Effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue

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Stallknecht, Bente, Jens J. Larsen, Kari J. Mikines, Lene Simonsen, Jens Bülow, and Henrik Galbo. Effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue. Am J Physiol Endocrinol Metab 279: E376–E385, 2000.—Training increases insulin sensitivity of both whole body and muscle in humans. To investigate whether training also increases insulin sensitivity of adipose tissue, we performed a three-step hyperinsulinemic, euglycemic clamp in eight endurance-trained (T) and eight sedentary (S) young men [insulin infusion rates: 10,000 (step I), 20,000 (step II), and 150,000 (step III) µU·min⁻¹·m⁻²]. Glucose and glycerol concentrations were measured in arterial blood and also by microdialysis in interstitial fluid in periumbilical, subcutaneous adipose tissue and in quadriceps femoris muscle (glucose only). Adipose tissue blood flow was measured by 133Xe washout. In the basal state, adipose tissue blood flow tended to be higher in T compared with S subjects, and in both groups blood flow was constant during the clamp. The change from basal in arterial-interstitial glucose concentration difference was increased in T during the clamp but not in S subjects in both adipose tissue and muscle [adipose tissue: step I (n = 8), 0.48 ± 0.18 mM (T), 0.23 ± 0.11 mM (S); step II (n = 8), 0.19 ± 0.09 (T), −0.09 ± 0.24 (S); step III (n = 5), 0.47 ± 0.24 (T), 0.06 ± 0.28 (S); (T: P < 0.001; S: P > 0.05); muscle: step I (n = 4), 1.40 ± 0.46 (T), 0.31 ± 0.21 (S); step II (n = 4), 1.14 ± 0.54 (T), −0.08 ± 0.14 (S); step III (n = 4), 1.23 ± 0.34 (T), 0.24 ± 0.09 (S); (T: P < 0.01, S: P > 0.05)]. Interstitial glycerol concentration decreased faster in T than in S subjects [half-time: T, 44 ± 9 min (n = 7); S, 102 ± 23 min (n = 5); P < 0.05]. In conclusion, training enhances insulin sensitivity of glucose uptake in subcutaneous adipose tissue and in skeletal muscle. Furthermore, interstitial glycerol data suggest that training also increases insulin sensitivity of lipolysis in subcutaneous adipose tissue. Insulin per se does not influence subcutaneous adipose tissue blood flow.

microdialysis; blood flow; glycerol; skeletal muscle; exertion

IN HUMANS, INSULIN-MEDIATED glucose uptake is increased by training in whole body (30, 33) and in skeletal muscle (9, 11). However, the increased glucose clearance in the trained muscles cannot fully account for the increase in whole body glucose clearance, and, accordingly, insulin action must increase in response to training in other tissues as well (9). In rats, insulin-stimulated 2-deoxyglucose uptake in adipose tissue is higher in trained compared with sedentary animals both in vitro (7) and in vivo (21). Furthermore, adipocytes from trained rats have a higher content of insulin-sensitive glucose transporters (GLUT-4) and a higher insulin-stimulated glucose transport in vitro than adipocytes from sedentary subjects (36). In humans, insulin-stimulated [14C]glucose uptake has been shown to be higher in adipocytes from trained compared with sedentary subjects in vitro (33). However, it is not known whether physical training increases insulin-stimulated glucose uptake in intact human adipose tissue in vivo.

During exercise, free fatty acids (FFA), derived from lipolysis of triglycerides (TG) in adipose tissue, are combusted. Between exercise sessions, adipose tissue TG stores must be replenished by esterification of FFA and glycerol 3-phosphate. The sources of FFA are intracellular FFA derived from previously hydrolyzed TG and FFA derived from hydrolysis of circulating lipoproteins and chylomicrons by lipoprotein lipase. Glycerol 3-phosphate is derived from glycolysis in the adipocyte. Accordingly, a training-induced enhancement of insulin-mediated glucose uptake in adipose tissue might enhance replenishment of the TG stores.

In the present study, we examined the effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue. Glucose and glycerol concentrations were measured in arterial blood and by microdialysis also in interstitial fluid of periumbilical, subcutaneous adipose tissue during a three-step hyperinsulinemic, euglycemic clamp in endurance-trained and sedentary young male subjects. To estimate glucose uptake and glycerol release, adipose tissue blood flow must be known also. It is controver-
ial whether insulin per se changes adipose tissue blood flow (15–17, 23, 34, 40), so adipose tissue blood flow was measured by 133Xe washout.

METHODS

Subjects. Eight sedentary males and eight male athletes (4 orienteers, 1 triathlete, 1 marathon runner, 1 canoeist, and 1 cyclist) gave their written consent according to the declaration of Helsinki II to participate in the study, which was approved by the Ethics Committee for Medical Research in Copenhagen (KF 01–089/95). None of the subjects had a family history of diabetes, and none was taking any medications. Subjects were regarded sedentary if they did not participate in any regular exercise and their peak O2 uptake (VO2peak) determined during cycling was <50 ml · kg⁻¹ · min⁻¹. Subjects were regarded trained if they competed in elite-class endurance sports and their VO2peak was >60 ml · kg⁻¹ · min⁻¹. Anthropometric data for the two groups are shown in Table 1. On the day before the experiment, subjects avoided strenuous physical activity, and, accordingly, subjects had not performed exercise for at least 40 h before the hyperinsulinemic clamp was started. After an overnight fast and abstention from coffee, tea, and tobacco, the subjects were brought to the laboratory at 0800 where they were weighed and had body height and abdominal skinfold thickness measured. The subjects voided (urine being discarded) and fasted supine at a room temperature of 21°C.

Microdialysis. Microdialysis was performed in principle as described previously (38). Microdialysis probes were made of dialysis fiber per se was 4 cm (adipose tissue) or 2 cm (skeletal muscle). A single fiber was glued at both ends to a nylon tube with a diameter of 0.50/0.63 mm (inner/outer). The dialysis fiber per se was 4 cm (adipose tissue) or 2 cm (muscle) long and had a diameter of 0.20/0.22 mm (inner/outer). At 0830, five microdialysis probes were placed in the abdominal subcutaneous adipose tissue transversal and in parallel ~1.5 cm apart with the most medial point ~4 cm laterally to the navel by use of 18-gauge cannulas. Of these probes, three were used for determination of interstitial glucose and lactate concentrations and two were used for determination of the interstitial glycerol concentration. In four trained and four sedentary subjects, two microdialysis probes were also inserted in the vastus lateralis portion of the quadriceps femoris muscle in a direction parallel to the muscle fiber orientation for determination of skeletal muscle interstitial glucose and lactate concentrations. The skin was anesthetized by 0.05 ml lidocaine (10 mg/ml) at the sites of perforation. After placement of probes, perfusion was started, and after 60 min the experiment was initiated. All probes were perfused at a rate of 1.5 µl/min using a high-precision syringe pump (CMA 100; Carnegie Medicine, Solna, Sweden), and dialysate was collected in 300-µl capped glass tubes at 30-min intervals. Dialysate sampling was delayed by 6 min relative to the rest of the experimental protocol to compensate for the transit time in the outlet tubing. The in vivo relative recovery (RR) was determined by the internal reference calibration technique (35). In probes used for determination of glucose and lactate, perfusate consisted of Ringer acetate with 3 mM glucose, 1 mM lactate, 6 nM [3H]glucose (specific activity: 1850 GBq/mmol; NEN), and 2.8 µM [14C]lactate (specific activity: 4.0 GBq/mmol; NEN). In probes used for determination of glycerol, perfusate consisted of sodium chloride with 25 µM glycerol and 1.5 µM [3H]glycerol (specific activity: 7.4 GBq/mmol; NEN). The RR was calculated as (dpmp - dmpd)/dpmp, where dpm and dpmd are disintegrations per minute in perfusate and dmpd are disintegrations per minute in dialysate. In probes in which RR was <2%, probes were excluded from the study. Throughout the entire study, this comprised three probes in adipose tissue and two probes in skeletal muscle. In adipose tissue, mean RR values for the remaining functioning probes were 34 ± 2% (mean ± SE, n = 38 probes) for glucose, 38 ± 2% (n = 38) for lactate, and 71 ± 2% (n = 21) for glycerol. In skeletal muscle, mean RR values were 28 ± 2% (n = 13) for glucose and 33 ± 2% (n = 13) for lactate. There were no significant differences between mean RR values in trained and sedentary subjects. The RR values for glucose and lactate did not change significantly with time either in adipose tissue or in muscle, but RR for glycerol in adipose tissue increased with time from 66 ± 3% in the basal period to 77 ± 3% at time 330–360 min (P < 0.0001). Interstitial concentrations were calculated as (Cd - Cp)/RR + Cp, where Cd is dialysate concentration and Cp is perfusate concentration.

Catheterization. In local analgesia (1 ml lidocaine, 10 mg/ml), a catheter (Ohmeda, Swindon, UK) was introduced percutaneously in the radial artery in the nondominant arm for blood sampling and continuous measuring of blood pressure and heart rate. The catheter was kept patent by isotonic saline delivered by an automatic flushing device (MX9504; Medex Medical, Lancashire, UK). Additionally, the subjects had a catheter (Venflon, Vigo, Sweden) inserted in a medial antecubital vein for later infusion of glucose and insulin.

Hyperinsulinemic, euglycemic clamp. Before the start of a three-step (designated steps I, II, and III) sequential hyperinsulinemic, euglycemic clamp, the basal plasma glucose concentration was determined as the mean of the glucose concentration in three arterial blood samples. For each subject, a 50-ml infusate had been prepared for each clamp step from rapidly acting insulin (100 IU/ml Actrapid; Novo Nordisk, Copenhagen, Denmark), isotonic saline, and 2 ml of the subject’s blood. At each clamp step, insulin was given as a 2-ml bolus followed by a constant infusion for 120 min at a rate of 258 µg/min using an IVAC P4000 syringe pump (IVAC, Hampshire, UK). Insulin infusion rates were 10,000 (step I) and 20,000 (step II) µU · min⁻¹ · m⁻² in the first three trained and first three sedentary subjects studied. These subjects, however, showed only modest decreases in interstitial glucose concentration at the physiological insulin concentrations thereby achieved. Consequently, a clamp step resulting in a supraphysiological insulin concentration (150,000 µU · min⁻¹ · m⁻², step III) was added to the protocol in the last five trained and last five sedentary subjects. During the clamp, arterial plasma glucose concentration was measured.

Table 1. Anthropometric data

<table>
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<tr>
<th>Age, yr</th>
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<th>Sedentary</th>
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<tr>
<td>25 ± 1</td>
<td>26 ± 1</td>
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<tr>
<td>Weight, kg</td>
<td>72 ± 2</td>
<td>79 ± 5</td>
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<tr>
<td>Height, cm</td>
<td>178 ± 1</td>
<td>180 ± 2</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>22.8 ± 0.5</td>
<td>24.3 ± 1.2</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>9 ± 1</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Abdominal skinfold, mm</td>
<td>8 ± 1</td>
<td>19 ± 3</td>
</tr>
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</table>

Values are means ± SE for 8 trained and 8 sedentary subjects. VO2peak: peak oxygen uptake during cycling. *P < 0.05 vs. sedentary subjects.
at least every 5 min by an automated glucose analyzer (YSI 2300; YSI, Yellow Springs, OH). On the basis of the glucose concentration and a modification (29) of the algorithm of DeFronzo et al. (8), a computer-adjusted glucose infusion (20%) was given using Harvard Peristaltic Pump 66 (Harvard Apparatus, Holliston, MA) to maintain plasma glucose concentration at the basal level throughout the clamp. The plasma glucose concentration had a coefficient of variation of 4 ± 1, 4 ± 1, and 8 ± 2% at the three clamp steps. Potassium was added to the glucose infusate (30 mmol/l) and also was given orally (Kaleorid; Leo, Ballerup, Denmark) at 0800 (20 mmol), at the start of the clamp (20 mmol), and at the start of each clamp step (40 mmol) to avoid hypokalemia. To check that serious hypokalemia did not develop, the plasma potassium concentration was measured during the experiment. At the end of each clamp step, the subjects tried to void, and urine was collected for determination of urea nitrogen. After the last clamp step, glucose had to be infused for another 1.25 h until glucose metabolism had normalized.

Blood flow. Subcutaneous adipose tissue blood flow was measured by the local 133Xe washout method (38) in the region contralateral to the region in which dialysis was performed. Adipose tissue blood flow was calculated using individual lambda values estimated from the thickness of the abdominal skinfold (3).

Indirect calorimetry. Whole body O2 uptake (V\(\dot{O}_2\)) and CO2 production (V\(\dot{CO}_2\)) were measured in the basal state and during the last 30 min of each clamp step using an open-circuit ventilated hood system (Oxycon Champion; Jaeger, Würzburg, Germany). Calibration of the system was done by combustion of a known amount of 99.6% ethanol. Calculations of glucose and lipid oxidation and glycogen and lipid synthesis were done from measurements of whole body V\(\dot{O}_2\) and V\(\dot{CO}_2\) and from urinary excretion of urea nitrogen (corrected for changes in the urea pool).

Sampling. Blood for determination of metabolites and hormones was sampled in iced tubes from the radial artery and immediately centrifuged. Blood was sampled at time 7 and 22 min of the 30-min basal period and at time 97 and 112 min of each clamp step. Blood for determination of glucose, lactate, glycerol, β-hydroxybutyrate, TG, and urea was stabilized with 20 IU heparin; blood for determination of FFA and insulin was stabilized with 500 kallikrein inhibitory units of aprotinin (Trasylol) and 1.5 mg EDTA; blood for determination of catecholamines was stabilized with 4 μmol reduced glutathione and 20 IU heparin per milliliter of blood. All blood, microdialysate, and urine samples were kept at −20°C until analysis, except samples for FFA and catecholamines, which were kept at −80°C.

Analyses. Plasma and microdialysate glucose and lactate concentrations were determined by a YSI 2300 glucose/lactate analyzer. Plasma concentrations were converted into plasma water concentrations by dividing by 0.94. After precipitation of plasma proteins with perchloric acid, plasma water and microdialysate glycerol concentrations were determined by fluorometry (12) adapted to a Monarch centrifugal analyzer (Instrumentation Laboratory; Warrington, Cheshire, UK). Plasma FFA concentrations were determined using a commercial enzymatic kit (Wako) adapted to the Monarch centrifugal analyzer, plasma TG concentrations were determined by spectrophotometry (20) adapted to the Monarch centrifugal analyzer, and plasma β-hydroxybutyrate concentrations were determined by fluorometry (13). Urea concentrations in plasma and urine were determined spectrophotometrically as previously described (10). Plasma insulin concentrations were determined using a commercial ELISA kit (DAKO Diagnostics, Cambridgeshire, UK). Cate-

cholamine concentrations were determined by a previously described single-isotope radioenzymatic assay (25). Plasma potassium concentration was determined by an ABL 505 (Radiometer).

Dual-energy X-ray absorptiometry scanning. On a separate day, subjects were scanned with dual-energy X-ray absorptiometry (DEXA) (Hologic 1000W, software version 5.61; Hologic, Waltham, MA) to determine body composition.

Statistics. The computer program SigmaStat for Windows version 1.0 (Jandel Scientific Software, San Rafael, CA) was used for statistical analysis. All data are presented as means ± SE. If assumptions for the test were met, a two-way repeated-measures ANOVA was used to test if data measured repeatedly differed between trained and sedentary subjects, if changes occurred with time, and if there was any interaction between group and time. If assumptions for a two-way repeated-measures ANOVA were not met, a one-way repeated-measures ANOVA was used to test if changes occurred with time in either the trained or the sedentary subjects. Dunnett’s test was used post hoc to test if values obtained during insulin infusion differed from values obtained in the basal state. A Student’s t-test for unpaired data (data normally distributed) or a Mann-Whitney rank sum test (data not normally distributed) was used to test if anthropometric data, mean relative recoveries, and half-life for interstitial glycerol concentration differed between trained and sedentary subjects. A significance level of 0.05 in two-tailed testing was chosen a priori.

RESULTS

Adipose tissue blood flow. In the basal state, abdominal subcutaneous adipose tissue blood flow tended to be higher in trained compared with sedentary subjects (P = 0.1; Fig. 1). Insulin infusion did not change adipose tissue blood flow significantly in either trained or sedentary subjects (Fig. 1).

Glucose concentrations. In both trained and sedentary subjects, the arterial plasma water glucose concentration was constant during the experiment except for a small transient drop (P < 0.05) in the beginning of the third clamp step (Fig. 2). Generally, the interstitial glucose concentration in adipose tissue was lower than the plasma water glucose concentration [basal concent-

Fig. 1. Blood flow in periumbilical, subcutaneous adipose tissue measured by local 133Xe washout technique in 8 trained and 8 sedentary young men during a 3-step hyperinsulinemic, euglycemic clamp. At step III, values are for only 5 trained and 5 sedentary subjects. Values are means ± SE.
trations: T: 4.87 ± 0.24 vs. 5.67 ± 0.09 mM (n = 8); S: 4.71 ± 0.27 vs. 5.79 ± 0.21 mM (n = 8); P < 0.001, indicating that glucose was taken up by adipose tissue (Fig. 2). In trained subjects, the interstitial glucose concentration was reduced 12 ± 4% (n = 8), 5 ± 2% (n = 8), and 5 ± 5% (n = 5), respectively, at the end of each clamp step relative to basal in the trained subjects and 10 ± 2% (n = 4), 0 ± 2% (n = 4), and 11 ± 8% (n = 4), respectively, at the end of each clamp step in the sedentary subjects. In trained subjects, the insulin-mediated decrease in interstitial glucose concentration achieved statistical significance at the end of the first clamp step, in the beginning of the second clamp step, and in the middle of the third clamp step. Because blood flow and arterial glucose concentration, and accordingly glucose supply, were constant, a decrease in interstitial glucose concentration indicates an increase in glucose uptake. In contrast, in sedentary subjects, the interstitial glucose concentration in adipose tissue did not change significantly during the experiment [compared with basal: −8 ± 3% (end of step I, n = 8), 0 ± 6% (end of step II, n = 8), +2 ± 7% (end of step III, n = 5)]. Moreover, the change from basal in arterial-interstitial glucose concentration difference increased in trained (P < 0.001) but not in sedentary subjects (P > 0.05) during the clamp (Fig. 2). This indicates that the fall in interstitial glucose concentration during the clamp in trained subjects reflects an increase in adipose tissue glucose uptake and does not reflect a fall in plasma glucose concentration.

Also, in skeletal muscle, the interstitial glucose concentration was lower than the arterial plasma water glucose concentration in both groups [basal concentrations: trained, 4.90 ± 0.26 vs. 5.63 ± 0.09 mM (n = 4); sedentary, 4.77 ± 0.13 vs. 5.85 ± 0.17 mM (n = 4); P < 0.001; Fig. 3]. The interstitial glucose concentration was reduced 31 ± 12% (n = 4), 25 ± 12% (n = 4), and 21 ± 6% (n = 4), respectively, at the end of each clamp step relative to basal in the trained subjects and 10 ± 2% (n = 4), 0 ± 2% (n = 4), and 11 ± 8% (n = 4), respectively, at the end of each clamp step in the sedentary subjects. In trained subjects, the insulin-mediated decrease in interstitial glucose concentration achieved statistical significance at the end of the first clamp step, in the second clamp step, and in the beginning of the third clamp step (Fig. 3). In sedentary subjects, the interstitial glucose concentration in skeletal muscle did not change significantly. In muscle, the change from basal in arterial-interstitial glucose concentration difference increased in trained (P < 0.01) and tended to increase in sedentary subjects (P = 0.08; Fig. 3).

Lactate concentrations. Generally, lactate concentrations were higher in the interstitial space of both adipose tissue and skeletal muscle than in arterial plasma water [basal concentrations: adipose tissue: trained 2.45 ± 0.40 vs. 1.16 ± 0.15 mM (n = 8), sedentary 2.60 ± 0.32 vs. 0.93 ± 0.09 mM (n = 8); skeletal muscle: trained 3.03 ± 0.39 vs. 1.04 ± 0.15 mM (n = 4), Fig. 3].

Fig. 2. Arterial plasma water and interstitial glucose concentrations in periumbilical, subcutaneous adipose tissue (A) and change from basal in arterial-interstitial glucose concentration (A-I) difference (B) during a 3-step hyperinsulinemic, euglycemic clamp in young men. Values are for 8 trained and 8 sedentary subjects except at step III at which values are for only 5 trained and 5 sedentary subjects. Values are means ± SE.

Fig. 3. Arterial plasma water and interstitial glucose concentrations in the vastus lateralis portion of the quadriceps femoris muscle (A) and change from basal in A-I difference (B) during a 3-step hyperinsulinemic, euglycemic clamp in young men. Values are for 4 trained and 4 sedentary subjects. Values are means ± SE.
sedentary 4.41 ± 1.32 vs. 0.83 ± 0.08 mM (n = 4); P < 0.001 and P < 0.01, respectively], indicating lactate output from both tissues (Figs. 4 and 5). In both trained and sedentary subjects, the interstitial lactate concentration in adipose tissue increased already at the first clamp step (P < 0.05), whereas the arterial plasma water lactate concentration increased only at the third clamp step (P < 0.05).

In trained subjects, the interstitial lactate concentration in skeletal muscle increased at the first clamp step and remained increased (P < 0.05), whereas it did not change in sedentary subjects (P > 0.05; Fig. 5).

Glycerol concentrations. Generally, the concentration of glycerol was higher in the interstitial fluid of adipose tissue than in arterial plasma water [basal concentrations: trained 184 ± 19 vs. 29 ± 7 μM (n = 7), sedentary, 175 ± 57 vs. 46 ± 6 μM (n = 5); P < 0.01], indicating glycerol output from adipose tissue (Fig. 6). In both trained and sedentary subjects, the interstitial glycerol concentration in adipose tissue decreased throughout the insulin infusion. The interstitial glycerol concentration decreased faster in trained than in sedentary subjects [half-time (T½): trained 44 ± 9 min (n = 7), sedentary 102 ± 23 min (n = 5); P < 0.05], suggesting that inhibition of lipolysis is more sensitive to insulin in trained than in sedentary subjects. Also arterial plasma water glycerol concentration decreased in both trained and sedentary subjects (P < 0.05), with no significant difference between groups.

Hormone concentrations. Infusion of insulin increased the arterial plasma insulin concentrations to ~105, 170, and 1,600 pM, respectively, during the three clamp steps (all P < 0.05 vs. basal; Table 2). Insulin concentrations did not differ significantly between trained and sedentary subjects. Generally, the arterial plasma catecholamine concentrations varied neither with time nor between trained and sedentary subjects (Table 2). At the third clamp step, however, there was a slight increase in norepinephrine concentration (P < 0.05).

FFA, β-hydroxybutyrate, and TG concentrations. Arterial plasma FFA and β-hydroxybutyrate concentrations decreased significantly during the first clamp step and remained low throughout the experiment in both trained and sedentary subjects (Table 2). Neither FFA nor β-hydroxybutyrate concentrations varied between trained and sedentary subjects (P > 0.05). Also, arterial plasma TG decreased slightly during the clamp and did not differ between trained and sedentary subjects (P > 0.05, Table 2).

Plasma glucose clearance. Plasma glucose clearance increased significantly between subsequent clamp steps in both trained and sedentary subjects [trained 3.5 ± 0.5 ml·kg body wt⁻¹·min⁻¹ (step I), 6.6 ± 1.0 (step II), 15.0 ± 0.8 (step III); sedentary 2.5 ± 0.4 (step I), 5.9 ±
Whole body fuel utilization. Nonprotein R value increased significantly with increasing insulin concentration and was at step III higher than 1 in both trained and sedentary subjects [trained 0.87 ± 0.04 (basal), 0.95 ± 0.03 (step I), 0.97 ± 0.03 (step II), 1.07 ± 0.03 (step III); sedentary 0.86 ± 0.02 (basal), 0.93 ± 0.02 (step I), 0.99 ± 0.02 (step II), 1.06 ± 0.02 (step III)]. Also, glucose oxidation and glucose deposition as glycogen and lipid increased significantly during the clamp, whereas lipid oxidation decreased (data not shown). No differences in whole body fuel utilization and storage were found between trained and sedentary subjects (P > 0.05, data not shown), with no interactions between time and group (P > 0.05, data not shown).

Cardiovascular parameters. The heart rate was significantly increased at the third clamp step compared with the basal state in both trained and sedentary subjects and was significantly lower in trained compared with sedentary subjects, with no significant interaction between time and group [trained 56 ± 2 (basal), 58 ± 2 (step I), 61 ± 2 (step II), 68 ± 3 (step III); sedentary 72 ± 4 (basal), 73 ± 4 (step I), 72 ± 4 (step II), 77 ± 5 (step III)]. Generally, systolic and diastolic blood pressures did not change with time or differ between groups, but the systolic blood pressure increased significantly at the third clamp step in trained subjects (data not shown). The hematocrit decreased slightly at the third clamp step (P < 0.05) and did not differ between groups (P > 0.05, data not shown). The plasma potassium concentration did not differ between groups (P > 0.05) but decreased slightly (P < 0.05) at clamp steps II and III compared with basal, with no significant interaction between time and group [trained 3.7 ± 0.0 mM (basal), 3.5 ± 0.0 (step II), 3.5 ± 0.0 (step III); sedentary 3.9 ± 0.1 (basal), 3.5 ± 0.1 (step II), 3.5 ± 0.0 (step III)].

Table 2. Arterial plasma hormone and metabolite concentrations during a hyperinsulinemic, euglycemic clamp

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<thead>
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<th></th>
<th>Clamp Step</th>
<th>Group Time Interaction</th>
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<td>Insulin, pM</td>
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<tr>
<td>Trained</td>
<td>29 ± 4</td>
<td>104 ± 10*</td>
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<tr>
<td>Sedentary</td>
<td>35 ± 4</td>
<td>109 ± 11*</td>
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<td>Epinephrine, nM</td>
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<tr>
<td>Trained</td>
<td>0.55 ± 0.11</td>
<td>0.60 ± 0.13*</td>
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<tr>
<td>Sedentary</td>
<td>0.46 ± 0.08</td>
<td>0.40 ± 0.04</td>
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<td>Norepinephrine, nM</td>
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<tr>
<td>Trained</td>
<td>1.05 ± 0.18</td>
<td>1.26 ± 0.25</td>
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<tr>
<td>Sedentary</td>
<td>1.33 ± 0.17</td>
<td>1.41 ± 0.17</td>
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<td>FFA, µM</td>
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<tr>
<td>Trained</td>
<td>390 ± 117</td>
<td>59 ± 6*</td>
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<tr>
<td>Sedentary</td>
<td>464 ± 55</td>
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<td>β-Hydroxybutyrate, µM</td>
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<tr>
<td>Trained</td>
<td>106 ± 35</td>
<td>40 ± 7*</td>
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<tr>
<td>Sedentary</td>
<td>147 ± 23</td>
<td>61 ± 4*</td>
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<td>Triglyceride, µM</td>
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<tr>
<td>Trained</td>
<td>982 ± 142</td>
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<tr>
<td>Sedentary</td>
<td>843 ± 159</td>
<td>739 ± 143*</td>
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Values are means ± SE for 8 trained and 8 sedentary subjects in the basal state and during clamp steps I and II and for 5 trained and 5 sedentary subjects during clamp step III. FFA, free fatty acid. *P < 0.05 vs. basal.

0.8 (step II), 13.2 ± 0.7 (step III). Plasma glucose clearance tended to be higher in trained compared with sedentary subjects (P = 0.07), with no interaction between time and group (P > 0.05).
DISCUSSION

The present study is the first to show that endurance training enhances insulin sensitivity of glucose uptake in subcutaneous adipose tissue in humans in vivo. Furthermore, interstitial glycerol data suggest that training also increases insulin sensitivity of lipolysis in subcutaneous adipose tissue. Moreover, the study has shown that insulin per se does not change subcutaneous adipose tissue blood flow in humans.

Interstitial concentrations of various substances can be estimated by the microdialysis technique (16, 27, 34, 37). If arterial concentrations are also measured, substances taken up by or released from a given tissue can be deducted. When blood flow is measured in addition, changes in rate of exchange of substances can be deducted. If certain assumptions are made, the absolute rate of exchange can be calculated (37, 38). Calculation of substance uptake or output is, however, associated with a high methodological variation when arteriovenous concentration differences are small (37). This may, when the number of subjects is small, make it difficult to show significant changes. In the present study, the experimental noise was considerably reduced when changes in interstitial glucose concentration were regarded as reflecting changes in glucose uptake. During the clamp, the interstitial glucose concentration fell significantly in trained subjects, but in sedentary subjects the interstitial glucose concentration did not change significantly. Because blood flow and arterial glucose concentration, and accordingly glucose supply, were constant, we can conclude that glucose uptake in adipose tissue was increased during insulin infusion in trained but not in sedentary subjects.

It has previously been shown that the glucose concentration in adipose tissue determined by microdialysis decreases little or not at all during a hyperinsulinenic, euglycemic clamp. In two studies, dialysate glucose concentration was found to decrease ~20% at plasma insulin concentrations of ~360 pM (14, 23). In contrast, in three studies, dialysate glucose concentration was found not to change in response to plasma insulin concentrations of 600 (34), 1,200 (27), and 1,300 pM (28), respectively. The discrepancy between these studies cannot be ascribed to differences in adipose tissue depot (all examined the periumbilical, subcutaneous adipose tissue) or age, sex, body mass index, and level of physical activity of studied subjects, because these variables did not differ much. Probably, the reason for the discrepancy is that any decrease in interstitial glucose concentration is so small that it is hard to detect. With the use of the subcutaneous, abdominal adipose tissue arteriovenous catheterization technique, it was found that during a hyperinsulinenic (320 pM), euglycemic clamp the arteriovenous difference over the tissue increased significantly from ~0.05 to 0.3 mM (6). Unfortunately, the arteriovenous catheterization technique is technically difficult to perform and applicable in far from all subjects.

In the present study, the arterial-interstitial glucose concentration difference in adipose tissue increased during the first clamp step, decreased during the second clamp step, but then increased dramatically during the third clamp step (Fig. 2). Plasma insulin concentrations during clamp steps I and II were in the physiological range, whereas the insulin concentration during step III was supraphysiological. We would like to suggest that the decrease in glucose uptake during step II was due to saturation of adipocytes with glucose at physiological insulin concentrations, but the supraphysiological insulin concentration was capable of overcoming the inhibition of glucose uptake.

In the present study, the change from basal in the arterial-interstitial glucose concentration difference in adipose tissue increased significantly during the clamp in trained but not in sedentary subjects, indicating that glucose uptake in adipose tissue is stimulated more by insulin in trained than in sedentary subjects (Fig. 2). Further support for this notion is found in previously published in vitro (7) and in vivo (21) studies in rats and in an in vitro study in humans (33) showing that insulin-stimulated glucose uptake in adipocytes is increased by training. Endurance training usually results in decreased adiposity, and also in the present study trained subjects were leaner than sedentary subjects (Table 1). The decreased adiposity, however, makes it difficult to distinguish between the effect of endurance training per se and the secondary effect of a training-induced reduction in body fat. Accordingly, we cannot exclude that the increased adipose tissue glucose uptake in the trained subjects was due to a training-induced reduction in adiposity.

Glucose may take different metabolic routes in the adipocytes. It may be used for reesterification of FFA, or it may be oxidized, stored, or degraded to lactate. The present results do not give conclusive answers about which of these processes is the most important. Because lactate production also increases (Fig. 4) when glucose uptake increases (Fig. 2), this may account for some of the glucose uptake. Another likely possibility is that the increased glucose uptake plays a role for the rate with which the adipose tissue TG stores are regenerated after an exercise bout.

In skeletal muscle, we found the same trend as in adipose tissue. Only in the trained group was a prolonged, persistent significant fall in the interstitial glucose concentration present during the insulin infusion (Fig. 3). We did not measure skeletal muscle blood flow, but studies performed with a protocol similar to the one used in the present study have found an increase in this variable during insulin infusion (9, 11). Furthermore, insulin-stimulated skeletal muscle blood flow was found to be increased by physical training (9, 11). Taking into account these previous findings, we can deduce from arterial and interstitial glucose concentrations that glucose uptake in skeletal muscle is increased during the clamp in both trained and sedentary subjects and that training increases insulin action on glucose uptake in human skeletal muscle. The same conclusion has appeared from studies using arterio-
venous differences (9, 11). The conclusion is also in agreement with the finding that, during insulin infusion, interstitial lactate concentration only increased in trained muscle (Fig. 5). One previous study has found a decrease in interstitial glucose concentration in muscle during a hyperinsulinemic, euglycemic clamp (34), whereas two other studies have not been able to demonstrate a decrease (27, 28).

In both adipose tissue and skeletal muscle, one of the metabolic routes that glucose can take is degradation to lactate. It is interesting to note that in both adipose tissue (Fig. 4) and muscle (Fig. 5) the increase from basal in interstitial-arterial lactate concentration difference, which is a marker of lactate output, was increased significantly during the clamp in trained subjects but little (Fig. 4) or none (Fig. 5) in sedentary subjects.

As would be expected (16), indicators of lipolysis, i.e., interstitial glycerol in adipose tissue and arterial plasma glycerol, FFA, and \( \beta \)-hydroxybutyrate concentrations, decreased after the start of insulin infusion in our study (Fig. 6; Table 2; see Refs. 4, 6, 14, 15, 23, 26, 34). The mentioned metabolites in plasma were decreased, and interstitial glycerol concentration was cut in half at a plasma insulin concentration of ~80 pM. This is in accordance with findings using the isotope dilution technique that the insulin concentration producing half-maximal suppression of lipolysis and rate of appearance of FFA is ~100 pM (4). Interestingly, the interstitial glycerol concentration and whole body lipid oxidation were correlated also \( (R = 0.61, P < 0.0001) \).

Basal interstitial glycerol concentration in periumbilical, subcutaneous adipose tissue was ~180 µM in the present study (Fig. 6). This value is similar to previously reported values that range from 130 (19) to 350 µM (38). We used the internal reference technique to determine RR of glycerol and found it to increase significantly from 66% in the basal period to 77% at \( \text{time 330–360 min} \). The mean RR of glycerol was 71%, which is high compared with a previous study in which, by the no-net-flux calibration technique, we found glycerol RR to be 38% (38). In contrast, mean RR of glucose and lactate did not differ notably between the present and the previous study (38; glucose: present study 34%, previous study 30%; lactate: present study 38%, previous study 38%). We do not have an explanation for the difference in glycerol RR between studies or for the increase in RR with time in the present study. RR of glycerol determined by internal reference and no-net-flux calibration techniques, respectively, has previously been found not to differ (19, 22). Furthermore, glycerol RR determined by the internal reference technique has been found to decrease slightly (rat epididymal adipose tissue) or not change (rat subcutaneous adipose tissue) during a hyperinsulinemic, euglycemic clamp (5). One explanation that could explain the apparently too high and gradually increasing RR of glycerol in the present study is binding of glycerol to the microdialysis membrane. In the present and in our previous study (38), we used a Hemophan membrane that, on delivery, was loaded with glycerol and thus before usage was rinsed to remove the glycerol. It could be that the \( [3H] \)glycerol after the rinsing procedure is attracted to the membrane and therefore lost from the perfusate, which would be interpreted as a too high RR of glycerol. The increasing RR could be explained by the decreasing endogenous glycerol production during insulin infusion and hence a decreasing competition of glycerol with \( [3H] \)glycerol for binding to the membrane.

In our study, the decrease in interstitial glycerol concentration was faster in trained than in sedentary subjects, indicating that the inhibition of lipolysis in subcutaneous adipose tissue is more sensitive to insulin in trained than in sedentary subjects (Fig. 6). In both the present (Fig. 6; Table 2) and in previous human studies (30), a tendency toward lower plasma glycerol, FFA, and \( \beta \)-hydroxybutyrate concentrations is found during insulin infusion, suggesting an increased lipolytic insulin sensitivity on the whole body level. The present study, however, is the first study to suggest an increased lipolytic insulin sensitivity directly in intact adipose tissue in trained subjects. Interestingly, the findings are in line with a previous study in rats showing a 60% decrease in plasma FFA in trained rats during a hyperinsulinemic, euglycemic clamp compared with a 27% decrease in sedentary animals (26). Similarly, insulin-induced inhibition of norepinephrine-stimulated lipolysis of rat adipocytes in vitro has been shown to be more pronounced in trained compared with sedentary animals (39).

In the present study, adipose tissue blood flow estimated by the \( ^{133} \)Xe washout technique was not changed by insulin at either physiological (steps I and II) or supraphysiological (step III) concentrations (Fig. 1). This is in line with a previous study of 38 healthy subjects in which fasting adipose tissue blood flow measured by the \(^{133} \)Xe washout technique did not vary with fasting insulin concentration (40). Whether a change in adipose tissue blood flow occurs during a hyperinsulinemic, euglycemic clamp has previously been examined in humans by the microdialysis technique. With the use of ethanol as the blood flow marker, three studies found no change in blood flow in response to hyperinsulinemia (15, 16, 23), whereas two studies, one using \(^{13} \)Curea and the other using ethanol as the blood flow marker, found an increase in blood flow (17, 34). The sensitivity of the microdialysis ethanol technique is reduced when microdialysis conditions are not optimized, which means that a change in blood flow can be missed if the type of microdialysis probe and the perfusion rate are not well chosen (18). Furthermore, at least in muscle, the ethanol technique is influenced by other factors than blood flow (32), making the interpretation of the data difficult. However, adipose tissue blood flow estimated by the microdialysis ethanol technique has consistently been found to increase during hyperinsulinemic, hypoglycemic clamps (1, 16, 31). Also, after oral glucose ingestion and resulting hyperinsulinemia and hyperglycemia, adipose tissue blood flow has been found to increase when estimated by both microdialysis ethanol (24) and \(^{133} \)Xe washout techniques (2). Possibly, the increased sym-
pathetic activity during hypoglycemia (1, 16, 31) and hyperglycemia (2) is responsible for the blood flow increase in the studies mentioned.

In conclusion, endurance training enhances insulin sensitivity of glucose uptake in subcutaneous adipose tissue in humans in vivo. Furthermore, it is suggested that training also enhances insulin sensitivity of lipolysis in subcutaneous adipose tissue. These adaptations will help to replenish and preserve TG stores in adipose tissue in trained subjects between exercise sessions. Subcutaneous adipose tissue blood flow is not influenced by hyperinsulinemia per se and does not differ between trained and sedentary young men.

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