Exercise training decreases in vitro stimulated lipolysis in a visceral (mesenteric) but not in the retroperitoneal fat depot of high-fat-fed rats

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The purpose of the present study was to determine the effects of an exercise training programme in high-fat-fed rats on in vitro lipolysis in a visceral (mesenteric) and a non-visceral fat depot (retroperitoneal) and its relationship to perilipin content. Two groups of female rats were fed a high-fat diet (42 % as energy) for 8 weeks, one remaining sedentary (Sed) and the other being exercise trained (Tr) for this entire period. Rats were killed after 2 and 8 weeks of their respective treatment. The significantly (P<0.01) higher levels in mesenteric and retroperitoneal fat pad weights, plasma leptin, NEFA and glucose observed with time in Sed high-fat-fed rats were significantly (P<0.05) attenuated in Tr animals. Isoproterenol-stimulated (10⁻⁵–10⁻³ M) lipolysis in the mesenteric, but not in the retroperitoneal tissue, was significantly (P<0.05) lower (about 57 %) in Tr than in Sed rats after 8 weeks of high-fat feeding. The isoproterenol-stimulated lipolysis in the mesenteric tissue of 8-week Tr high-fat-fed rats was lowered to the level measured in 2-week fat-fed rats although mesenteric fat accumulation was still significantly (P<0.01) higher in 8- than in 2-week Tr rats. Perilipin content (Western blot) was not affected by the exercise training programme. These results indicate that exercise training resulted in a reduction in the high-fat diet-induced elevated levels of lipolysis in the mesenteric tissue. This response appears to be independent of the perilipin content.

Non-esterified fatty acids: Perilipin: Isoproterenol-induced lipolysis: Leptin: Fat mass

When present in excess, plasma NEFA are involved directly in the pathogenesis of metabolic disturbances leading to insulin resistance and the metabolic syndrome (1). It has been suggested in animal as well as human studies that increased fat accumulation inside the adipocyte results in an increased amount of NEFA reaching the portal system. The increased NEFA mobilisation from visceral fat cells is accelerated due to increased basal and spontaneous or total body fat (13). Catecholamine-induced rate of lipolysis, especially in the visceral compartment (i.e. omental, mesenteric) is usually associated more strongly with parallel metabolic disturbances observed in upper-body obesity (14). The main purpose of the present study was to test the hypothesis that exercise training in high-fat-fed animals is associated with a reduction in adipocyte lipolysis. This was tested in a visceral (mesenteric) and in a non-visceral (retroperitoneal) fat tissue. Visceral fat (i.e. omental, mesenteric) is usually associated more strongly with metabolic abnormalities such as dyslipidaemia, hypertension, insulin resistance and type 2 diabetes than is subcutaneous or total body fat (13). Catecholamine-induced rate of NEFA mobilisation from visceral fat cells is accelerated due to elevated rate of lipolysis in human obesity, mainly because of an increased α-3-adrenergocceptor function, and a decreased α-2-adrenergocceptor function. This promotes an increased release of NEFA to the portal system, which may contribute to the increased release of NEFA to the portal system, which may contribute to the parallel metabolic disturbances observed in upper-body obesity (14). A reduction in the elevated lipolysis, especially in the mesenteric tissue with exercise training in high-fat-fed rats would, therefore, constitute an asset in reducing the increased amount of NEFA reaching the portal system.

In recent years, a new component regulating the activity of hormone-sensitive lipase (HSL)-mediated lipolysis has been identified. Perilipins are phosphoproteins in adipocytes...
Lipolysis in trained high-fat-fed rats

Methods

Animal care

Female Sprague–Dawley strain rats (n = 45) (Charles River, St-Constant, PQ, Canada), weighing 180–200 g upon their arrival were housed individually and had ad libitum access to food and tap water. Their environment was controlled in terms of light (12 h–12 h light–dark cycle starting at 06.00 hours), humidity and room temperature (20–23°C). All experiments described in this report were conducted according to the directives of the Canadian Council on Animal Care.

Diet and exercise protocol

A few days after their arrival, all animals were assigned to a high-fat diet while remaining sedentary (Sed) or being submitted to an exercise training programme (Tr) for 2 or 8 weeks (nine rats per group). Another group of rats were immediately killed to provide basal values (T = 0; n = 6–9). The high-fat diet consisted of 42 % lipid (80 % lard, 20 % maize oil), 36 % carbohydrate, and 22 % protein (by energy content) and was provided in small pellets from ICN Pharmaceuticals (Plainview, NY, USA). Details of this diet have been presented elsewhere (1). The exercise training programme was introduced concomitantly to the high-fat diet. Exercise training consisted of continuous running on a motor-driven rodent treadmill (Quinton Instruments, Seattle, WA, USA) five times per week for the duration of the experiment. During the first 2 weeks, rats were progressively run from 15 min/d at 15 m/min, 0 % slope up to 30 min/d at 26 m/min, 4 % slope. Thereafter, the training programme was progressively increased to reach 60 min at 26 m/min, 10 % slope for the last 4 weeks. All Tr animals were restrained from training 48 h before killing.

Blood and tissue sampling

At the end of their respective experimental condition, all animals were weighed and killed between 08.30 and 10.30 hours. Food was removed from the animals at least 2 h before killing. After complete anaesthesia (sodium pentobarbital, 50 mg/kg, intraperitoneal), the abdominal cavity was rapidly opened following the median line of the abdomen. Blood was rapidly (45 s) drawn from the abdominal vena cava (about 4 ml) into syringes pre-treated with EDTA (15 %). Blood was centrifuged (4000 rpm for 10 min, 4°C) and the plasma kept for NEFA, glucose, insulin and leptin determinations. The mesenteric and retroperitoneal fat depots were, thereafter, rapidly excised and weighed. The mesenteric fat pad consisted of adipose tissue surrounding the gastrointestinal tract from the gastro-oesophageal sphincter to the end of the rectum with special care taken in distinguishing and removing pancreatic cells. The retroperitoneal fat pad was taken as that distinct deposit behind each kidney along the lumbar muscles. About 700 mg of the depots was immediately used for determination of lipolysis. The remaining portions of the mesenteric and retroperitoneal depots as well as plasma samples were stored at −78°C until analyses.

Preparation of isolated adipocytes

Adipocytes were isolated according to the method of Rodbell (2). Tissue fragments (700 mg) from the mesenteric and the retroperitoneal tissue were minced in polyethylene vials containing Krebs–Ringer bicarbonate buffer in the presence of collagenase P (1 mg/ml) (Roche 1213865; Mannheim, Germany). Krebs–Ringer bicarbonate buffer also contained fatty acid-free bovine serum albumin (4 %) and glucose (0.5 g/l) (Krebs–Ringer bicarbonate buffer + albumin; KRBA). Thereafter, the atmosphere of the flasks was saturated with 95 % O2 and 5 % CO2. Adipocytes were then incubated in a shaking water-bath at 37°C for 30 min with a shaking frequency of 100 strokes/min. The digested pieces were filtered through a nylon mesh for separation of fat cells from the stroma. The filtrate was collected in a graduated polyethylene tube to avoid cell breakage. The aqueous phase was aspirated with polyethylene pipettes. The floating adipocytes were then re-suspended in 5 ml of fresh KRBA. This process was repeated three times to eliminate the remaining collagenase. The adipocytes were stored in the KRBA for 2 min between two washes.

Incubation of adipocytes and measurement of lipolysis

Basal and stimulated lipolysis was determined in isolated adipocytes (50 μl) incubated with a 10 μl solution of KRBA buffer and ascorbic acid (basal) and with isoproterenol adjusted to give concentrations of 10−7, 10−5 and 10−4 M. Isoproterenol was used because it is a pure β-agonist and as such it elicits the greatest lipolytic response (3). Isoproterenol produced a dose-dependent increase in lipolysis, with the maximal responses (plateau) reached at 10−2 and 10−5 M. In agreement with other groups, adenosine deaminase was not added to the incubation medium to block the anti-lipolytic action of adenosine (21, 22). The incubation was performed at 37°C under a 95 % O2–5 % CO2 atmosphere with gentle shaking (eighty strokes per min) for 2 h. The reaction was stopped by adding 50 μl HCl (1 M) and then 50 μl NaOH (1 M) to neutralise the medium. The vials were vortexed and put on ice for 10 min. After a centrifugation of 5 s at 250 g, the aqueous phase was removed and stored at −78°C until glycerol determination.

Lipolytic activity was measured by glycerol quantification with the use of commercially available kits from Roche Diagnostics (Mannheim, Germany). Glycerol quantification was expressed in millions of cells/h after counting the number of cells in each tested sample.
Cell count

The number of cells in each individual sample was determined by first diluting 100 μl of the isolated adipocyte preparation into 9 ml KRBA (100 μl) and then dropping 50 μl of this solution in 25 μl of trypan blue (0.4% normal saline). The total number of cells in this final suspension was determined with the use of a Nikon-optiphot microscope (10×) and with Image-Pro plus version 4.5 (Media Cybernetics; Silver Spring, MD, USA).

Measurement of perilipin content

Mesenteric fat depots (about 100 mg) were homogenised in EDTA–sucrose buffer and centrifuged at 12 000 g, 4°C for 20 min. The infranatant was collected with a blunt-tipped Pasteur pipette and stored at −80°C until perilipin determination. Perilipin A content in adipocytes was determined by Western blotting. All samples (25 μg of proteins) were separated on a 10% SDS–polyacrylamide gel followed by protein transfer to a polyvinylidene difluoride membrane by electroblotting. The membrane was blocked in 5% (w/v) skimmed milk in TBS. The membrane was washed three times for 10 min each time in TBS–Triton 0.5% before a chemiluminescence substrate (enhanced chemiluminescence; Amersham, Baie D’Urfe, PQ, USA) was applied to the membrane. The resulting signal was detected on enhanced chemiluminescence film (Amersham), scanned with the use of Agfas Arcus II, and quantified with Imagepro 4.5 software (Media Cybernetics, Silver Spring, MD, USA) and expressed as arbitrary units.

Analytical procedures

Plasma glucose concentration was determined with the use of a glucose analyser (Yellow Springs Instruments 2300; Yellow Springs, OH, USA). Plasma insulin levels were measured with commercially available kits from MP Biomedicals, LLC (Orangeburg, NY, USA). Plasma NEFA levels were measured with commercially available kits from Roche Diagnostics (Mannheim, Germany). Leptin levels were measured with commercially available kits from Linco (St Charles, MO, USA).

Statistical analysis

Values are expressed as mean values with their standard errors. Statistical analyses were performed by a two-way ANOVA for non-repeated-measures design with exercise training and time as main effects at times 2 and 8 weeks, excluding time point 0 (since no diet treatment was given at that time). In a second step, the effect of time was analysed separately in Sed and Tr rats using a one-way ANOVA for non-repeated measures over all time points using the same values at time 0 for both groups. This second statistical analysis was performed to better characterise the effect of time in each condition including comparison with time 0. Fisher’s post hoc test was used in the event of a significant (P < 0.05) F ratio.

Results

Changes in body composition and metabolic characteristics of Tr and Sed rats are presented in Table 1. The increase in body weight over time (P < 0.01) was similar in Tr and Sed rats. The increase in the weight of the mesenteric and the retroperitoneal fat pad was also similar in Tr and Sed rats. The increase in body weight was greater in Sed than in Tr rats (P < 0.05). The increase in body fat mass was greater in Sed than in Tr rats (P < 0.05). The increase in plasma NEFA levels was greater in Sed than in Tr rats (P < 0.05). The increase in plasma insulin levels was greater in Sed than in Tr rats (P < 0.05).

Table 1. Body composition and metabolic characteristics of trained (Tr) and sedentary (Sed) rats at time week 0 (baseline values) and in response to 2 and 8 weeks of high-fat feeding (seven to nine rats/group)§

<table>
<thead>
<tr>
<th>High-fat feeding period</th>
<th>0 weeks</th>
<th>2 week</th>
<th>8 weeks</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>Sed 236</td>
<td>3.0</td>
<td>277**</td>
</tr>
<tr>
<td></td>
<td>Tr 3.33</td>
<td>0.24</td>
<td>283**</td>
</tr>
<tr>
<td>Mesenteric fat pad (g)</td>
<td>Sed 3.24</td>
<td>0.14</td>
<td>3.6*</td>
</tr>
<tr>
<td></td>
<td>Tr 3.13</td>
<td>0.25</td>
<td>3.13*</td>
</tr>
<tr>
<td>Retroperitoneal fat pad (g)</td>
<td>Sed 1.27</td>
<td>0.21</td>
<td>2.44†</td>
</tr>
<tr>
<td></td>
<td>Tr 7.31</td>
<td>0.21</td>
<td>7.31**</td>
</tr>
<tr>
<td>Leptin (ng/l)</td>
<td>Sed 7.5*</td>
<td>0.21</td>
<td>7.5*</td>
</tr>
<tr>
<td></td>
<td>Tr 0.29</td>
<td>0.01</td>
<td>0.29**</td>
</tr>
<tr>
<td>Plasma NEFA (mm)</td>
<td>Sed 5.15</td>
<td>0.45</td>
<td>7.05**</td>
</tr>
<tr>
<td></td>
<td>Tr 7.31</td>
<td>0.21</td>
<td>7.31**</td>
</tr>
<tr>
<td>Glucose (mm)</td>
<td>Sed 69.3</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Tr 238**</td>
<td>18</td>
<td>240**</td>
</tr>
</tbody>
</table>

Mean value was significantly different from that at baseline (time 0 weeks): *P < 0.05, **P < 0.01.
Mean value was significantly different from that at time 2 weeks: †P < 0.01.

§ All rats were fed a high-fat diet.
fad pads, however, was higher ($P<0.05$) in Sed than in Tr rats after 8 weeks of high-fat feeding. The same observation was made for plasma leptin levels, with higher ($P<0.05$) values found in Sed than in Tr animals (Table 1). Plasma NEFA and glucose levels were also higher ($P<0.01$) with time in both Tr and Sed rats, but to a significantly ($P<0.05$) larger extent in Sed than in Tr animals. Plasma insulin levels were higher ($P<0.01$) with time in both Tr and Sed rats (8 weeks).

Basal as well as stimulated lipolysis levels were higher with time ($P<0.01$) in the mesenteric tissue of Sed animals (Fig. 1). In Tr rats, basal lipolysis in the mesenteric tissue was not affected with time, while stimulated lipolysis was higher ($P<0.01$) compared with time 0 but to a lesser extent than what was observed in Sed animals (Fig. 1). As a result, the elevation in stimulated lipolysis ($10^{-5} - 10^{-4} \text{M}$) in the mesenteric tissue was lower ($P<0.05$) in Tr than in Sed animals (week 8; Fig. 1). In the retroperitoneal tissue, however, stimulated lipolysis ($10^{-5} - 10^{-4} \text{M}$) in Tr animals was increased to the same level as in Sed animals after 8 weeks, thus resulting in an absence of effects of training on lipolysis (Fig. 2).

Perilipin content in the mesenteric tissue expressed in arbitrary units was higher ($P<0.05$) after 2 weeks in both Sed and Tr animals and decreased to initial values after 8 weeks (Fig. 3 (A)). This response was not significantly affected by the exercise training programme. When perilipin content was expressed per unit of fat pad weight, significantly lower ($P<0.01$) levels were found after 8 weeks compared with basal levels in both Sed and Tr animals (Fig. 3 (B)).

Discussion

In agreement with previous studies conducted in high-fat-fed rats$,^{4,6,7}$ exercise training in the present study resulted in a significant reduction in fat accumulation in the mesenteric and the retroperitoneal tissues along with lower levels of plasma NEFA and leptin (week 8; Table 1). In line with these observations, the most novel finding of the present study is that the high-fat-induced increase in isoproterenol-stimulated lipolysis in the mesenteric tissue was reduced by 8 weeks of concurrent exercise training. This response is rather surprising considering that exercise training is generally associated with an increase, and not a decrease, in $\text{in vitro}$ and $\text{in vivo}$ stimulated adipose tissue lipolysis in normal dietary conditions$^{10,11,23}$. The fact that the elevation in stimulated lipolysis was reduced by training in high-fat-fed-rats might, at first glance, simply reflect a reduction in fat mass in the mesenteric tissue. As mentioned by other studies$^{23}$, this will not decrease the interest of the study since the reduction in fat mass is an effect of training. Most interestingly, however, stimulated lipolysis in the mesenteric tissue of 8-week
Tr rats was lowered to the level measured in 2-week-old rats even though fat accumulation was still about 75% higher in the former. This indicates that the large reduction in stimulated lipolysis in 8-week Tr rats cannot solely be attributed to the reduction in fat mass. Exercise training may act upon stimulated lipolysis in the mesenteric tissue of high-fat-fed rats so as to contribute to limit the amount of circulating NEFA resulting from daily sympathetic nerve activity. We previously reported that concurrent exercise conducted in high-fat-fed rats reduced plasma NEFA levels and completely prevented the high-fat diet-induced hepatic steatosis(6). Taking into account that plasma NEFA levels reflect whole-body lipolysis, the present data extend these findings by being the first to indicate that under conditions of high-fat feeding exercise training reduced the elevation in isoproterenol-stimulated lipolytic activity of the mesenteric adipose tissue.

The interest of the present finding that exercise training reduced the elevation in stimulated lipolysis in the mesenteric tissue of high-fat-fed rats is exacerbated by the fact that the same observation was not made in the retroperitoneal tissue. Stimulated lipolysis in the retroperitoneal tissue was increased by about 95% (10^{-5} M) following 8 weeks of high-fat feeding. However, these increased lipolytic values were not affected by the exercise training programme (Fig. 2). In interpreting these data, it is important to recall that the retroperitoneal tissue, although considered an intra-abdominal tissue, is not a visceral tissue as is the intraperitoneal mesenteric depot(24). Accordingly, the high-fat feeding protocol increased stimulated lipolytic activity to a greater extent in the mesenteric (about ten-fold) than in the retroperitoneal tissue (about two-fold; Figs. 1 and 2) although relative fat accumulation was higher in the retroperitoneal than in the mesenteric...

**Fig. 2.** Adipocyte lipolysis in retroperitoneal adipose tissue of sedentary (A) and trained (B) rats at time 0 weeks (baseline values; C) and in response to 2 and 8 weeks of high-fat feeding. Values are expressed as glycerol production per million cells per h in the basal state (A) and in response to isoproterenol (10^{-7} M (B); 10^{-5} M (C); 10^{-4} M (D) after 2 h of incubation. Values are means for seven to nine rats per group (n 6 at week 0), with standard errors represented by vertical bars. * Mean value was significantly different from that at baseline (time 0 weeks) (P < 0.05). † Mean value was significantly different from that at 2 weeks (P < 0.05).
Fig. 3. Perilipin content (A) and perilipin:fat ratio (B) measured in mesenteric tissue from sedentary (●) and trained (○) rats at time 0 weeks (baseline values; III) and in response to 2 and 8 weeks of high-fat feeding. Perilipin was measured by Western blotting and expressed in arbitrary units (AU). Perilipin:fat ratio was obtained by dividing the perilipin content by the absolute weight (g) of the mesenteric depot. Values are means for eight to nine rats per group (n 6 at week 0), with standard errors represented by vertical bars. Mean value was significantly different from that at baseline (time 0 weeks): *P < 0·05, **P < 0·01. Mean value was significantly different from that at 2 weeks: †P < 0·05, ††P < 0·01.

Exercise and visceral adipose tissue in humans

It is well documented that visceral fat constitutes an important risk factor for the development of metabolic abnormalities and CVD in humans (25). Exercise training decreases visceral fat and this is associated with an improvement in glucose disposal and insulin sensitivity (25). Recent evidence suggests that the improvement in insulin sensitivity with exercise training may be explained by a decreased availability of NEFA (22). A reduction in lipolytic activity of the visceral fat depot with exercise training could, therefore, contribute to the reduced availability of NEFA to the portal system and to the improvement in insulin sensitivity in obese individuals.

Lipolysis and perilipin

It is not readily apparent as to what mechanism might explain the exercise training-induced attenuation in the lipolytic activity of the mesenteric tissue in the present high-fat-fed rats. Plasma glucose and insulin were increased to a similar extent in Sed and Tr animals after 8 weeks of high-fat feeding. Nomura et al. (19) observing that basal activity of HSL was greater in Tr normally fed rats, but basal lipolysis was not, recalled the suggestion of a dual mechanism of lipolytic activation involving some factor on the surface of the lipid droplets (i.e. perilipin) that may be necessary for the hormonal stimulation of lipolysis (27). In an attempt to explore this mechanistic avenue, perilipin content was measured in the mesenteric tissue of the present Tr and Sed animals. Interestingly, our data indicate a transient increase in perilipin content in absolute values after 2 weeks that decreased to initial values after 8 weeks (Fig. 3 (A)). This resulted in low perilipin content in relation to fat mass accretion after 8 weeks (Fig. 3 (B)). Since perilipin has been reported to be involved in the mechanism of stimulation of lipolysis (22,28), the low level of perilipin expressed per unit of fat mass after 8 weeks may explain the increase in lipolysis in the present high-fat-fed rats kept in
the Sed state. It is not clear what caused the decrease in perilipin content after 8 weeks of high-fat feeding. It could be related to the increased plasma leptin levels. Mice overexpressing leptin show a reduction in perilipin gene expression in white adipose tissue. On the other hand, and most importantly, exercise training did not significantly affect perilipin content in the mesenteric tissue, whether reported in absolute or in relative values. The association between perilipin content and lipolysis is, however, complicated by the fact that other factors in addition to HSL and perilipin may contribute to the lipolytic response. The new enzyme adipose TAG lipase, that catalyses the initial step in TAG hydrolysis in mammalian adipose tissue, along with the adipocyte lipid-binding proteins that interact with HSL, favouring the translocalisation from cytosol to lipid droplet, are potential candidates\(^{31,32}\).

In summary, results of the present study indicate that exercise training lowers the elevation in stimulated in vitro lipolytic activity in the mesenteric tissue of rats concurrently fed with a high-fat diet. This effect was not observed in a non-visceral tissue (retroperitoneal) and seems to be independent of the perilipin content. Whether exercise training may decrease the lipolytic activity of the visceral adipose tissue of established obese individuals and thus constitute a health-promoting effect remains to be established.

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