Effect of exercise training on in vivo insulin-stimulated glucose uptake 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

Enevoldsen, L. H., B. Stallknecht, J. D. Fluckey, and H. Galbo. Effect of exercise training on in vivo insulin-stimulated glucose uptake in intra-abdominal adipose tissue in rats. Am. J. Physiol. Endocrinol. Metab. 278: E25–E34, 2000.—Intra-abdominal obesity may be crucial in the pathogenesis of the insulin-resistance syndrome, and training may alleviate this condition. We compared insulin-mediated glucose uptake in vivo in three intra-abdominal adipose tissues (ATs; retroperitoneal, parametrial, and mesenteric) and in subcutaneous AT and also studied the effect of training. Rats were either swim trained (15 wk, n = 9) or sedentary (n = 16). While the rats were under anesthesia, a hyperinsulinemic (≈900 pM), euglycemic clamp was carried out and local glucose uptake was measured by both the 2-deoxy-o-[^3H]glucose and microdialysis techniques. Blood flow was measured by microspheres. Upon insulin stimulation, blood flow generally decreased in AT. Flow was higher in mesenteric tissue than in other ATs, whereas insulin-mediated glucose uptake did not differ between ATs. Training doubled the glucose infusion rate during hyperinsulinemia, in part, reflecting an effect in muscle. During hyperinsulinemia, interstitial glucose concentrations were lower, glucose uptake per 100 g of tissue was higher in AT in trained compared with sedentary rats, and training influenced glucose uptake identically in all ATs. In conclusion, differences between ATs in insulin sensitivity with respect to glucose uptake do not explain that insulin resistance is associated with intra-abdominal rather than subcutaneous obesity. Furthermore, training may be beneficial by enhancing insulin sensitivity in intra-abdominal fat depots.

Microdialysis; skeletal muscle; blood flow; lactate

Modern Western lifestyle is associated with frequent simultaneous occurrence of a series of diseases grouped under the name the insulin-resistance syndrome (25). This syndrome includes hypertension, hyperlipidemia, obesity, and type II diabetes and is characterized by reduced insulin sensitivity. The syndrome is associated with increased morbidity due to cardiovascular diseases, including myocardial infarction and stroke. In the pathogenesis of the syndrome, the intra-abdominal fat is thought to play a major role. This view is built on the basis that the occurrence of the syndrome correlates with the amount of intra-abdominal fat (15) and that the intra-abdominal adipose tissue is especially lipolytically active (1). A large intra-abdominal fat mass and lipolysis can indirectly have a negative impact on insulin-mediated glucose metabolism in both liver and periphery (2). Whether a difference in insulin-mediated glucose uptake between intra-abdominal and other adipose tissues might contribute directly to whole body insulin resistance has not been clarified in vivo.

Physical training increases whole body insulin-mediated glucose uptake (22) and has been recommended in the treatment of the insulin-resistance syndrome (3). The effect of training on whole body insulin sensitivity and responsiveness has mainly been attributed to an increase in glucose uptake and metabolism in skeletal muscle (10). However, the increased glucose uptake in skeletal muscle cannot account completely for the effect of training on whole body glucose uptake (9). In line with this, training has been shown to enhance insulin-stimulated glucose uptake in rat parametrial adipose tissue in vitro (4) and in rat epididymal adipose tissue in vivo (14). Furthermore, in human subcutaneous adipose tissue, a stimulation of glucose uptake by insulin can be demonstrated by microdialysis in vivo in trained but not in sedentary subjects (Stallknecht, unpublished data). The influence of training on insulin-mediated glucose uptake in intra-abdominal adipose tissue in vivo is not known.

In the present study, we have compared insulin-stimulated glucose uptake in subcutaneous tissue and in various intra-abdominal adipose tissues in vivo. Furthermore, we have studied the influence of physical training on insulin-stimulated glucose uptake in these tissues. In order to give an optimal evaluation of glucose uptake, we measured this with both a classical, well-established method, i.e., the 2-deoxy-o-[^3H]glucose (2-DG) technique, and a more recent method, i.e., the microdialysis technique, the two methods being based on different assumptions. The study was performed in rats because at present subcutaneous adipose tissue is the only accessible adipose tissue for in vivo studies in humans.

Materials and Methods

Experimental groups. Twenty-five female Wistar rats weighing 96 ± 1 g (means ± SE) were randomly divided into two
groups, which either participated in a 15-wk swimming program (n = 9) or served as sedentary controls (n = 16).

Swim training. The rats were trained by swimming after a protocol accepted by the Danish Animal Experiments Inspectorate in accordance with the animal experimentation guidelines approved by the Council of the American Physiological Society. They swam in tepid water maintained at 36°C (35.5–36.5°C). The duration of the daily training (5 days/wk) was gradually increased up to 6 h/day during the first 10 wk. All rats swam 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.

Experimental protocol. Forty hours after the last training session and after an overnight fast, the rats were brought to the laboratory at 9 AM. After being weighed, rats were anesthetized by halothane gas, combined with 50% O₂ and 50% N₂O. Each rat was then placed on a heated table under a heating lamp to ensure a mean rectal temperature of 37°C. Subsequently, the rats had tridodecylmethylammonium chloride, heparin-complex 2% (Polysciences, Warrington, PA) catheters inserted into the left jugular vein (for infusion of glucose and insulin), into the left carotid artery (for arterial blood sampling and analysis), and into the tail artery (for microsphere reference blood samples). The abdomen was opened for insertion of microdialysis fibers. To prevent evaporation, polyethylene foil was placed over the opened neck and abdomen. Operation of animals and 60 min of microdialysis fiber perfusion without sampling (to reach steady state) were followed by a 30-min basal period (0–30 min). Then a 120-min hyperinsulinemic, euglycemic clamp (30–150 min) was started. At 120 min, the animals were given an intra-arterial bolus of 80 µCi of 2-DG. Blood samples for determination of 2-DG were obtained at 122, 125, 130, 140, and 150 min. Within 2 min after the last blood sample, the heart was cut out. Then tissue biopsies were taken, freeze-damped with aluminum tongs precooled in liquid nitrogen, and stored at –80°C until analysis. In the basal period and during the clamp, microdialysate was collected from adipose and muscle tissue in 300-µl capped glass tubes at 30-min intervals. Blood samples were drawn at 10 and 20 min (basal period) and 100 and 110 min (hyperinsulinemic, euglycemic clamp) for comparison of arterial plasma water and interstitial glucose and lactate concentrations. Dialysate sampling was delayed 8 min relative to sampling of arterial blood to compensate for the transit time in the microdialysis outlet tubing. This placed blood samples in the middle of the period in which the dialysate passed the dialysis membrane.

Microdialysis. Dialysis fibers were obtained from artificial dialysis kidneys (GFE18, Gambro, Lund, Sweden) with a molecular cutoff at 5,000 Da. A single fiber was glued at both ends to a nylon tube of 0.50 mm inner diameter and 0.63 mm outer diameter. The dialysis fiber per se was 1 cm long with a 0.20 mm inner diameter and a 0.22 mm outer diameter. With the use of 18-gauge cannulas, six fibers were placed in the left retroperitoneal, left parametrial, mesenteric, and neck subcutaneous adipose tissues and in the left tibialis anterior and left gastrocnemius muscles, respectively. The fibers were perfused at a rate of 1.5 µl/min with a high-precision pump (CMA 100, Carnegie Medicine, Solna, Sweden). The perfusate consisted of Ringer acetate with a glucose concentration of 3.0 mM and a lactate concentration of 0.5 mM. Because the exchange over the microdialysis membrane does not reach equilibrium, in vivo relative recovery (RR) for the fibers was determined by the internal reference technique by adding [³H]glucose and [¹⁴C]lactate to the perfusate (28). The glucose and lactate concentrations of dialysate were analyzed by a YSI 2300 (YSI, Yellow Springs, OH), and [³H]glucose and [¹⁴C]lactate were measured by liquid scintillation counting (2200CA, Packard Instrument) and corrected for background and cross talk. The RR was calculated as (dpmₐ - dpmₚ)/ dpmₚ, where dpmₚ is disintegrations per min in 10 µl of perfusate and dpmₚ is disintegrations per min in 10 µl of dialysate. RRs (means ± SE) in retroperitoneal, parametrial, mesenteric, and subcutaneous adipose tissue and in m. tibialis anterior and m. gastrocnemius were: 0.14 ± 0.02 (n = 9), 0.14 ± 0.02 (n = 9), 0.12 ± 0.02 (n = 8), 0.16 ± 0.02 (n = 8), 0.16 ± 0.02 (n = 9), and 0.15 ± 0.02 (n = 8) for trained rats and 0.16 ± 0.06 (n = 14), 0.18 ± 0.07 (n = 12), 0.18 ± 0.03 (n = 14), 0.17 ± 0.04 (n = 14), 0.20 ± 0.06 (n = 14), and 0.22 ± 0.07 (n = 12) for sedentary rats; lactate: 0.17 ± 0.02 (n = 9), 0.14 ± 0.02 (n = 9), 0.19 ± 0.02 (n = 8), 0.18 ± 0.03 (n = 8), 0.20 ± 0.02 (n = 9), and 0.19 ± 0.03 (n = 8) for trained rats and 0.18 ± 0.06 (n = 14), 0.21 ± 0.03 (n = 12), 0.18 ± 0.06 (n = 14), 0.19 ± 0.04 (n = 14), 0.24 ± 0.06 (n = 14), and 0.25 ± 0.06 (n = 12) for sedentary rats. RR did not differ significantly either between trained and sedentary rats or between tissues. Furthermore, RRs did not change significantly with time. Interstitial concentrations were calculated as [(Cₐ - Cₚ)/R] + Cₚ, where Cₐ is dialysate concentration and Cₚ is perfusate concentration.

Hyperinsulinemic, euglycemic clamp. The euglycemic clamp was performed as previously described (16). Insulin (Actrapid, Novo Nordic, Bagsvaerd, Denmark) was infused at a constant rate of 1.67 mU·kg body wt⁻¹·min⁻¹ via the venous catheter for 120 min by a high-precision pump (CMA 100, Carnegie Medicine). Blood glucose was maintained at the basal level by adjusting the rate of infusion of a 30% glucose solution in isotonic saline with pH adjusted to 7.7 according to blood glucose measurements performed at regular intervals (10 min) in samples taken from the arterial catheter. Blood samples (0.5 ml) were obtained for insulin determination in all experiments at 10, 60, 90, and 150 min. Blood samples for determination of hematocrit were obtained at 40, 90, and 120 min.

The blood drawn was replaced by red blood cells obtained by cardiac puncture of an anesthetized donor rat. Donor blood was 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 metabolites and hormones was sampled by heparinized syringes into iced tubes from the aortic arch and centrifuged immediately. Blood for determination of insulin was stabilized with 500 kallikrein-inhibitor units of aprotinin (Trasyrol) and 4 µmol EDTA/ml of blood. All plasma and microdialysate samples were kept at –20°C until analysis.

Glucose and lactate concentrations were determined by YSI 2300 (YSI). Insulin concentrations were determined by a commercially available RIA kit with rat insulin as standard (Linco Research, St. Charles, MO). Hematocrit was determined by an ABL 625 (Radiometer, Roedour, Denmark).

Blood flow. Blood flow was determined by use of the radioactive microsphere technique (18). Microspheres (mean size: 15.5 ± 0.1 µm; DuPont de Nemours, Mechelen, Belgium) labeled with either ¹⁴Ce (specific activity: 17.79 mCi/g) or ¹¹⁵Sn (specific activity: 8.62 mCi/g) in a suspension of 10% dextran containing 0.01% Tween-80 surfactant were mixed in an ultrasonicator (Branson 3200, Buch & Holm A/S, Herlev, Denmark) for 10 min and then on a vortex for 1–2 min before infusion to assure a uniform distribution of spheres as
determined by microscopic observations. Bolus injections (100 µl ¹⁴Ce and 50 µl ¹¹³Sn) were performed at 15 and 105 min, respectively. For each blood flow measurement, ~130,000 spheres (6.25 µCi ¹⁴Ce and 3.15 µCi ¹¹³Sn) were injected into the aortic arch and followed by a 0.5-ml isotonic saline wash. Injections lasted 30 s. 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 continued for 80 s. To verify that the microspheres were equally distributed, the coefficient of variation for blood flows in the right and left kidney was calculated. In the basal period, the coefficient of variation was 20 ± 4% in trained and 16 ± 3% in sedentary rats. During insulin infusion, the coefficient of variation was 11 ± 3% in trained and 13 ± 6% in sedentary rats. At the end of the experiment, animals were euthanized by the heart being cut out, and the following tissues were excised, weighed, and placed in counting vials and transferred to a gamma counter (Packard Auto-Gamma 5650, Packard Instrument): left retroperitoneal, left parametrical, and mesenteric intra-abdominal adipose tissues and in the neck subcutaneous adipose tissue and the tibialis anterior and gastrocnemius muscles of the left side. Reference blood samples were also counted at this time. Absolute blood flow (ml·100 g⁻¹·min⁻¹) to each tissue was calculated as \((dpm_f \times 0.05 \text{ ml·min}^{-1} \cdot \text{dpm}^{-1}) \times 100 \text{ wt(g)}\), where \(dpm_f\) is disintegrations per minute in tissue, \(dpm_{RBS}\) is disintegrations per minute in reference blood sample, and \(wt\) is tissue weight.

Determination of glucose metabolism in tissues by the 2-DG method. Right retroperitoneal, right parametrical, and neck subcutaneous adipose tissues and right tibialis anterior and right gastrocnemius muscles were weighed, homogenized in 2 ml of distilled water on ice with a polytron (PT 3100, Buch & Holm A/S), and boiled for 5 min to inactivate enzymes. The boiled homogenate was centrifuged, and 500 µl of the supernatant was resuspended in 200 µl of H₂O and 10 ml of Instagel in a polyethylene column containing 1 g of an anion exchange resin (Dowex 2 × 8, 100–200 mesh, Sigma) had been washed before the application of the test sample as described by Vissing et al. (39). The resin with test sample was eluted with 5 ml of distilled water followed by 10 ml of 0.2 M formic acid and 0.5 M ammonium acetate yielding 2-DG and 2-DG-6-phosphate (2-DG-6-P). Each chromatography column containing 1 g of an anion exchange resin (Dowex 2 × 8, 100–200 mesh, Sigma) was washed before the application of the test sample as described by Vissing et al. (39). The resin with test sample was eluted with 5 ml of distilled water followed by 10 ml of 0.2 M formic acid and 0.5 M ammonium acetate yielding 2-DG and 2-DG-6-P in the former and latter eluate, respectively. The first eluate was counted in a liquid scintillation analyzer as described for plasma samples. The second eluate was counted with 10 ml of Instagel (Packard Instrument). Plasma activities of 2-DG were determined by counting evaporated plasma samples (25 µl) resuspended in 200 µl of H₂O and 10 ml of Instagel in a liquid scintillation analyzer (2200CA, Packard Instrument).

Calculations. Adipose and muscle tissue glucose uptakes determined by microdialysis and microspheres were calculated from plasma and interstitial glucose concentrations and blood flow. Concentrations in venous plasma water \((C_{Ca})\) were calculated by the principle described by Intaglietta and Johnson (13). The calculation is based on Fick's law of diffusion for a thin membrane: \(J = -PS(C_C - C_a)\) where \(J\) is the substrate flux, \(P\) is the membrane permeability of the substrate, \(S\) is the membrane surface area, \(C_C\) is the concentration of substrate in the capillary, and \(C_a\) is the substrate concentration in interstitial water. If this equation is integrated over the entire length of a capillary, the following expression is obtained: \(C_{Ca} = [C_C - C_a]e^{-PSQ}\) giving: \(C_{Ca} = [C_C - C_a]e^{-PSQ} + C_a\), \(C_a\) is concentration in arterial plasma water, and \(Q\) is the blood water flow in which the metabolite is distributed. The PS product was set to 2 ml·100 g⁻¹·min⁻¹ for glucose in adipose tissue, because values in this range have been found for molecules of similar sizes (19). The PS product was set to 5 ml·100 g⁻¹·min⁻¹ in skeletal muscle (7). The PS products were assumed to be constant within the range of blood flow variations registered and not to differ between trained and sedentary rats. Glucose uptake was calculated as the product of the difference between \(C_C\) and \(C_a\) and the blood water flow in which the metabolite is distributed. This blood water flow is the plasma water flow plus \(k\) erythrocyte flow, where \(k\) was taken to be 0.67 for glucose (30).

Glucose metabolic rate in individual tissues was determined by 2-DG as previously described (39): \(R_g = C_f \sum(C_f/C_a) dt\), where \(R_g\) is glucose metabolic rate \((\mu\text{mol·}100\text{ g}^{-1} \cdot \text{min}^{-1})\). \(C_f\) is accumulated 2-DG-6-P in individual tissues \((\text{dpm} / 100\text{ g wet wt})\) at \(t = 30\text{ min}\), \(C_a\) is plasma 2-DG activity \((\text{dpm} / \text{ml})\), and \(C_p\) is plasma glucose concentration \((\mu\text{mol} / \text{ml})\).

Statistics. All data are presented as means ± SE. A two-way repeated measures ANOVA was used to test if arterial plasma insulin, glucose infusion rate, arterial blood glucose, RR, and interstitial and plasma metabolite concentrations and blood flow differed with time or between trained and sedentary rats. The Student-Newman-Keuls test was used as post hoc test. A one-way repeated measures ANOVA was used to test if interstitial and plasma metabolite concentrations, glucose uptake rates, and blood flow in the basal period and during insulin infusion at 90–120 min differed among different tissues. The Student-Newman-Keuls test was used as post hoc test. Due to microdialysis catheter failure over time and premature death of some rats, the number of rats decreased over time. This was compensated for in the statistics by using a general linear model approach. 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 if anthropometric data and glucose uptake rates differed between trained and sedentary rats and if glucose uptake differed between the 2-DG and microdialysis methods. A significance level of 0.05 in two-tailed testing was chosen a priori.

RESULTS

Characteristics of experimental animals. Body weight did not differ between trained and sedentary rats either before or after 15 wk of swim training \((P > 0.05; \text{Table 1})\). Heart weight-to-body weight ratio was, however, significantly higher in trained than in sedentary rats. Weights of retroperitoneal, parametrical, and mesenteric adipose tissues were significantly lower in trained compared with sedentary rats. Water content did not

Table 1. Anthropometric data for rats

<table>
<thead>
<tr>
<th></th>
<th>Trained</th>
<th>Sedentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, before, g</td>
<td>96 ± 1</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>BW, after, g</td>
<td>236 ± 7</td>
<td>247 ± 6</td>
</tr>
<tr>
<td>HW, g</td>
<td>0.82 ± 0.03†</td>
<td>0.75 ± 0.02</td>
</tr>
<tr>
<td>HW/BW, %</td>
<td>0.35 ± 0.01*</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>Retroperitoneal AT wt, mg</td>
<td>146 ± 25</td>
<td>355 ± 31</td>
</tr>
<tr>
<td>Parametrial AT wt, mg</td>
<td>164 ± 25</td>
<td>377 ± 35</td>
</tr>
<tr>
<td>Mesenteric AT wt, mg</td>
<td>86 ± 13*</td>
<td>141 ± 21</td>
</tr>
</tbody>
</table>

Values are means ± SE for 9 trained and 16 sedentary rats. BW, before and BW, after: body weights before and after swim training or equivalent period of sedentary life, respectively; HW: heart weight after these periods; AT: adipose tissue. Retroperitoneal and parametrial AT weights are for right fat pads. *P < 0.05 vs. sedentary rats. †P = 0.08 vs. sedentary rats.
differ between trained (n = 10) and sedentary (n = 10) rats either in muscle (0.77 ± 0.01 vs. 0.75 ± 0.01 ml/g, respectively; P > 0.05) or in adipose tissue (0.28 ± 0.05 vs. 0.16 ± 0.02 ml/g, respectively; P > 0.05). Hematocrit was lower in trained compared with sedentary rats (P = 0.04) but did not change (P > 0.05) during the course of the experimental protocol in any of the groups [trained: 40 min: 40 ± 1 (n = 9), 90 min: 41 ± 1 (n = 7), and 120 min: 38 ± 1% (n = 7); sedentary: 40 min: 42 ± 1 (n = 14), 90 min: 43 ± 1 (n = 11), and 120 min: 42 ± 2% (n = 3)].

Euglycemic, hyperinsulinemic clamp. Arterial blood glucose concentration was constant during clamping and identical in trained and sedentary rats (Table 2). At no time was there any difference in insulin concentration between trained and sedentary rats (Table 2). During insulin infusion, glucose infusion rate increased with time in both trained and sedentary rats (P < 0.05) and was significantly higher in the former than in the latter (Table 2).

Interstitial and plasma water glucose concentrations and glucose uptake calculated from microdialysis. Glucose concentrations in arterial plasma water did not differ significantly either with time or between groups (Fig. 1). Interstitial glucose concentrations decreased significantly after start of insulin infusion in all adipose tissues in trained rats, but in sedentary rats the interstitial glucose concentration decreased significantly only in subcutaneous adipose tissue. Accordingly, in all adipose tissues, interstitial glucose concentrations attained lower values in trained compared with untrained rats during insulin infusion; however, significance was only achieved in mesenteric adipose tissue. In the basal period, glucose uptake per 100 g of adipose tissue did not differ significantly between trained and sedentary rats [retroperitoneal adipose tissue: 7 ± 1 (n = 8), parametrial adipose tissue: 7 ± 2 (n = 8), mesenteric adipose tissue: 7 ± 2 (n = 6), and subcutaneous adipose tissue: 6 ± 1 µmol·100 g⁻¹·min⁻¹ (n = 5) in trained rats and 7 ± 1 (n = 12), 6 ± 1 (n = 10), 4 ± 1 (n = 12), and 2 ± 1 (n = 14) in sedentary rats]. However, during insulin infusion in all adipose tissues, glucose uptake per 100 g of adipose tissue tended to be at least twofold higher in trained than in sedentary rats (P < 0.01, Fig. 2, top), but significance was reached only in retroperitoneal adipose tissue. Also, in muscle the insulin-induced decrease in interstitial glucose concentration tended to be higher in trained than in sedentary rats. However, both in the basal period (m. tibialis anterior: 4 ± 2 (n = 8) and m. gastrocnemius: 4 ± 1 µmol·100 g⁻¹·min⁻¹ (n = 6) in trained rats and 5 ± 1 (n = 13) and 2 ± 1 (n = 13) in sedentary rats, respectively) and during insulin infusion (Fig. 2, top) glucose uptake per 100 g of muscle tissue did not differ significantly between trained and sedentary rats. Glucose uptake per 100 g of adipose and muscle tissue did not differ between the basal and insulin infusion periods in either trained or sedentary rats (P > 0.05).

In the basal period, the interstitial glucose concentration was higher in subcutaneous adipose tissue than in other adipose tissues (P < 0.05), and during insulin infusion the interstitial glucose concentration was higher in mesenteric and subcutaneous adipose tissues than in parametrial adipose tissue (P < 0.05; Fig. 1). Interstitial glucose concentrations did not differ between muscles either in the basal period or during insulin infusion (P > 0.05).

2-DG uptake rates. Disappearance of 2-DG from plasma was faster in trained compared with sedentary rats (decrease of 2-DG in plasma from 5 to 30 min after injection: trained: 82 ± 3%; sedentary: 52 ± 7%; P < 0.005). In all examined adipose (retroperitoneal, parametrial, and subcutaneous) and muscle (tibialis anterior and gastrocnemius) tissues, glucose uptake per 100 g of tissue during insulin infusion was ~450% higher in trained than in sedentary rats (P < 0.05; Fig. 2, bottom). No significant differences were found in glucose uptake rates between adipose tissues.

Table 2. Arterial plasma insulin and blood glucose concentrations

<table>
<thead>
<tr>
<th></th>
<th>Basal (n)</th>
<th>60 min (n)</th>
<th>90 min (n)</th>
<th>150 min (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin, pM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained</td>
<td>120 ± 18 (9)</td>
<td>1115 ± 252† (9)</td>
<td>1248 ± 327† (8)</td>
<td>1233 ± 280† (7)</td>
</tr>
<tr>
<td>Sedentary</td>
<td>123 ± 14 (16)</td>
<td>804 ± 138† (13)</td>
<td>1830 ± 732† (8)</td>
<td>908 ± 410† (6)</td>
</tr>
<tr>
<td><strong>Glucose, mM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained</td>
<td>4.8 ± 0.2 (9)</td>
<td>5.0 ± 0.2 (9)</td>
<td>4.8 ± 0.2 (8)</td>
<td>5.0 ± 0.2 (7)</td>
</tr>
<tr>
<td>Sedentary</td>
<td>4.6 ± 0.1 (15 or 16)</td>
<td>5.3 ± 0.4 (13)</td>
<td>5.0 ± 0.3 (10)</td>
<td>4.8 ± 0.4 (6)</td>
</tr>
<tr>
<td>GI R, µmol·100 g⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained</td>
<td>4.0 ± 0.5* (6)</td>
<td>6.5 ± 0.9‡ (7)</td>
<td>7.2 ± 1.5*‡</td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>2.7 ± 0.3</td>
<td>4.2 ± 0.7†</td>
<td>3.7 ± 0.9‡</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of arterial plasma insulin and blood glucose concentrations for trained and sedentary rats in basal period (glucose: mean of 5 samples) and of arterial plasma insulin, blood glucose concentrations, and glucose infusion rates (GI R) for trained and sedentary rats during insulin infusion (60, 90, and 150 min). *P < 0.05 vs. sedentary rats. †P < 0.05 vs. the basal period. ‡P < 0.05 vs. 60 min.
Blood flow. Blood flow per 100 g of adipose tissue was generally higher in trained than in sedentary rats both in the basal period and during insulin infusion (Fig. 4). Furthermore, adipose tissue blood flow decreased during insulin infusion in both trained and sedentary rats. Blood flow per 100 g of muscle tissue did not differ between trained and sedentary rats (P > 0.05) and did not change significantly with time.

In the basal period, mesenteric adipose tissue blood flow was significantly higher than blood flow in other adipose tissues and muscles in both trained and sedentary rats. During insulin infusion, mesenteric adipose tissue blood flow was significantly higher than blood flow in retroperitoneal and subcutaneous adipose tissues and in m. tibialis anterior of trained rats.

**DISCUSSION**

A major new finding of the present study is that exercise training enhances insulin-stimulated glucose uptake per 100 g of adipose tissue in vivo in a number of adipose tissues, including intra-abdominal adipose tissues (Fig. 2). Furthermore, insulin-stimulated glucose uptake does not differ between the various adipose tissues (Fig. 2). Finally, insulin decreases blood flow in most adipose tissues examined, whereas training increases basal blood flow in all and insulin-stimulated blood flow in most intra-abdominal adipose tissue depots (Fig. 4).

The insulin infusion used in the present study resulted in a plasma insulin concentration that previously has been found to be nearly maximally effective with respect to glucose disposal in adipose tissue (17). In the present study, during insulin infusion, the glucose infusion rate necessary to maintain euglycemia was higher in trained than in sedentary rats. Thus, in accordance with previous findings (14), trained rats had a higher whole body response to insulin than sedentary rats. As judged from findings with the 2-DG...
method, this was, as one would expect (10, 14), partly due to a higher response to insulin in skeletal muscle of trained compared with sedentary rats (Fig. 2, bottom). However, it is a new finding that training also increases insulin-stimulated glucose uptake per 100 g of tissue in vivo in many adipose tissues, including intra-abdominal adipose tissues. Both 2-DG uptake measurements (Fig. 2, bottom) and microdialysis-microsphere data (Fig. 2, top) support this conclusion. During insulin infusion, interstitial glucose concentrations in adipose tissue were lower in trained compared with sedentary rats (Fig. 1). A low interstitial glucose concentration can either be due to a high glucose uptake in the tissue or to a low supply of glucose by the blood. Glucose supply equals blood flow multiplied by arterial glucose concentration. Because adipose tissue blood flow per 100 g of tissue was higher in trained compared with sedentary rats (Fig. 4) and because arterial glucose concentrations did not differ between trained and sedentary rats (Fig. 1; Table 2), trained rats did not have a lower supply of glucose by the blood than sedentary rats. Accordingly, the raw microdialysis data indicate that glucose uptake in adipose tissues is higher in trained compared with sedentary rats. This simple reasoning was corroborated by calculations of glucose uptake based on microdialysis and flow data and requiring various assumptions (Fig. 2, top; Refs. 13, 33, 35). Moreover, the fact that the interstitial glucose concentration decreases more in trained than in sedentary rats during insulin infusion despite a higher blood flow in the trained rats shows that glucose delivery becomes less well matched to glucose uptake with training (Figs. 1 and 4).

Only a few studies have previously been performed in which the effect of exercise training on insulin action in
adipose tissue was examined in vivo. James et al. (14) found that during a hyperinsulinemic, euglycemic clamp, insulin-stimulated glucose uptake determined by the 2-DG method was approximately twofold higher in epididymal adipose tissue in trained compared with sedentary rats. The effect of exercise training on insulin action in inguinal, subcutaneous adipose tissue in rats has previously been studied by the microdialysis technique (41). In that study, only dialysate lactate concentrations were measured. In accordance with our study, dialysate lactate concentration increased with insulin and did not differ between trained and sedentary rats. However, the present study has added the information that arterial and interstitial lactate concentrations do not differ during the conditions studied (Fig. 3). The lack of coordination with glucose uptake rates leads to the conclusion that it must be questioned whether dialysate lactate concentration is a reliable measure of local adipose tissue metabolism.

It may not be possible to predict glucose uptake in vivo from in vitro findings. However, in agreement with the present findings, it has been shown in vitro that exercise training increases insulin action in both epididymal (37, 38) and parametrial (4, 11) adipose tissue. The various steps at which training increased insulin-mediated glucose uptake (up to 6-fold; Ref. 4) included insulin binding to adipocytes (up to 2.5-fold; Refs. 4, 38), glucose transport (up to 3-fold; Refs. 11, 31, 38), amount of GLUT-4 protein (up to 4-fold; Refs. 11, 31) and mRNA (3-fold; Ref. 31), glucose oxidation (up to 6-fold; Ref. 4), and glucose incorporation into lipids (up to 6-fold; Ref. 38).

In the present study, insulin-stimulated glucose uptake expressed per 100 g of adipose tissue did not differ
between the various adipose tissue depots (Fig. 2). Accordingly, a difference in insulin sensitivity between adipose tissues does not seem to contribute to the fact that the insulin-resistance syndrome is associated with intra-abdominal rather than subcutaneous obesity (15). However, the finding that training increases insulin sensitivity also in intra-abdominal adipose tissue supports the view that training may be beneficial during conditions in which the intra-abdominal fat mass is increased (3).

Only few studies have examined if insulin action differs between various adipose tissue depots. Total in vitro insulin-stimulated glucose metabolism (sum of CO₂, triglyceride, and lactate production) has been found to be higher in mesenteric and retroperitoneal than in subcutaneous and epididymal adipocytes in rats weighing 140–180 g (23). The antilipolytic effect of insulin was lower in omental and mammary adipocytes than in subcutaneous adipocytes in vitro (26). In line with our findings (Fig. 2, bottom), a recent study (27) has reported no difference in insulin-stimulated 2-DG uptake between retroperitoneal and epididymal adipose tissue in vivo, whereas insulin-stimulated 2-DG uptake was approximately seven times higher in muscles (including tibialis and gastrocnemius muscles) than in adipose tissues (27).

There are uncertainties connected with all methods used in research. In the present study, we used two different methods for measuring glucose uptake, the 2-DG and the microdialysis-microsphere methods, to make conclusions more robust. Absolute glucose uptake rates were lower when determined by the 2-DG than by the microdialysis-microsphere method (Fig. 2, bottom). The quantitative difference between the two methods is probably, in particular, due to the fact that microdialysis calculations are based on many assumptions and variables, each of which may be inaccurately assessed (13, 33, 35). The most important assumption is that the PS for glucose is known. In accordance with this view, evaluation of the microdialysis technique for metabolic studies in adipose tissue has led to the conclusion that it may be advisable to make interpretations from the directly measured variables, e.g., interstitial and arterial plasma concentrations and blood flow, rather than relying on the composite uptake-output calculations (33). In contrast to the 2-DG method (Fig. 2, bottom) in the present study, the microdialysis technique was not able to detect a training-induced increase in insulin-stimulated glucose uptake in muscle (Fig. 2, top). When calculating glucose uptake in muscle, we used a PS of 5 ml·100 g⁻¹·min⁻¹ in both trained and sedentary rats. Endurance training, however, increases capillary surface area (12) and possibly also increases PS in muscle. If PS was higher in muscle from trained compared with muscle from sedentary rats, then the microdialysis-microsphere technique would have also found a training-induced increase in insulin-stimulated glucose uptake in muscle. In line with such an
increase (10), insulin-mediated lactate output was higher in trained compared with untrained tibialis anterior muscle as judged from interstitial and arterial lactate concentrations and blood flow (Figs. 3 and 4).

Basal parametrial adipose tissue blood flow measured by the microsphere method in sedentary rats (9.4 ± 2.0 ml·100 g⁻¹·min⁻¹) was very close to previously reported values obtained by the ¹³³Xenon-washout method (8.2 ± 0.6 ml·100 g⁻¹·min⁻¹; Ref. 20). Furthermore, in agreement with previous studies on conscious rats, basal mesenteric adipose tissue blood flow was two-to-three times as high as blood flow in other adipose tissues (6). 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 (21). In contrast, we found with the same method that trained rats have a higher basal adipose tissue blood flow per 100 g of adipose tissue than sedentary rats (Fig. 4). This finding is in accordance with previous observations with the ¹³³Xenon-washout method in human abdominal subcutaneous adipose tissue (35). In the present study, adipose tissue blood flow decreased during insulin stimulation in both trained and sedentary rats (Fig. 4). In agreement with this, it has previously been demonstrated with the ¹³³Xenon-washout method that hyperinsulinemia accompanied by hyperglycemia caused a reduction in adipose tissue blood flow by 38% in fasted rats (20). Indicating that the effect of insulin on adipose tissue blood flow differs between rat and man abdominal subcutaneous adipose tissue, blood flow does not change during a hyperinsulinenic, euglycemic clamp in humans (34). In line with this, in response to a meal, adipose tissue blood flow decreases in rats (40) but increases in humans (29). Insulin has been shown to enhance muscle blood flow in humans (8). The fact that insulin did not influence muscle blood flow in the present study of rats may also reflect a species difference or may reflect that the rats were anesthetized.

A swimming protocol similar to the one used in the present study has in previous studies proven to be effective with respect to endurance training of rats by increasing heart weight/relative heart weight (24, 31, 32, 36), cytochrome c oxidase activity in skeletal muscle (24), and adipose tissue (36) and 3-O-methylglucose transport capacity in skeletal muscle (24) and adipose tissue (31). In the present study, relative heart weight was increased and absolute heart weight tended to be increased in trained compared with sedentary rats (Table 1). We do not believe that the rats are stressed by the water exposure because we previously have found that adrenal gland weight did not increase in rats handled like swim-trained rats but swimming for only 2 min/day (sham training) (32). Rats could be exposed to cold either in the water or during drying after swimming. We have previously examined if rats trained by our swimming protocol were exposed to cold by measuring rectal temperature before and after the swimming session. We found that rectal temperature did not change either during swimming or drying (36).

A further indication that the increased glucose uptake in adipose tissue found in the present study is due to exercise training per se and not to stress or cold exposure is that we previously have found that enzyme activities in adipose tissue are increased by our training protocol but not by sham training or cold stress (4°C for 10 wk) (36).

One of the adaptations to physical training is morphological changes in adipose tissue. In a previous study on male rats in which we used a training protocol similar to the one used in the present study (31), adipocyte volume was 176 ± 5 pl in swim-trained rats compared with 668 ± 36 pl in sedentary rats. Also, in female rats, as were used in the present study, Craig et al. (5) found adipocyte volumes of swim-trained rats to be less than adipocyte volumes of sedentary rats (98 ± 9 vs. 275 ± 19 pl). Both in the present (Table 1) and in a previous study (31), weight of adipose tissue was lower in trained than in sedentary rats. Whether the increased adipose tissue blood flow (Fig. 4) and the increased insulin-stimulated glucose uptake (Fig. 2) in trained compared with sedentary rats were due to training per se or the morphological changes created by the training cannot be elucidated from the present study. In the present study, blood flow and glucose uptake are expressed per 100 g of tissue. Differences between trained and sedentary rats would probably be diminished if data were expressed per number of adipocytes.

In conclusion, insulin-stimulated glucose uptake is similar in subcutaneous and various intra-abdominal adipose tissues. Accordingly, differences between adipose tissues in insulin sensitivity with respect to glucose uptake cannot explain that insulin resistance is associated with intra-abdominal rather than subcutaneous obesity. Training increases insulin-stimulated glucose uptake per 100 g of adipose tissue in both subcutaneous and intra-abdominal adipose tissues. The effect of training on the latter fat stores may be important in the treatment of the insulin-resistance syndrome. Finally, insulin decreases blood flow in rat adipose tissue while training increases intra-abdominal adipose tissue blood flow per 100 g of adipose tissue both in the basal state and during hyperinsulinemia.

We thank Regitze Kraunsæ and Lisbeth Kall for skilled technical assistance.

The study was supported by grants from the Danish National Research Foundation (504–14), the Novo Nordisk Foundation, the Danish Diabetes Association, Weimann’s Foundation, and the Copenhagen Hospital Corporation.

Address for reprint requests and other correspondence: B. Staliknekht, Department of Medical Physiology, The Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark (E-mail: B.Staliknecht@nfit.ku.dk).

Received 21 January 1999; accepted in final form 24 August 1999.

REFERENCES


EFFECT OF TRAINING ON INTRA-ABDOMINAL ADIPOSE TISSUE


