Effect of exercise training on in vivo lipolysis in intra-abdominal adipose tissue in rats

L. H. ENEVOLDSEN, B. STALLKNECHT, J. D. FLUCKEY, AND H. GALBO
Department of Medical Physiology, The Panum Institute, University of Copenhagen and Copenhagen Muscle Research Centre, National University Hospital, DK-2200 Copenhagen, Denmark

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Enevoldsen, L. H., B. Stallknecht, J. D. Fluckey, and H. Galbo. Effect of exercise training on in vivo lipolysis in intra-abdominal adipose tissue in rats. Am J Physiol Endocrinol Metab 279: E585–E592, 2000.—Intra-abdominal obesity is associated with cardiovascular disease and non-insulin-dependent diabetes mellitus, and physical training has been suggested to alleviate these conditions. We compared epinephrine-stimulated lipolysis in vivo in three intra-abdominal adipose tissues (ATs: retroperitoneal, parametrial, and mesenteric) and in subcutaneous AT, and we also studied the effect of physical training. Moreover, we studied the effect of physical training on epinephrine-stimulated lipolysis in muscle in vivo. Female rats were either swim trained (15 wk, n = 8) or sedentary (n = 7). Under anesthesia, a two-stage intravenous epinephrine infusion (60 min of 80 and 200 ng·kg\(^{-1}\)·min\(^{-1}\), respectively) was carried out, and local interstitial glycerol concentration was measured by the microdialysis technique. Blood flow was measured by microspheres. Training increased blood flow in all ATs [on average: 73 ± 12 (trained) vs. 14 ± 4 (sedentary) ml·100 g\(^{-1}\)·min\(^{-1}\), P < 0.05]; nevertheless, epinephrine-stimulated interstitial glycerol concentrations were increased or unchanged. Interstitial glycerol concentration was higher in intra-abdominal than in subcutaneous AT in both trained and sedentary rats. In skeletal muscle, interstitial glycerol concentration and blood flow did not differ between trained and sedentary rats. In conclusion, in vivo lipolysis is higher both in the basal state and during epinephrine-stimulation in intra-abdominal than in subcutaneous AT, and training may be beneficial in alleviating intra-abdominal obesity by enhancing lipolysis in intra-abdominal fat depots.

EXCESSIVE DEPOSITION of visceral adipose tissue is associated with major diseases such as cardiovascular disease and non-insulin-dependent diabetes mellitus (4). Physical training enhances whole body fat oxidation during submaximal work (23) and has been recommended in the treatment of diseases associated with visceral obesity (4). During physical work, adipose tissue lipolysis is stimulated by catecholamines. Furthermore, it has previously been shown that subcutaneous abdominal fat cells from trained subjects have a higher epinephrine-stimulated lipolysis in vitro than those from untrained subjects (7). However, in vivo enhanced lipolytic sensitivity to epinephrine could not be demonstrated in subcutaneous abdominal adipose tissues by microdialysis in trained compared with sedentary subjects (35).

Previous in vitro studies in humans (12, 14, 28) and in rats (36, 37) and an in vivo study of mesenteric adipose tissue (AT) in rats (16) have provided evidence that lipolysis caused by β-adrenergic agonists is higher in intra-abdominal compared with subcutaneous AT. Furthermore, training has been shown to increase rat parametrial adipocyte responsiveness to epinephrine in vitro (5). However, the influence of training on epinephrine-stimulated lipolysis in intra-abdominal AT in vivo is not known. The effect of training on whole body fat oxidation during exercise may not solely reflect an increase in catecholamine-stimulated lipolysis in AT. Thus evidence has been presented that, during exercise, intramuscular triglyceride breakdown is enhanced in trained compared with untrained muscle (15). However, this is not accepted by all (17).

The aim of the present study was to extend existing knowledge on the relationship between in vivo lipolytic sensitivity to epinephrine in various intra-abdominal and subcutaneous adipose tissues, respectively. Furthermore, the influence of physical training on epinephrine-stimulated lipolysis in these tissues as well as in skeletal muscle was studied. Lipolysis was determined by microdialysis in rats, because, at present, intra-abdominal AT is not accessible for in vivo studies in humans. Female rats were used because, compared with male rats, they show much smaller weight reduction, including reduction in muscle mass, in response to severe endurance training. This means that, in female rats, effects of deficient energy intake (malnutrition) will not significantly interfere with effects of training per se. Assuming no major species differences between humans and rats, we thought that a study of rats would also provide direction to the endeavor of understanding regulation of human adipose tissue metabolism.

Address for reprint requests and other correspondence: L. H. Enevoldsen, Dept. of Medical Physiology, The Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark (E-mail: lenevoldsen@mfi.ku.dk).

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EFFECT OF TRAINING ON INTRA-ABDOMINAL ADIPOSE TISSUE

MATERIALS AND METHODS

Experimental groups. Fifteen female Wistar rats weighing 96 ± 1 g were randomly assigned to two groups, which either participated in a 15-wk swimming program (n = 8) or served as sedentary controls (n = 7).

Swim training. The rats were trained by swimming following a protocol accepted by the Danish Animal Experiments Inspectorate and in accordance with the animal experimentation guidelines approved by the Council of the American Physiological Society. They swim in tepid water maintained at 36°F (35.5–36.5°C). The duration of the daily training (5 days/wk) was gradually increased to 6 h/day during the first 10 wk. All rats swam simultaneously in a tank with a water depth of 58 cm and an average surface area of 200 cm²/rat. This ensured that the rats were in constant activity during the training sessions. After each training session, rats were dried in a towel and placed under a lamp at 31°C for 1 h with ad libitum food access. A swimming protocol similar to the one used in the present study has been shown in previous studies to increase heart weight and cytochrome c oxidase activity in skeletal muscle (27).

Experimental protocol. Forty hours after the last training session and after an overnight fast, the rats were brought to the laboratory at 0900. After being weighed, rats were anesthetized by 3% halothane gas and subsequently kept anesthetized by 1% halothane gas combined with 50% O₂-50% N₂O. Each rat was then placed on a heated lamp under a lamp at 31°C to ensure a mean rectal temperature of 37°C. Subsequently, the rats had TDMAC-heparinized (tridodecylmethylammonium chloride-heparin complex 2%, Polysciences, Warrington, PA) catheters inserted in the left jugular vein (for infusion of epinephrine), the aortic arch via the right common carotid artery (for blood sampling and infusion of microspheres), and in the tail artery (for microsphere sampling). The catheterizations were performed under ketamine-xylazine anesthesia (Saffan; Intervet, Solna, Sweden). Catecholamine concentrations were determined by a previously described single isotope radioenzymatic assay (19). Hemato-cale were obtained for interstitial concentrations of glycerol were calculated as \( [\text{C}_d - \text{C}_p/\RR] \), where \( \text{C}_d \) is dialysate concentration and \( \text{C}_p \) is perfusate concentration. In two control experiments in which microdialysis was performed as described above but without epinephrine infusion, arterial plasma glycerol concentrations did not change with time in either adipose or muscle tissue (P > 0.05; data not shown).

Blood sampling and analysis. Blood for determination of metabolites and hormones was sampled from the aortic arch by heparinized syringes into iced tubes and centrifuged immediately. Blood samples were obtained for catecholamine determination (0.3 ml) in all experiments at 10, 20, 45, 80, 105, and 140 min and for arterial plasma glycerol determination (0.2 ml) at 10, 20, 45, 70, 80, 105, 130, and 140 min. Blood samples for determination of hematocrit (0.1 ml) were obtained at 10, 70, and 130 min. The blood drawn was replaced with red blood cells at 20 and 80 min (1 ml, 1:1). Donor blood was obtained by cardiac puncture of an anesthetized donor rat and centrifuged, and red blood cells were resuspended in 4% bovine serum albumin in isotonic saline with pH adjusted to 7.4. Blood for determination of catecholamines was stabilized with a dilute trichloroacetic acid stock solution, and blood samples for catecholamine determination were kept at −20°C until analysis, and plasma samples for catecholamine determination were kept at −80°C.

Plasma glycerol concentrations were determined with a commercial enzymatic kit (Wako Chemicals, Neuss, Germany) adapted to a Monarch centrifugal analyzer (Instrumental Laboratory, Warrington, Cheshire, UK). Catecholamine concentrations were determined by a previously described single isotope radioenzymatic assay (19). Hematocrit was determined by an ABL 625 (Radiometer, Roedovde, Denmark).

Blood flow. Local blood flow was determined by the radioactive microsphere technique (21). Microspheres (mean size 15.5 ± 0.1 μm; Du Pont de Nemours, Mechelen, Belgium) labeled with either \(^{14}\text{Ce}\) (specific activity 17.79 mCi/g) or \(^{113}\text{Sn}\) (specific activity 8.62 mCi/g) in a suspension of 10% dextran containing 0.01% Tween-80 surfactant were mixed...
Table 1. Anthropometric data for rats

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<th>Trained</th>
<th>Sedentary</th>
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<tr>
<td>BW, before, g</td>
<td>96 ± 1</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>BW, after, g</td>
<td>233 ± 8</td>
<td>265 ± 9</td>
</tr>
<tr>
<td>HW, g</td>
<td>0.90 ± 0.03</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>HW/BW, %</td>
<td>0.38 ± 0.00</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Retroperitoneal AT weight, mg</td>
<td>138 ± 19</td>
<td>362 ± 70</td>
</tr>
<tr>
<td>Parametrial AT weight, mg</td>
<td>124 ± 33</td>
<td>245 ± 69</td>
</tr>
<tr>
<td>Mesenteric AT weight, mg</td>
<td>76 ± 13</td>
<td>140 ± 17</td>
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Values are means ± SE for 8 trained and 7 sedentary rats. BW before and BW after, body weights before and after swim training or equivalent period of sedentary life; HW, heart weight after these periods; AT, adipose tissue. Retroperitoneal and parametrial AT weights are for right fat pads. *P < 0.05 compared with sedentary rats.

in an ultrasonicator (Branson 3200, Buch and Holm, Herlev, Denmark) for 10 min and then on a vortex mixer (VORTEX-GENIE 2, Buch and Holm) for 1–2 min before infusion to assure a uniform distribution of spheres, as verified by microscopic observations. Bolus injections were performed at 15 (141Ce, 100 μl) and 75 min (113Sn, 50 μl), respectively. For each blood flow measurement, ~130,000 spheres (6.25 μCi 141Ce or 3.15 μCi 113Sn) were injected into the aortic arch, followed by 0.5 ml of isotonic saline. Injections were administered over a 30-s period. The reference blood samples were withdrawn from the tail artery at a rate of 50 μl/min by a high-precision pump (CMA 100, Carnegie Medicine) starting 10 s before the microsphere injection and were continued for 80 s. At the end of the experiment, animals were killed by removing the heart, and the following tissues were excised, weighed, placed in counting vials, and transferred to a high-precision pump (CMA 100, Carnegie Medicine) starting 10 s before the microsphere injection and were continued for 80 s. At the end of the experiment, animals were killed by removing the heart, and the following tissues were excised, weighed, placed in counting vials, and transferred to a gamma counter (Packard Auto-Gamma 5650, Packard Instruments): right retroperitoneal, right parametrial, and mesenteric AT, neck subcutaneous AT, the right tibialis anterior and gastrocnemius muscles, and both kidneys. Reference blood samples were also counted at this time. Absolute blood flow (ml·100 g⁻¹·min⁻¹) to each tissue was calculated as [dpm x (0.05 ml·min⁻¹·dpm wt⁻¹) x 100/wt (g)], where dpm, is disintegrations per minute in tissue, dpm wt is disintegrations per minute in reference blood sample, and wt is tissue weight. To evaluate whether the microspheres were equally distributed, the coefficients of variation for blood flows in the right and left kidneys were calculated. In the basal period, the coefficient of variation was 11 ± 2% in trained and 17 ± 9% in sedentary rats. During epinephrine infusion, the coefficient of variation was 16 ± 7% in trained and 23 ± 13% in sedentary rats.

Statistics. All data are presented as means ± SE. A two-way repeated-measures ANOVA was used to test whether arterial catecholamine concentrations, arterial plasma and interstitial glycerol concentrations, relative recovery of glycerol, and blood flow differed with time or between trained and sedentary rats, and whether interstitial glycerol concentrations differed between arterial plasma and interstitium. A one-way repeated-measures ANOVA was used to test whether relative recovery, interstitial glycerol concentrations, and blood flow in the basal period and during low epinephrine infusion differed between different tissues. The Student-Newman-Keuls test was used as a post hoc test. Student’s t-test for unpaired data (when data were normally distributed) or the Mann-Whitney rank-sum test (when data were not normally distributed) was used to test whether anthropometric data differed between trained and sedentary rats. A significance level of 0.05 in two-tailed testing was chosen a priori.

RESULTS

Characteristics of experimental animals. After swim training, body weights were significantly lower, and heart weight-to-body weight ratios were significantly higher, in trained compared with sedentary rats. Weights of retroperitoneal, parametrial, and mesenteric AT were significantly lower in trained than in sedentary rats (Table 1). Hematocrit did not differ between groups (P > 0.05) but decreased significantly during the experiment [trained: 10 min, 45 ± 1 (n = 6); 70 min, 42 ± 1 (n = 7); 130 min, 39 ± 1% (n = 7); and sedentary: 10 min, 49 ± 2 (n = 4); 70 min, 44 ± 1 (n = 7); 130 min, 40 ± 1% (n = 7)].

Catecholamine concentrations. During low and high epinephrine infusions, epinephrine concentrations in arterial plasma were increased to ~8 and 16 nM, respectively, and did not differ between trained and sedentary rats. Norepinephrine concentration in arterial plasma increased significantly during the low epinephrine infusion in both trained and sedentary rats (P < 0.05) but did not differ between groups at any time (Table 2).

Arterial plasma glycerol concentrations. Epinephrine infusion increased glycerol concentrations in arterial plasma in both trained and sedentary rats (P < 0.05), and glycerol concentrations never differed between groups (P > 0.05) (Fig. 1).

Interstitial glycerol concentrations. In trained rats, during both the basal period and epinephrine infusion, interstitial glycerol concentrations in all intra-abdominal AT were significantly higher than arterial plasma glycerol concentrations (Fig. 2, A–D). However, in trained rats, interstitial glycerol concentrations in subcutaneous AT did not differ from plasma glycerol concentrations at any time (P > 0.05), and muscle interstitial glycerol concentrations were lower than arterial

Table 2. Arterial plasma catecholamine concentrations

<table>
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<tr>
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<th>Basal</th>
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<th>Low</th>
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<th>High</th>
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<tbody>
<tr>
<td></td>
<td>Trained</td>
<td>Sedentary</td>
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<td>Trained</td>
<td>Sedentary</td>
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<td>Trained</td>
</tr>
<tr>
<td>Epinephrine, nM</td>
<td>1.53 ± 0.31</td>
<td>2.75 ± 0.33</td>
<td></td>
<td>6.81 ± 1.04†</td>
<td>8.86 ± 1.00†</td>
<td></td>
<td>14.13 ± 2.33‡</td>
</tr>
<tr>
<td>Norepinephrine, nM</td>
<td>2.18 ± 0.45</td>
<td>2.95 ± 0.74</td>
<td></td>
<td>3.12 ± 0.88‡</td>
<td>4.72 ± 1.51‡</td>
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<td>2.87 ± 0.8</td>
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</table>

Values are means ± SE for 7 trained and 7 sedentary rats in the basal period (mean of two samples drawn 10 min apart starting 10 min after start of experiment), during low epinephrine infusion (mean of two samples drawn 35 min apart starting 15 min after start of infusion), and during high epinephrine infusion (mean of two samples drawn 35 min apart starting 15 min after start of high infusion). *P < 0.05 compared with sedentary rats. †P < 0.05 compared with the basal period. ‡P < 0.05 compared with the low epinephrine infusion period.
plasma glycerol concentrations \((P < 0.05)\) (Fig. 2, E and F). In sedentary rats, only in retroperitoneal AT were interstitial glycerol concentrations significantly higher than plasma glycerol concentrations (Fig. 2), and only in gastrocnemius muscle were interstitial glycerol concentrations significantly lower than plasma glycerol concentrations (Fig. 2). During both the basal period and epinephrine infusion, the interstitial glycerol concentration was generally higher in intra-abdominal AT than in subcutaneous AT and muscles in both trained and sedentary rats. Interstitial glycerol concentrations increased significantly during epinephrine infusion in mesenteric AT in trained but not in sedentary rats (Fig. 2). Furthermore, the mean interstitial glycerol concentrations from all AT increased significantly with time in trained but not in sedentary rats (Table 3). After the start of epinephrine infusion, interstitial glycerol concentrations in tibialis anterior muscle increased similarly in both trained and sedentary rats (Fig. 2).

During epinephrine stimulation, the mean glycerol gradient between interstitial space and arterial plasma was significantly higher in AT from trained compared with sedentary rats (Table 3).

**Blood flow.** Blood flow per 100 g never differed significantly between the various adipose tissues and did not change with epinephrine infusion in either adipose tissue or muscle (Fig. 3). Blood flow per 100 g of the various adipose tissues was more than fourfold higher in trained than in sedentary rats, both in the basal period and during epinephrine infusion \((P < 0.05)\). Blood flow per 100 g of muscle tissue did not differ between trained and sedentary rats \((P > 0.05)\). In trained rats, blood flow tended to be higher in all ATs than in the two muscles studied, and the difference was significant for parametrial and mesenteric AT and also for retroperitoneal AT vs. gastrocnemius muscle (Fig. 3).

**DISCUSSION**

Major new findings of the present study are that in vivo basal and epinephrine-stimulated lipolysis estimated per 100 g tissue weight is higher in a number of intra-abdominal adipose tissues compared with subcutaneous adipose tissue. Furthermore, training markedly enhances blood flow and lipolytic response to epinephrine of all adipose tissues in vivo. Finally, training does not increase blood flow and epinephrine-stimulated lipolysis in tibialis anterior and gastrocnemius muscles.

During epinephrine infusion, the arterial epinephrine concentrations achieved were similar in the two groups and also similar to those seen in rats running at moderate intensity (29). In vitro studies of basal and epinephrine-stimulated subcutaneous adipocytes in humans (7, 8) and parametrial and epididymal adipocytes in rats (5, 25) have shown that training increases lipolysis by a postreceptor mechanism. In accordance with these in vitro findings, we found that, during epinephrine infusion, interstitial glycerol concentrations in adipose tissue increased in trained but not in sedentary rats (Fig. 2 and Table 3). An increase in interstitial glycerol concentration can be caused either by an increase in glycerol release from the adipocytes or by a decrease in removal of glycerol from adipose tissue by the blood, glycerol removal generally being considered to be flow dependent (34). Because arterial plasma glycerol was similar in the two groups and adipose tissue blood flow was fourfold higher in trained compared with sedentary rats (Fig. 3), trained rats had a higher, not a lower, removal of glycerol by the blood than sedentary rats. The finding that, during epinephrine stimulation, the average glycerol gradient between interstitial space and arterial plasma was higher in adipose tissues from trained compared with untrained rats supports this view (Table 3). Accordingly, the microdialysis and blood flow data indicate that in vivo epinephrine-stimulated lipolytic activity in a number of adipose tissues, including intra-abdominal adipose tissues, is higher in trained compared with sedentary rats.

This finding in female rats is apparently in conflict with a previous in vivo study in which epinephrine-stimulated subcutaneous adipose tissue lipolysis was not increased in trained compared with sedentary young men (35). However, the discrepancy may reflect sex differences in lipolytic responses to training (8, 11, 23). In young women, endurance training increases the oxidation of free fatty acids (FFA) derived from plasma both during exercise performed at the same absolute workload and during exercise performed at the same relative workload as before training (11). In contrast, in young men, plasma FFA oxidation is lower at a given absolute workload after compared with that before endurance training (23), suggesting that women respond to endurance training with a greater reliance on adipose tissue lipolysis during exercise than men do. In line with this, Crampes et al. (8) have shown that...
endurance training results in a larger increase in epinephrine-stimulated glycerol release from periumbilical adipocytes in vitro in women than in men because of a higher efficiency of the $\beta$-adrenergic pathway, along with a lower efficiency of the $\alpha_2$-adrenergic pathway, in women compared with men. In addition to sex differences, the discrepancy between the present study and a previous study in young men (35) may reflect species differences or differences in training, the rats of the present study having been trained much more

Table 3. Mean interstitial glycerol concentrations and mean glycerol gradient between interstitial space and arterial plasma ($\mu$M) in all adipose tissues

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Trained ($n$)</th>
<th>Sedentary ($n$)</th>
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<tbody>
<tr>
<td>Interstitial Glycerol Concentration, $\mu$M</td>
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<tr>
<td>0–30 min</td>
<td>298 ± 27 (29)</td>
<td>345 ± 51 (28)</td>
<td>137 ± 29 (25)</td>
<td>156 ± 49 (28)</td>
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<tr>
<td>Low (30–60 min)</td>
<td>320 ± 29 (27)</td>
<td>281 ± 27 (28)</td>
<td>156 ± 30† (23)</td>
<td>86 ± 28 (28)</td>
</tr>
<tr>
<td>Low (60–90 min)</td>
<td>378 ± 37* (28)</td>
<td>276 ± 30 (26)</td>
<td>174 ± 39† (25)</td>
<td>44 ± 34 (26)</td>
</tr>
<tr>
<td>High (90–120 min)</td>
<td>398 ± 35* (21)</td>
<td>348 ± 36 (27)</td>
<td>188 ± 32† (17)</td>
<td>68 ± 40 (27)</td>
</tr>
<tr>
<td>High (120–150 min)</td>
<td>396 ± 40* (25)</td>
<td>360 ± 44 (26)</td>
<td>182 ± 41† (25)</td>
<td>86 ± 51 (26)</td>
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<tr>
<td>Interstitial Minus Arterial Plasma Glycerol, $\mu$M</td>
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<td>0–30 min</td>
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<td>Low (30–60 min)</td>
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<td>High (120–150 min)</td>
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Values are means ± SE of all adipose tissues (retroperitoneal, parametrial, mesenteric, and subcutaneous) for 8 trained and 7 sedentary rats before (0–30 min) and during a low (30–90 min, 80 ng·kg$^{-1}$·min$^{-1}$) and a high (90–150 min, 200 ng·kg$^{-1}$·min$^{-1}$) epinephrine infusion. Numbers in parentheses are nos. of observations. *$P < 0.05$ compared with the basal period. †$P < 0.05$ compared with sedentary rats.
intensively and from a relatively younger age than what is possible in human studies.

Interestingly, despite differences in lipolysis estimated per 100 g of adipose tissue, whole body lipolysis did not differ between trained and sedentary rats as reflected by plasma glycerol. This apparently reflects the fact that the reduction of overall mass of adipose tissue with training outbalanced the increase in lipolytic activity per 100 g. It would be desirable to express the data as the amount of total adipose tissue for each region. However, our considerations concerning adipose tissue lipolysis are qualitative, because calculations of lipolysis from microdialysis in quantitative terms are based on many assumptions and variables, each of which may be assessed inaccurately (34). Furthermore, the retroperitoneal, parametrial, and mesenteric fat pads can be cut out easily and weighed, but subcutaneous adipose tissue is widely distributed and would be impossible to dissect out.

Because the total mass of subcutaneous adipose tissue is difficult to determine correctly, we cannot be sure that it was reduced relatively less by training compared with intra-abdominal adipose tissue. If it were, however, subcutaneous adipose tissue would contribute relatively more to whole body lipolysis in the trained compared with the sedentary state, and in that case, the supply of FFA to the portal circulation in the basal state and during epinephrine stimulation would be decreased by training. It is generally recognized that accumulation of intra-abdominal adipose tissue can lead to greater fluxes of FFA to the portal circulation and, in turn, to liver insulin resistance (18). In accordance with this view, it has been shown that a decrease in FFA fluxes to the portal circulation alleviates the obesity-linked insulin resistance syndrome (1, 2). Accordingly, one might speculate that training by diminishing overall FFA flux to the portal vein has a health-promoting effect. Measurement of interstitial FFA concentrations to verify these interpretations is not technically possible, because only water-soluble molecules can be detected by microdialysis. Alternatively, the relationship between morbidity and a large mass of intra-abdominal adipose tissue reflects other pathogenic mechanisms, in which case our findings are compatible with the interpretation that the training-induced increment of lipolysis per 100 g of adipose tissue is beneficial by reducing the total mass of intra-abdominal fat.

In the present study, it is not possible to conclude whether the effect of training on lipolysis reflects an effect of training per se or is the result of reduced fat mass. However, this does not diminish the interest of the study, because the reduction in fat mass is an effect of training. Given a difference in adipose tissue mass between trained and sedentary rats, one might speculate that differences in plasma insulin levels between groups influenced results. However, in a comparable recent study, in which swim-trained and sedentary female rats were used, we found no differences in plasma insulin and glucose concentrations between groups in the basal state (10). It will never be possible, in an in vivo study, to exclude the fact that the influence of an intervention, e.g., training or epinephrine infusion, on a given variable is indirect, being mediated via another variable. However, no matter what the mechanisms, our study indicates that in vivo adipose tissue lipolysis during epinephrine stimulation is increased with training.

Estimation of interstitial glycerol concentrations could be influenced by differences in the diffusion of glycerol from interstitial space to microdialysis probe resulting from morphological differences in adipose tissue between trained and sedentary rats. In fact, relative recovery of glycerol was significantly higher in trained compared with sedentary rats in both the retroperitoneal and the mesenteric adipose tissues (see
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REMARKS AND METHODS). However, because recovery of glycerol was determined in all rats and was used in the calculation of interstitial glycerol concentrations, the estimated concentrations should be correct. In the present study, interstitial glycerol concentrations were higher in intra-abdominal adipose tissues compared with subcutaneous adipose tissue in trained and sedentary rats (Fig. 2). Because blood flow did not differ between subcutaneous and intra-abdominal adipose tissues (Fig. 3), differences in interstitial glycerol concentration may be ascribed to a higher glycerol release, suggesting a higher rate of lipolysis in intra-abdominal adipose tissue. In line with this, one in vivo study of a single adipose tissue (16) and many in vitro (12, 14, 28, 36, 37) studies have provided evidence that lipolysis caused by $\beta$-adrenergic stimulation is higher in intra-abdominal compared with subcutaneous adipose tissue.

It has previously been found by the microsphere method that training does not change either the retroperitoneal or the epididymal adipose tissue blood flow in rats at rest (24). In contrast, we found with the same method that blood flow per 100 g of adipose tissue was higher in the basal period as well as during epinephrine stimulation in trained than in sedentary rats (Fig. 3). The present findings are in accordance with previous observations with the $^{133}$Xe washout method in human abdominal subcutaneous adipose tissue (35). Furthermore, in the present study, blood flow in intra-abdominal adipose tissue did not differ from that in subcutaneous adipose tissue either in the basal period or during epinephrine infusion (Fig. 3). This finding is in accordance with a previous study in which blood flow with and without local isoproterenol stimulation did not differ between mesenteric and subcutaneous adipose tissue (16).

In the present study, adipose tissue blood flow did not change during epinephrine stimulation (Fig. 3). In line with this, no effect of local sympathectatic stimulation on mesenteric, subcutaneous, and epididymal adipose tissue blood flow, estimated by microdialysis outflow-to-inflow ratio of ethanol, was found in recent studies of rats (6, 16). In contrast, abdominal subcutaneous adipose tissue blood flow increased up to sixfold during epinephrine infusion in humans (30, 33, 35). Again, a species difference between rats and humans might explain the difference. In line with this, adipose tissue blood flow decreases in rats (38) but increases in humans in response to a meal (32). Furthermore, epinephrine is a vasodilator in human subcutaneous adipose tissue (22, 30, 33, 35) but is a vasoconstrictor in dog adipose tissue (3, 13).

Interstitial glycerol concentrations in muscle increased in response to epinephrine in tibialis anterior but not in gastrocnemius muscle, and concentrations did not differ between trained and sedentary rats (Fig. 2). Because blood flow in the two muscles did not change with epinephrine infusion and did not differ between trained and sedentary rats (Fig. 3), these data indicate that epinephrine may not stimulate lipolysis in all muscles and that epinephrine-induced lipolysis in muscle is not affected by training. Whether muscle triglyceride is an important source of energy during exercise is at present not clear; also, the effect of training on lipolysis in muscle during exercise is uncertain (15, 17). Assuming no interaction between effect of epinephrine and exercise, the findings of the present study indicate that epinephrine-induced mobilization of muscle triglyceride is not essential for metabolism in exercising untrained or trained muscle. Epinephrine infusion has been shown to enhance muscle blood flow sixfold in humans (33). The findings of the present study confirm that muscle blood flow changes much less in response to epinephrine in rats compared with humans (26). In the present study, the interstitial glycerol concentration in muscle (Fig. 2) was, in fact, lower than the arterial plasma glycerol concentration (Fig. 1), suggesting uptake of glycerol in muscle. This is in conflict with the current opinion that glycerol is taken up mainly in the liver and not in skeletal muscle. However, in 6-h fasted subjects, Landau et al. (20) found that only approximately one-half of glycerol released to plasma was taken up by the splanchnic bed and kidneys. In addition, studies applying arteriovenous forearm catheterization have shown that glycerol may actually be taken up in muscle (9). However, a methodological explanation for the apparent glycerol uptake in skeletal muscle in the present study cannot be rejected. Relative microdialysis recoveries estimated by the internal reference technique in the present study were relatively high compared with relative recoveries previously obtained for glycerol with the no-net-flux technique (35). A too-high relative recovery would result in underestimation of the interstitial glycerol concentration.

In conclusion, training increases blood flow and basal as well as epinephrine-stimulated lipolysis per 100 g of a variety of adipose tissues in vivo. Furthermore, in vivo lipolysis is higher both in the basal state and during epinephrine stimulation in intra-abdominal adipose tissue than in subcutaneous adipose tissue. Lipolysis in muscle is not affected by training. A perspective of the present study is that the effect of training on intra-abdominal adipose tissue may be important in the treatment of diseases associated with visceral obesity.

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