutamine and proteraterol, respectively) on lipolysis. A significant training-induced enhancement was found for the β1-adrenergic and not for the β2-adrenergic pathway; this suggests that training selectively increases β1-adrenergic activity.

In obese subjects, the α2-AR-mediated antilipolytic effect of catecholamines is high (19), and the present study suggests that endurance training can decrease α2-adrenergic sensitivity. This lower α2-adrenergic antilipolytic effect might contribute to the training-induced rise of the lipolytic effect of epinephrine (Fig. 1A).

Besides catecholamines, insulin is a powerful hormone in the control of lipolysis in adipocytes. Therefore, the influence of training on the antilipolytic action of insulin was assessed. The training resulted in its decrease. The only study known to date which deals with training effects on insulin antilipolytic activity in vitro in adipose tissue in obese subjects (women) did not find any change due to training (16). Whereas the duration and volume of training were identical in this study, gender differences could explain the different results (similar to gender-dependent effects of training on the lipolytic action of catecholamines). It is noteworthy that the effect of training on the action of insulin differs with respect to the target tissue: whereas the sensitivity to insulin action on glucose transport in muscle is enhanced in obese subjects (1, 16), the antilipolytic effect in adipose tissue appears to be decreased.

When lipolysis was stimulated with agents acting at a postreceptor level ( forskolin, cAMP, IBMX, theophylline), no differences in pre- and posttraining states were found. This is in contrast to our previous results (9) and other results (20) in nonobese subjects. The reason may be the very different training status of the groups in other studies, which contrasts with the mild training-induced increase in physical capacity in the present study.

It is important to note that the results of this study are specific to the gender of the subjects and the region of adipose tissue deposit. The gender-specific effect of exercise training on lipolysis (9, 11) and body fat reduction (1) has been repeatedly demonstrated. Regional differences have also been reported in the training-induced lipolysis modifications (20). The variability of exercise-induced lipolysis with respect to region and gender has also been demonstrated during a single bout of exercise (2). Therefore, it remains to be shown whether the beneficial effect of exercise is preserved in obese women and in other fat depots.

In conclusion, the present study demonstrates that 12 wk of aerobic training induces an enhancement of in vitro catecholamine-stimulated lipolysis in abdominal subcutaneous tissue of obese male subjects, without substantially changing body fat mass. The basal rates of lipolysis and HSL activity were decreased after 12 wk of training. The increase in lipolytic response seems to be related to an increased response of the β-adrenergic pathway. Decreases in the antilipolytic action of α2-AR and insulin also contribute to the improvement of the lipolytic activity of adipose tissue in trained states.

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Lipolysis Changes After Training in Obese Subjects