Endurance training changes in lipolytic responsiveness of obese adipose tissue

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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) but not those of procaterol (β2-adrenoceptor agonist). The antilipolytic effects of α2-adrenoceptor and insulin were significantly decreased (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.

adipose tissue biopsy; catecholamines; insulin; hormone-sensitive lipase

OBSERVATIONS

Obesity is associated with increased morbidity and especially coronary heart disease risk. Weight loss through dietary energy restriction is the most common form of treatment for the obese patient. However, hypocaloric diet alone leads to a decrease in fat mass, with a parallel decrease in fat-free mass. The decrease in muscle mass can be prevented by adding exercise training to the diet (3).

During aerobic exercise, nonesterified fatty acids (NEFA) constitute an important fuel oxidized by working muscle. The NEFA are released into the circulation as a product of triacylglycerol hydrolysis in the adipose tissue. This lipolysis is a key step in the metabolic process leading to the decrease of fat mass. Lipolysis is mainly regulated by catecholamines, which are lipolytic through β-adrenoceptors (AR) and antilipolytic through α2-AR and insulin, which is an antilipolytic hormone (17). Increasing the sensitivity of adipose tissue to the lipolytic action of catecholamines can facilitate lipid mobilization from fat stores. In healthy nonobese subjects, many studies have shown that aerobic exercise training induces an increase in catecholamine-stimulated lipolysis in isolated adipocytes in both cross-sectional (8, 9, 20) and longitudinal (10) designs.

A recent study examined the effect of training combined with diet restriction on in vitro lipolysis in obese subjects and found that training blunted the dietary-induced decline of lipolysis (21). To our knowledge, no study has shown the effect of aerobic training on lipolysis in obese subjects without a concurrent nutritional intervention. Moreover, alterations of catecholamine-stimulated lipolysis have been reported in obesity (19, 22); therefore, it is of particular interest to determine whether the disturbed lipolytic sensitivity can be influenced by training.

The present study was undertaken to explore the effect of endurance training without modification of diet on adipose tissue metabolism in obese subjects to assess the mechanisms underlying the effects of training in overweight therapy. Adipose tissue sensitivity to catecholamines and insulin was studied in vitro before and after 12 wk of aerobic training in 13 obese male subjects.

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
Another four tubes with 20 µl KRBA were immediately placed in a shaking water bath for 4 h. The quantity of glycerol released into the medium was measured with an ultrasensitive bioluminescence technique to estimate adipocyte lipolysis (13). 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 basal level of 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 propranolol (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 monophosphate 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/l of 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)Holeoyl-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 the substrate has only one hydroxyable 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 (Sanofi 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 increase being significant for the total VO$_{2\text{max}}$ (Fig. 1, not shown), were significantly different after training (Table 2).

**RESULTS**

The aerobic training program increased VO$_{2\text{max}}$ by 5.7%, the increase being significant for the total VO$_{2\text{max}}$ (P < 0.05) and not significant when related to either body weight or fat-free mass (Table 1). No significant change of body weight (decrease by 1.3%) or fat-free body weight or fat-free mass (Table 1). No significant change was observed in plasma glycerol and NEFA concentrations (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 (HDL) concentrations did not change after training (Table 2).

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

The dose-response curves of epinephrine and the β-AR agonist isoprenaline were significantly different, showing that the β-adrenergic lipolytic effect was higher after than before training (Fig. 1A and B). The dose-response curves of the β$_2$-AR agonist (dobutamine), but not those of the β$_1$-AR agonist (propranolol) (not shown), were significantly different after training (Fig. 1C).

Table 2. Plasma parameters before and after training

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Training</th>
<th>After Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmmol/l</td>
<td>6.2 ± 0.3</td>
<td>5.3 ± 0.2*</td>
</tr>
<tr>
<td>HDL cholesterol, mmmol/l</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides, mmmol/l</td>
<td>2.8 ± 0.5</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>NEFA, µmol/l</td>
<td>553.7 ± 64.7</td>
<td>757.8 ± 70.7</td>
</tr>
<tr>
<td>Glycero, µmol/l</td>
<td>77.9 ± 6.9</td>
<td>84.9 ± 5.8</td>
</tr>
<tr>
<td>Glucose, mmmol/l</td>
<td>5.54 ± 0.51</td>
<td>5.01 ± 0.70</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>24.3 ± 2.6</td>
<td>21.2 ± 2.7</td>
</tr>
</tbody>
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Values are means ± SE; n = 13. HDL, high-density lipoprotein; NEFA, nonesterified fatty acids. *P < 0.01 after vs. before training.

maximal inhibitory effect in the presence of adenosine deaminase) were significantly different, indicating that the antilipolytic β$_2$-AR-adrenergic effect was decreased by training (Fig. 2A). The antilipolytic action of insulin was also decreased after training (Fig. 2B).

To study the mechanism of the observed training-induced increase of β-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 (Table 3).

To investigate whether the changes in lipolytic activity are mediated by changes in body fat mass, the correlation (Pearson's product-moment correlation coefficient using linear regression) between the training-induced increase 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 (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 (assessed by maximum aerobic capacity). It did not produce a change in body weight, whereas there
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

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**Fig. 1.** Lipolytic effects of adrenergic agonists before (○) and after (●) training. Lipolysis was induced by epinephrine (A), by nonselective β-adrenergic agonist isoproterenol (B), and by selective β_1-adrenergic agonist dobutamine (C). Glycerol concentrations are expressed after subtraction of basal lipolysis. Values are means ± SE. *P < 0.05; **P < 0.01; ***P < 0.001.

**Fig. 2.** α_2-Adrenergic and insulin antilipolytic effects before (○) and after (●) training. Antilipolysis was induced by epinephrine in presence of 10^{-5} M of the β-adrenergic antagonist propranolol (A) and by insulin (B). Antilipolysis experiments were performed in the presence of 2 µg/ml adenosine deaminase. Values are percent change of adenosine deaminase-induced lipolysis after subtraction of basal lipolysis. Values are means ± SE. *P < 0.05; **P < 0.01.
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 subpopulations of β-AR mediate the training-induced effect, we explored the stimulatory effects of selective β1- and β2-AR agonists (dobutamine and procaterol, 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 status 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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REFERENCES


