Effect of training on insulin-mediated glucose uptake in human muscle

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Dela, Flemming, Kári J. Mikines, Michael von Linstow, Niels H. Secher, and Henrik Galbo. Effect of training on insulin-mediated glucose uptake in human muscle. Am. J. Physiol. 263 (Endocrinol. Metab. 26): E1134–E1143, 1992.—During insulin stimulation whole body glucose uptake is increased in trained compared with untrained humans. However, it is not known which tissue is responsible. Seven young male subjects bicycle trained one leg for 10 wk at 70% of maximal O₂ consumption (VO₂ max). Sixteen hours after last exercise bout, a three-step euglycemic hyperinsulinemic clamp (clamp I) was performed (insulin levels, means ± SE: 9 ± 1.53 ± 3, 174 ± 5, and 2,323 ± 80 = μU/ml), with measurement of arteriovenous differences and blood flow in both legs. After 6 days of detraining subjects were restudied, having exercised the untrained leg 16 h before. VO₂ max for trained (T) and untrained (UT) legs was 52 ± 2 vs. 44 ± 2 ml·min⁻¹·kg⁻¹ (P < 0.05). In clamp I glucose uptake in T and UT legs was 1.0 ± 0.2 vs. 0.5 ± 0.1 mg·min⁻¹·kg⁻¹ (basal), 9.7 ± 2.3 vs. 6.7 ± 1.7 (P < 0.05) (step I), 19.2 ± 2.8 vs. 14.3 ± 2.0 (P < 0.05) (step II), and 22.8 ± 2.3 vs. 18.6 ± 2.2 (P < 0.05) (step III). During insulin infusion lactate release (P < 0.05) [8.9 ± 1.8 vs. 2.9 ± 0.9 μmol·min⁻¹·kg⁻¹ (step I), 24.6 ± 3.1 vs. 12.5 ± 2.6 (step III)] and glycogen storage (P < 0.1) calculated by indirect calorimetry [6.7 ± 2.3 vs. 5.0 ± 1.7 μmol·min⁻¹·kg⁻¹ (step I), 16.8 ± 2.1 vs. 14.1 ± 1.8 (step III)] were always higher in T than in UT legs. Release of glycerol, free fatty acids, and tyrosine and clearance of insulin were not influenced by training. Insulin-mediated glucose uptake was not increased after detraining or a single bout of exercise. In conclusion, training increases sensitivity and responsiveness of insulin-mediated glucose uptake in human muscle by local mechanisms. Glycolysis and glycogen storage are equally enhanced. The training effect represents a genuine adaptation to repeated exercise but is short lived. Insulin clearance in muscle is not influenced by training.

Whole body studies have shown that the effect of insulin on glucose disappearance from plasma is increased in trained compared with untrained humans (6, 27, 33, 41). The difference exceeds the effect of a single bout of exercise (34). Furthermore, the training-induced enhancement of insulin action, in contrast to the enhancement by acute exercise, may not be completely gone after 5 days of normal activity (34). Indirect calorimetry has revealed that the training-induced enhancement of insulin action reflects an increased glucose storage as glycogen (33). However, the site of additional glycogen synthesis has not been clearly defined. Apart from direct conversion of glucose to glycogen in muscle or liver, the possibility exists that glucose is metabolized to lactate, which in turn serves as substrate for hepatic glycogen synthesis (33, 34).

In rats training has been shown to enhance the effect of insulin on glucose uptake in both muscle (12, 14, 19, 24) and adipose tissue (11, 47). In trained rat muscle insulin-stimulated glucose uptake is primarily channeled into the glycolytic pathway (12, 14, 19, 24). In humans training also increases insulin-stimulated glucose uptake in adipocytes (41). Furthermore, training may increase the suppressive action of insulin on hepatic glucose production in humans (33, 41). The influence of training on insulin action in human muscle has not been directly studied. However, because muscle is the quantitatively most important insulin target tissue in untrained subjects (15), it has been postulated that in humans the training-induced improvement in insulin action includes increased insulin effect in muscle also (41).

From a metabolic point of view the leg consists predominantly of muscle (43). Therefore to directly study the influence of training on human muscle we have now measured insulin-mediated uptake and metabolism of glucose in both legs after 10 wk of one-legged bicycle training. Furthermore, to separate possible effects of last exercise bout from genuine adaptations to repeated exercise and to elucidate the duration of any adaptation, the subjects were restudied after 6 days of detraining, having carried out a single exercise bout with the untrained leg on the 5th day. The protocol allowed us to also study the influence of training on insulin clearance in muscle. We found this of interest because insulin is degraded after binding to its receptor and because some studies have reported increased insulin binding in trained rat muscle (5, 18). Furthermore, studies of humans and of isolated rat liver have indicated that training increases hepatic insulin clearance (17, 48).

Materials and Methods

Subjects

Seven healthy male medical students (mean 23 yr; range 21–24) participated in the study, which was approved by The Municipal Ethics Committee for Copenhagen and Frederiksberg. None of the subjects had a family history of diabetes or other endocrinological disorder, and none were taking any medication. They did not participate in sports on a regular basis, and all were nonsmokers. The subjects weighed 68.9 ± 1.5 kg (mean ± SE) before and 69.5 ± 1.0 kg after the training period (P > 0.05) and their height was 182.5 ± 1.5 cm. Body fat estimated from skinfold measurements (37) was 9.0 ± 0.8% before and 7.8 ± 0.8% after the training period (P < 0.05).

Experimental Design (Fig. 1)

Training regimen. Within 2 wk before the start of a 10-wk training program (see below), the following measurements were made for both legs: isokinetic, concentric strength of quadriceps...
femoris (KIN-COM model 500-11, Chattecx) at angular velocities of 30, 60, 120, and 180 °/s; circumference and cross-sectional areas measured planimetrically on computer tomographic scans (CT area) of the thigh and the calf 15 cm proximal and distal, respectively, to the patella; leg volume by water displacement; skinfold thickness on the lateral side of the leg 15 cm proximal and distal to the left of the knee joint. Furthermore, during one-legged graded bicycle tests until exhaustion, pulmonary ventilation (VE) and O\(_2\) uptake (VO\(_2\)), concentration of lactate in finger-tip capillary blood, and heart rate were also measured. Thereafter, the leg (left or right) to be trained was chosen by lot. The one-legged training program consisted of bicycle ergometer exercise 30 min/day, 6 days/wk for 10 wk at a work load of \(\sim 70\%\) of one-legged maximal \(O_2\) consumption (VO\(_{2\ max}\)). During training subjects wore a bicycle shoe locked to the pedal on one side while the other foot rested on a stool. All training sessions were supervised by the first author. At all training sessions heart rate was monitored continuously (Sports Tester, Polar Elektro, Finland), and during the training sessions were performed simultaneously from the arterial and venous catheters. The catheters were connected to a recorder and through an analog-to-digital converter also to a computer (Fig. 2). After 45 min of recumbent rest, basal blood samples were drawn simultaneously from the arterial and venous catheters. This was done twice, with an interval of 10 min. Immediately after blood sampling, blood flow was measured by use of a thermoradiation technique. The method has been described elsewhere (40), but in brief, we used an infusion pump (Harvard Instruments, Millis, MA) connected to a stainless steel coil (90 ml) that was placed in ice water. The coil was connected to the venous catheters by an insulated polyethylene tube. Temperature of the infused saline was 2.3°C, and infusion rate was usually 60 ml/min. However, in some instances, when the subjects reported that they felt cold in the inguinal region, a rate of 40 ml/min was used. When blood temperature had dropped to a new constant level (which could be seen on the temperature recorder), infusion of cold saline was continued for another 10–15 s. One minute before and during every blood sampling and blood flow measurement, pneumatic cuffs placed around the ankles were inflated to a systolic pressure of \(+50\) mmHg. This was done to minimize contributions from the feet, which have

<table>
<thead>
<tr>
<th>Training Leg</th>
<th>Sedentary Leg</th>
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<tbody>
<tr>
<td>UT-leg</td>
<td>Muscle biopsy</td>
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<tr>
<td>Ex UT-leg</td>
<td></td>
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<tr>
<td>Clamp 1.</td>
<td>Euglycemic, hyperinsulinemic</td>
</tr>
<tr>
<td>Clamp 2.</td>
<td>Euglycemic, hyperinsulinemic</td>
</tr>
<tr>
<td>Detraining</td>
<td></td>
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</tbody>
</table>

Fig. 1. Study design: 16 young male subjects trained 1 leg on a bicycle ergometer for 10 wk. Sixteen hours after last exercise bout, a sequential 3-step euglycemic hyperinsulinemic clamp was performed (clamp 1). Five days later, procedure and measurements were repeated (clamp 2). Between 2 clamps, training leg (T leg) did not perform daily exercise (i.e., it became detrained, dT leg). However, 16 h before clamp 2, the previously untrained leg (UT leg) performed 1 bout of exercise (i.e., UT leg became an exercised untrained leg, exUT leg). VO\(_{2\ max}\), \(O_2\) consumption.

1.00 mm \(\times\) 45 mm, Viggo) was inserted in the left radial artery for drawing blood samples. This cannula was connected to a pressure monitoring kit with an automatic flushing device (Baxter). Teflon catheters were then inserted in both femoral veins 6–8 cm below the inguinal ligament (Seldinger technique). The catheters were conic, with the hole at the tip being just wide enough for a thermistor to pass through. Four small (0.3 mm diam) side holes were drilled, 1.5 cm from the tip of the catheter, to allow blood drawing and infusion of ice-cold saline (see below). The catheters were advanced proximally so that their tips were located ~2 cm distally to the inguinal ligament. Through each catheter a thermistor (Edslab probe 94-030-2.5 F, Baxter) was inserted and advanced 6–8 cm beyond the catheter tip. The thermodilution probe was connected to a recorder and through an analog-to-digital converter also to a computer (Fig. 2). After 45 min of recumbent rest, basal blood samples were drawn simultaneously from the arterial and venous catheters. This was done twice, with an interval of 10 min. Immediately after blood sampling, blood flow was measured by use of a thermodilution technique. The method has been described elsewhere (40), but in brief, we used an infusion pump (Harvard Instruments, Millis, MA) connected to a stainless steel coil (90 ml) that was placed in ice water. The coil was connected to the venous catheters by an insulated polyethylene tube. Temperature of the infused saline was 2.3°C, and infusion rate was usually 60 ml/min. However, in some instances, when the subjects reported that they felt cold in the inguinal region, a rate of 40 ml/min was used. When blood temperature had dropped to a new constant level (which could be seen on the temperature recorder), infusion of cold saline was continued for another 10–15 s. One minute before and during every blood sampling and blood flow measurement, pneumatic cuffs placed around the ankles were inflated to a systolic pressure of \(+50\) mmHg. This was done to minimize contributions from the feet, which have
relatively little muscle tissue and many arteriovenous shunts. Expiratory air was collected in Douglas bags through a mouthpiece. Subjects were accustomed to the mouthpiece for at least 4 min before collection of air (~10 min). Muscle biopsies (~350 mg wet wt) from both legs were then taken from the vastus lateralis of the quadriceps femoris, 15 cm above the patella, using a Bergstrom needle. Biopsies were quickly freed from blood and connective tissue, and within 10–15 s frozen in liquid nitrogen. They were stored at −80°C for subsequent analysis.

Before a three-step (designated I, II, and III) sequential hyperinsulinemic euglycemic clamp was initiated, the subjects voided. The urine volume was measured, and an aliquot was stored at −20°C for subsequent analysis. Before a three-step (designated I, II, and III) sequential hyperinsulinemic euglycemic clamp was initiated, the subjects voided. The urine volume was measured, and an aliquot was stored at −20°C for subsequent analysis. Before a three-step (designated I, II, and III) sequential hyperinsulinemic euglycemic clamp was initiated, the subjects voided. The urine volume was measured, and an aliquot was stored at −20°C for subsequent analysis.

Analytical Procedures

Blood for determination of metabolites and hormones were collected in iced tubes and either immediately centrifugated at 4°C or precipitated in perchloric acid for analysis of plasma or whole blood, respectively. Blood for determinations of glucose, FFA, glycerol, alanine, tyrosine, lactate, β-hydroxybutyrate, urea, hematocrit, sodium, and potassium was stabilized with 10 IU heparin/ml blood. Blood for determinations of insulin, cortisol, and growth hormone (GH) was stabilized with 500 kalikrein inhibitory units aprotinin (Trasylol) and 1.5 mg EDTA/ml blood. Blood for determination of epinephrine and norepinephrine was stabilized with 5 μmol ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and 4 μmol reduced glutathione in 20 μl 0.6 N sodium hydroxide/ml blood. Commercial radioimmunoassay (RIA) kits were used for determination of concentrations in plasma of insulin (Novo), GH (Pharmacia), and cortisol (CIS Bio International) (intraassay coefficients of variation: 3.3, 3.6, and 8.8%, respectively; interassay coefficients of variation: 6.8, 1.8, and 12.5%, respectively). All samples from one subject were analyzed in the same assay run. Catecholamines were assayed by a previously described single-isotope radioenzymatic assay (28). Concentrations in plasma of FFA were determined by a microfluorometric...
assay (35) [intra-assay coefficient of variation: 3.2 and 6.9%, respectively]. Glycerol, β-hydroxybutyrate, alanine, tyrosine, and lactate in blood were determined by use of enzymatic fluorometric techniques (intra-assay coefficients of variation: 4.0, 3.2, 2.1, 3.7, and 1.1%, respectively; interassay coefficients of variation: 3.8, 3.3, 4.1, 3.4, and 3.1%, respectively). Plasma urea was determined spectrophotometrically as previously described (17). Blood for determination of sodium, potassium, O₂, and CO₂ was drawn anaerobically in heparinized syringes, placed on ice, and analyzed within 1 h on a K+-Na+ analyzer (KNA, Radiometer) and on an automatic blood gas analyzer (ABL 4, Radiometer). Concentrations of CO₂ in whole blood were calculated from hematocrit, pH, O₂ saturation in blood, and CO₂ tension in plasma, assuming normal concentrations of 2,3-diphosphoglycerate in erythrocytes (45). Hematocrit was measured by the microhematocrit method. Capillary blood from finger tips was kept on ice and within 30 min analyzed for content of lactate (GM-7, Analox Instruments). Concentrations in urine of glucose and urea were determined in the same way as concentrations in plasma. Expiratory air content of O₂ and CO₂ was analyzed by paramagnetic (Servomex OA 189) and infrared (Capnograph Godard 146) electronic analyses, respectively. The expiratory volume was measured in a giant spirometer.

Muscle biopsies were stored at -80°C. Subsequently, they were divided into smaller pieces for analysis (in duplicate) of glycogen, hexokinase, and cytochrome-c oxidase. Samples were weighed on a precision balance at -20°C. The biopsies were lyophilized at -40°C for 48 h and were subsequently studied in a room with a constant relative humidity of 20% and a temperature of 20°C. For every 14 samples, the weight of one sample was monitored during equilibration with room air to calculate the relative increase in weight due to absorption of H₂O from the air. This relative increase together with the weight at 20% humidity was used to calculate the dry weight of the other samples. Samples were inspected under a stereomicroscope and muscle fibers were dissected free of fat, blood, and fascia, weighed, and analyzed.

Glycogen was determined by a hexokinase method (25) and expressed as millimole glucose units per kilogram dry muscle. Hexokinase was determined fluorometrically according to Bergmeyer (4). Cytochrome-c oxidase was determined spectrophotometrically by a modification of the method described previously (46).

Calculations

Volume of the leg was calculated as total leg volume minus volume of the foot. Leg weight was calculated from leg volume, assuming a specific gravity of 1. CT areas of the thigh and calf muscle were calculated as total area minus area of skin, bone, fat, and connective tissue. Isokinetic strength at a given angular velocity was defined as the maximal value obtained during three successive tests, and the value is expressed as newtons times meters (torque).

Glucose infusion rates were averaged for 10 min periods. Whole body glucose uptake was calculated as the mean of steady state glucose infusion rates during the last 30 min of each step. Uptake and release of substrates and metabolites in the legs were calculated as the mean of arteriovenous differences times blood flow measured at 85, 100, and 115 min at each clamp step. However, Because FFA and insulin in the blood are found only in the plasma phase, balances for these substances were calculated using concentrations in plasma and plasma flow instead of concentrations in blood and blood flow [plasma flow = blood flow × (1 - arterial hematocrit)].

To estimate sensitivity for insulin-stimulated glucose uptake, the insulin concentration that elicits half-maximal stimulation of glucose uptake was determined from individual dose-response curves that were extrapolated to a hypothetical zero value of plasma insulin concentration. This y-intercept was taken to be the insulin-independent glucose uptake and was subtracted from the highest glucose uptake to obtain the maximal insulin-stimulated value. Calculations of whole body glucose oxidation and storage, lipid oxidation, and lipid synthesis were done according to Mikines et al. (30). In brief, calculations were based on measurements of VO₂, CO₂ production, and urinary excretion of urea nitrogen and glucose. Protein combustion was calculated as 100/16 × urea nitrogen excretion corrected for changes in the urea pool, assuming a distribution volume for urea of 60.2% of body weight (1). Calculation of substrate oxidation and storage in the legs was based on concentrations of O₂ and CO₂ in arterial and femoral venous whole blood and on the assumption that protein combustion was whole body protein combustion × VO₂ (leg)/VO₂ (whole body).

Statistical evaluation was made by means of Wilcoxon's non-parametric ranking test for paired data. Correlation analysis was done by the Spearman rank test. P < 0.05 (2-tailed testing) was considered significant.

RESULTS

Changes in Performance and Composition of Legs

During the training period VO₂ max, maximal heart rate (HR max: 193 ± 4 to 199 ± 2 beats/min; P < 0.05), and maximal work load increased significantly for the training leg, whereas maximal blood lactate was unchanged (7.8 ± 0.6 to 8.0 ± 0.8 mM) (Table 1). Responses of VE, heart rate, and blood lactate to submaximal exercise decreased during training (Table 1). Apart from a tendency to a diminished heart rate at submaximal exercise no change in performance was seen for the sedentary leg during the training period (Table 1; HR max: 195 ± 3 to 193 ± 3 beats/min; maximal blood lactate: 7.4 ± 0.5 to 7.2 ± 0.6; P > 0.05).

The volume of the training leg increased 9 ± 2% during training (P < 0.05, Table 2), and this was mainly due to an increase in thigh volume (Table 2). The increase in volume represented an increase in muscle mass as indicated by CT images and skinfold measurements (Table 2). During the detraining period (i.e., between clamps 1 and 2), the leg volume decreased 4 ± 2% (P < 0.05; data not shown). No significant changes in anthropometric measures of the sedentary leg were seen.

Isokinetic strength was identical in the two legs before training, decreasing with increasing angular velocity (P < 0.05). Differences between values obtained after vs. before training: $t P < 0.05; t'$ P < 0.01.

Table 1. Response to one-legged exercise before and after 10 wk of one-legged training

<table>
<thead>
<tr>
<th></th>
<th>Trained Leg</th>
<th>Untrained Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂ max, ml·min⁻¹·kg⁻¹</td>
<td>46 ± 2</td>
<td>52 ± 2*</td>
</tr>
<tr>
<td>Work load at fatigue, W</td>
<td>186 ± 7</td>
<td>249 ± 10*</td>
</tr>
<tr>
<td>VE at 160 W, l/min</td>
<td>50 ± 5</td>
<td>53 ± 5*</td>
</tr>
<tr>
<td>Heart rate at 160 W, beats/min</td>
<td>179 ± 5</td>
<td>166 ± 5*</td>
</tr>
<tr>
<td>Lactate at 180 W, mmol/l</td>
<td>5.8 ± 0.6</td>
<td>4.1 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Seven healthy young male subjects performed 1-legged graded bicycle exercise to exhaustion with both legs. This was done before and in the 9th-10th wk of a 10-wk period, in which one leg was endurance trained. VO₂ max, maximal O₂ consumption; VE, pulmonary ventilation. * Differences between trained and untrained legs, P < 0.05. Differences between values obtained after vs. before training: $t P < 0.05; t'$ P < 0.01.
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TRAINING AND INSULIN ACTION

Table 2. Anthropometric measures of legs

<table>
<thead>
<tr>
<th></th>
<th>Trained Leg</th>
<th>Untrained Leg</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Leg volume, liter</td>
<td>9.8±0.3</td>
<td>10.7±0.4†</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>2.9±0.1</td>
</tr>
<tr>
<td></td>
<td>Thigh</td>
<td>6.9±0.3</td>
</tr>
<tr>
<td>CT area, cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>77.6±2.8</td>
<td>80.9±2.4*</td>
</tr>
<tr>
<td>Thigh</td>
<td>147.1±1.2</td>
<td>159.6±6.5*†</td>
</tr>
<tr>
<td>Circumference, cm</td>
<td>37.4±0.8</td>
<td>37.5±0.6</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>48.6±0.7</td>
</tr>
<tr>
<td></td>
<td>Thigh</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>Skinfold, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>10.5±1.0</td>
<td>9.0±1.3‡</td>
</tr>
<tr>
<td>Thigh</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE. Seven healthy young male subjects trained 1 leg 30 min/day, 6 days/wk for 10 wks. Before and after anthropometric measurements were done. Leg volumes were measured by water displacement; total leg volume is volume after subtraction of foot volume; circumference and CT area (cross-sectional area of muscle measured planimetrically on computer tomographic scans) were measured both 15 cm above and beneath the patella; skinfold thickness was measured on the lateral side of the leg, 15 cm proximal and distal to the knee joint. Differences between values obtained after vs. before training: † P < 0.05; ‡ P < 0.01. Difference between trained and untrained leg, * P < 0.05.

0.05). Strength was unaltered by the endurance-training program. Torque at angular velocities of 30, 60, 120, and 180°/s after training was 249 ± 19, 226 ± 16, 188 ± 13, and 170 ± 10 N·m (T leg) and 247 ± 16, 235 ± 16, 196 ± 9, and 168 ± 7 N·m (UT leg).

Plasma Insulin Concentration and Glucose Uptake Rates

At submaximal insulin concentrations insulin clearance in both whole body and leg was constant, whereas it decreased at the highest insulin level (Table 3). Leg insulin clearance was not influenced by training, detraining, or acute exercise (Table 3). In the basal state (P < 0.1) as well as at the various clamp steps (P < 0.05) leg glucose uptake expressed per leg was higher in T than in UT legs (data not shown). This difference could be expected as leg volume and, with that, leg blood flow were increased in T compared with UT legs (Table 4). However, at all insulin levels, glucose uptake was also higher in T compared with UT legs when expressed per unit leg weight (Fig. 3). This difference predominantly reflected that glucose extraction was higher in T than in UT legs (Table 4), because blood flow per kilogram leg did not differ (P > 0.05) between states of training. With increasing insulin level both glucose extraction and blood flow always increased significantly. After 6 days of detraining glucose uptake in the T leg did no longer differ from that of the UT leg (Fig. 3). Glucose uptake in the UT leg was not influenced by a single bout of exercise (Fig. 3).

The insulin concentration resulting in half-maximal increase in glucose uptake was lower in T (69 ± 13 μU/ml) than in UT (91 ± 14 μU/ml), dT (94 ± 13 μU/ml), and exUT (100 ± 16 μU/ml) (P < 0.05). On the whole body level, the plasma insulin concentration eliciting half-maximal glucose uptake was not different between clamps 1 and 2 (70 ± 12 and 71 ± 8 μU/ml, respectively; P > 0.05). Maximal insulin-stimulated whole body glucose uptake did not differ between clamps 1 and 2 (14 ± 1 and 15 ± 1 mg·min⁻¹·kg body wt⁻¹, respectively; P > 0.05).

Substrate and Electrolyte Exchange Across Legs

At basal, lactate was released from all legs, with no difference between T and UT legs (pooled data: 3.7 ± 1.1 μmol·min⁻¹·kg⁻¹). During the clamp lactate release

Table 3. Insulin clearance

<table>
<thead>
<tr>
<th>Clamp Step:</th>
<th>Basal</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>poled data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin Level, μU/ml</td>
<td>9±1</td>
<td>54±3</td>
<td>174±5</td>
<td>2,323±80</td>
<td></td>
</tr>
<tr>
<td>Whole body (clamps 1 + 2,</td>
<td>1,070±118</td>
<td>977±52</td>
<td>400±24†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained leg</td>
<td>31±13</td>
<td>37±8</td>
<td>36±7</td>
<td>17±6*</td>
<td></td>
</tr>
<tr>
<td>Untrained leg</td>
<td>18±7</td>
<td>35±4</td>
<td>31±7</td>
<td>19±6*</td>
<td></td>
</tr>
<tr>
<td>Deterained leg</td>
<td>46±12</td>
<td>41±12</td>
<td>47±27</td>
<td>11±6†</td>
<td></td>
</tr>
<tr>
<td>Exercised untrained leg</td>
<td>20±9</td>
<td>29±8</td>
<td>30±7</td>
<td>18±5</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SE of measurements in 7 subjects. Insulin levels were identical in 2 series of insulin infusions (clamps 1 and 2) and pooled values are shown. For protocol see text. Insulin clearance in whole body was calculated as insulin infusion rate/arterial plasma insulin concentration. Insulin clearance in legs was calculated as arteriovenous concentration difference × plasma flow/arterial plasma insulin concentration. Plasma flow was calculated as blood flow × (1 - arterial hematocrit). Difference from preceding insulin level: * P < 0.1; † P < 0.05.

Table 4. Blood flow and glucose extraction in and lactate release from trained and untrained legs before and during a three-step hyperinsulinemic euglycemic clamp

<table>
<thead>
<tr>
<th>Clamp Step:</th>
<th>Basal</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin Level, μU/ml</td>
<td>9±1</td>
<td>54±3</td>
<td>174±5</td>
<td>2,323±80</td>
</tr>
<tr>
<td>Blood flow, ml/min⁻¹</td>
<td>472±80</td>
<td>637±80*</td>
<td>806±80*</td>
<td>846±70*</td>
</tr>
<tr>
<td>Glucose extraction, mmol/l</td>
<td>0.13±0.02</td>
<td>0.87±0.19*</td>
<td>1.40±0.16*</td>
<td>1.63±0.20*</td>
</tr>
<tr>
<td>Lactate release, μmol·min⁻¹·kg⁻¹</td>
<td>3.6±0.8</td>
<td>8.9±1.8†</td>
<td>23.5±2.7†</td>
<td>24.6±3.1†</td>
</tr>
</tbody>
</table>

Values are mean ± SE of measurements in 7 subjects. For protocol see text. Blood flow was measured by use of thermomil dilution technique T, trained; UT, untrained. Difference from UT leg: * P < 0.1; † P < 0.05.
always increased with plasma insulin concentration reaching a plateau at step II (Fig. 4, similar data from dT and exUT legs not shown). During insulin infusion lactate release was always higher ($P < 0.05$) in T compared with UT legs (Table 4). After 6 days of detraining (dT) lactate release still was higher compared with UT leg (step I: 6.7 ± 1.0 ($P < 0.1$); step II: 13.3 ± 2.7 ($P < 0.1$); step III: 22.2 ± 3.8 ($P < 0.05$) μmol·min$^{-1}$·kg$^{-1}$). The release of lactate from exUT leg was not different ($P > 0.05$) from that of UT leg (basal: 1.8 ± 1.4; step I: 2.5 ± 0.8; step II: 10.0 ± 4.3; step III: 11.2 ± 3.0 μmol·min$^{-1}$·kg$^{-1}$). During insulin infusion, the release of lactate in percentage of the glucose taken up was always higher in T than in UT legs ($P < 0.05$), and the percentage was constant with increasing insulin level (step I: 11 ± 3 vs. 6 ± 2%; step II: 13 ± 2 vs. 9 ± 2%; step III: 11 ± 2 vs. 7 ± 2%) (Fig. 4). It still tended to be higher in the dT compared with the UT leg (step I: 13 ± 3 vs. UT ($P < 0.1$); step II: 11 ± 2 vs. UT (NS); step III: 10 ± 1% vs. UT ($P < 0.1$).

At basal, FFA and glycerol were always released from the legs, whereas β-hydroxybutyrate was taken up (pooled data: 1.7 ± 0.4, 1.4 ± 0.2, and 2.5 ± 0.3 μmol·min$^{-1}$·kg$^{-1}$, respectively). During insulin infusion both arterial and venous concentrations declined ($P < 0.05$), resulting in marked decreases ($P < 0.05$) in release of glycerol (to 0.4 ± 0.04 μmol·min$^{-1}$·kg$^{-1}$ at step I) and FFA [in T and UT legs even a net uptake ($P < 0.05$) was seen at steps I and II; pooled data: 1.3 ± 0.2 μmol·min$^{-1}$·kg$^{-1}$], whereas β-hydroxybutyrate uptake ceased. No influence of training, detraining, or acute exercise was seen.

At basal, alanine was always released by the legs, with no difference among the T, UT, dT, or exUT leg (pooled data: 1.6 ± 0.3 mmol·min$^{-1}$·kg$^{-1}$). During insulin infusion alanine release tended to be higher in the T than in the UT leg (step I: 0.9 ± 0.4 vs. 0.8 ± 0.4; step II: 2.2 ± 0.2 vs. 1.1 ± 0.2, $P < 0.1$; and step III: 2.1 ± 0.3 vs. 1.5 ± 0.4 μmol·min$^{-1}$·kg$^{-1}$, $P < 0.05$). The same was true after 6 days of detraining (step I: 1.4 ± 0.3 (dT) vs. UT, $P < 0.1$; step II: 2.2 ± 0.5 (dT) vs. UT, $P < 0.1$; step III: 2.0 ± 0.3 μmol·min$^{-1}$·kg$^{-1}$ (dT) vs. UT, $P < 0.05$). Arterial tyrosine concentration (measured only at clamp I) was halved during insulin infusion (70 ± 4 μmol/l at basal and 35 ± 2 μmol/l at step III; $P < 0.05$), but net balances across the legs did not differ significantly from zero (not shown).
At basal, sodium was always released from the legs (with no difference between legs of different training status (T: 22 ± 4; UT: 33 ± 6; exUT: 24 ± 4; DT: 20 ± 9 μmol·min⁻¹·kg⁻¹). At step I, the release was maintained in T and DT legs (20 ± 7 and 18 ± 8 μmol·min⁻¹·kg⁻¹, respectively), but was not significantly different from zero in UT and exUT legs. During step II sodium balance never differed from zero, whereas at step III pmol El140 with no difference between legs of different training status from zero in UT and exUT legs. During step II sodium balance never differed from zero, whereas at step III all legs again released sodium (T: 23 ± 7; UT: 24 ± 5; exUT: 19 ± 8; DT: 42 ± 14 μmol·min⁻¹·kg⁻¹). Potassium balance across the legs never differed significantly from zero.

Hormones

Basal arterial plasma concentrations of catecholamines (epinephrine: 0.6 ± 0.1 vs. 0.4 ± 0.1 nmol/l; norepinephrine: 1.5 ± 0.2 vs. 1.9 ± 0.2 nmol/l), cortisol (450 ± 102 vs. 299 ± 31 nmol/l), and GH (12.4 ± 3.8 vs. 0.8 ± 4.1 μU/ml) did not differ significantly between clamps I and II, and concentrations did not change (P > 0.05) during the clamps (data not shown).

$V_{O_{2}p}$, Indirect Calorimetry, and Fate of Glucose During Clamps

Whole body $V_{O_{2}}$, $V_{CO_{2}}$, and urea excretion did not differ between clamps I and II, and the results from whole body indirect calorimetry are presented as pooled data. $V_{O_{2}}$ increased with increasing insulin level from 3.1 ± 0.3 ml·min⁻¹·kg⁻¹ at basal to 3.5 ± 0.4 at step II (P < 0.05) and 3.9 ± 0.4 at step III. Glucosyl units oxidized increased at each insulin level (P < 0.05; basal: 0.99 ± 0.23 mg·min⁻¹·kg⁻¹; step I: 2.31 ± 0.35; step II: 2.96 ± 0.49; step III: 4.38 ± 0.62). No glucose was stored as glycogen in the basal state; however, storage increased with increasing insulin level, and at step III 70 ± 2% of whole body glucose uptake from plasma was stored as glycogen. Whole body lipid oxidation decreased with increasing insulin level (basal: 0.83 ± 0.06; step I: 0.44 ± 0.09, P < 0.05; step II: 0.26 ± 0.08; step III: 0.15 ± 0.04 mg·min⁻¹·kg⁻¹, P < 0.05 vs. step I).

At basal, $V_{O_{2}}$ uptake in T and UT legs did not differ (2.1 ± 0.2 and 2.5 ± 0.3 ml·min⁻¹·kg⁻¹, respectively; P > 0.05), and $V_{O_{2}}$ increased in parallel during the clamp and was significantly higher (P < 0.05) at the highest insulin level than at basal [step I: 3.0 ± 0.5 (T), 2.5 ± 0.3 (UT); step II: 3.4 ± 0.5 (T), 3.0 ± 0.4 (UT); step III: 3.3 ± 0.2 (T), 3.2 ± 0.4 (UT) ml·min⁻¹·kg⁻¹]. Oxidation of glucosyl units in T and UT legs did not differ at basal (Fig. 4) and always increased at step I, the more so in T compared with UT legs (Fig. 4). From step II, glucosyl oxidation was constant and did not differ between legs (Fig. 4). Glucose storage as glycogen increased with increasing insulin level in both T and UT legs (P < 0.05), and the absolute amounts tended to be higher in T compared with UT legs (Fig. 4). However, the fraction of glucose uptake stored was the same for T and UT legs at given insulin concentrations [step I: 57 ± 10 (T) and 57 ± 18% (UT); step II: 67 ± 7 (T) and 67 ± 6% (UT); step III: 73 ± 3 (T) and 76 ± 4% (UT)]. At basal lipid was oxidized to the same extent in T and UT legs (0.7 ± 0.1 vs. 0.8 ± 0.1 mg·min⁻¹·kg⁻¹, respectively). At the lowest insulin level (step I) lipid oxidation was unaltered in both legs compared with basal, but in both legs lipid oxidation decreased in steps II and III to levels that could not be distinguished from zero.

Muscle Enzymes and Metabolites

The activity of cytochrome-c oxidase was 51 ± 9% higher in the T compared with the UT leg (P < 0.05; Table 5). The hexokinase enzyme activity and the preclamp glycogen concentration were similar in T and UT legs (Table 5).

Heart Rate and Blood Pressure

Heart rate and blood pressure did not differ between clamps I and II, and the results are presented as pooled data. Heart rate remained constant at submaximal insulin levels, but increased at the highest insulin level (P < 0.05) (basal: 62 ± 4, step I: 63 ± 3, step II: 64 ± 4, and step III: 72 ± 5 beats/min). A similar pattern was seen for systolic blood pressure, whereas the diastolic blood pressure did not change during the clamps (basal: 138 ± 3/75 ± 2, step I: 141 ± 2/77 ± 3, step II: 142 ± 4/74 ± 2, and step III: 151 ± 4/74 ± 3 mmHg).

DISCUSSION

The present study has shown that in humans physical training increases sensitivity and responsiveness of insulin-mediated glucose uptake in skeletal muscle by local mechanisms. Glucose storage (P < 0.1) as well as glycolysis (P < 0.05) are increased after training. At submaximal insulin levels increases in both lactate production and glucose oxidation contribute to the augmented glycolysis, whereas at high insulin concentrations only lactate production is higher in trained than in untrained muscle. Also, in proportion to glucose uptake lactate production is higher in trained than in untrained insulin-stimulated muscle. The effect of training on insulin-mediated glucose disposal in muscle is a genuine adaptation to repeated exercise and not merely a consequence of the last exercise bout. However, the effect is short lived, being insignificant after 6 days of detraining. Finally, insulin clearance in muscle is not influenced by training.

We used a one-legged exercise protocol, and the finding of increased vastus lateralis cytochrome-c oxidase concentration (Table 5) and one-legged $V_{O_{2}max}$ and decreased plasma lactate and cardiorespiratory responses to submaximal exercise (Table 2) after training testified to marked adaptations in muscle of the trained leg. The fact that a slight reduction in heart rate response was

Table 5. Enzyme activity and glycogen concentration for vastus lateralis in trained and untrained legs

<table>
<thead>
<tr>
<th></th>
<th>Trained Leg</th>
<th>Untrained Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome-c oxidase, nmol·s⁻¹·mg⁻¹</td>
<td>2.0 ± 0.01†</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Hexokinase, μmol·min⁻¹·mg⁻¹</td>
<td>1.78 ± 0.17</td>
<td>1.62 ± 0.17</td>
</tr>
<tr>
<td>Glycogen, mmol·kg⁻¹</td>
<td>465 ± 26</td>
<td>405 ± 20</td>
</tr>
</tbody>
</table>

Values are mean ± SE per unit dry muscle weight. Biopsies were taken from vastus lateralis of quadriceps in basal state from 7 subjects who had performed 10 wk of endurance training with 1 leg (trained leg) while the other leg remained sedentary (untrained leg). † Different from untrained leg, P < 0.05.
seen even during work with the sedentary leg (Table 1) probably reflected central circulatory adaptations rather than alterations within the muscles of this leg (8). In accordance with previous findings heavy bicycle endurance training also resulted in some muscle hypertrophy (Table 2) that was not accompanied by a significant change in isokinetic strength (42). Estimated from CT scans and skinfold measurements the amount of fat tissue did not differ between the trained and the untrained leg (Table 2). This agrees with the view that possible generalized effects of training on fat and muscle, e.g., caused by hormonal changes, would be expected to result in identical changes in the two legs. From the presented evidence it can be concluded that the difference in insulin effect between the two legs reflected a difference between trained and untrained muscle. Furthermore, this training induced adaptation was caused by local contraction-dependent mechanisms.

In the rat, training has previously been shown to increase both insulin sensitivity and responsiveness of skeletal muscle (14, 24). Similar studies of trained human muscle have not been presented until now. However, in studies of pronounced physical inactivity, a condition in extreme contrast to the trained state, reduced glucose uptake in human muscle, has been found both at submaximal and supraphysiological insulin concentrations (31, 39). Studies of whole body glucose disposal in humans agree that training increases insulin sensitivity (26, 27, 33, 34). However, some studies have failed to demonstrate a significant training-induced increase in whole body insulin responsiveness (27), whereas other studies, in accordance with what would be expected from both muscle and fat cell (11, 41, 47) findings, did show such an effect (33, 34).

The higher apparent insulin sensitivity in the trained compared with the untrained leg might be due to higher insulin binding to receptors in the former leg. A higher insulin binding could result from an increased number or affinity of insulin receptors in trained muscle or from an increased muscle capillarization (2) reducing the insulin concentration gradient between plasma and sarcolemma. However, as evidence against a significant role of such mechanisms, insulin clearance was the same in the trained and the untrained leg at submaximal as well as at saturating insulin concentrations (Table 3). Insulin clearance in human muscle is also unaltered after inactivity (31, 39). In contrast to these findings in muscle, measurements of arterial concentrations of insulin and C-peptide in humans (17) as well as of insulin uptake in rat liver (48) have indicated that training increases hepatic insulin clearance. However, direct measurement of hepatic insulin binding has revealed no influence of training (5). Studies of the effect of training on insulin binding to rat skeletal muscle and fat cells have been conflicting, reporting for both tissues no increase (23, 49), a slight increase (10, 12, 47), and a significant increase (5, 18). In accordance with the present finding of unaltered insulin clearance in muscle after training, we have recently found that insulin binding to human muscle is not affected by training (Dela, unpublished data). Also indicating that the effect of training on glucose uptake instead reflects adaptations in postreceptor events, we found that glucose extraction from plasma was higher in the trained than in the untrained leg at both submaximal and maximal insulin concentrations. In this context it is interesting to note that in rat muscle training has been shown to increase the amount of glucose-transporting protein and the maximal insulin-stimulated glucose transport (36). Furthermore, physical inactivity is accompanied by changes in muscle opposite to those seen with training, i.e., reduced insulin-mediated transport (21) and extraction (31, 39) of glucose and diminished glucose transporter number (20).

In the present study glucose storage and glycolysis were increased in parallel at given insulin concentrations in trained compared with untrained muscle. This is in contrast to previous studies of trained rat muscle in which insulin-stimulated glucose uptake was primarily channeled into the glycolytic pathway (12, 14, 19, 24). This discrepancy may reflect that the earlier studies were carried out later after the last exercise bout than the present study. Accordingly, before experiments muscle glycogen levels had in the earlier studies probably become supranormal, resulting in inhibition of further glycogen synthesis (14, 24). However, because the fraction of glucose uptake undergoing glycolysis always decreased with increasing glucose uptake in the present study (Fig. 4), comparisons at given glucose uptake rates do, in agreement with the rat studies, in fact indicate that training facilitates glycolysis in proportion to glycogenesis. At submaximal insulin levels increases in both lactate production and glucose oxidation contributed to the augmented overall glycolysis after training, whereas at high insulin concentrations glucose oxidation was limited by metabolic rate and only lactate production was higher in trained than in untrained muscle (Fig. 4). Interestingly, at both submaximal and maximal insulin levels lactate production made up a higher fraction of glycolysis as well of overall glucose uptake in trained compared with untrained muscle (Fig. 4). This may be due to an increase in lactate dehydrogenase activity during training (44). Compatible with a training-induced enhancement of the glycolytic pathway, compared with the untrained state, release of lactate and alanine tended to be increased during insulin stimulation even after 6 days of detraining, at which time overall glucose uptake was not significantly enhanced. Again supplementing the present findings, physical inactivity has been shown to diminish insulin-mediated storage and oxidation of glucose as well as lactate production in human muscle (31).

In the untrained leg a single exercise bout did not influence the effect of insulin on glucose uptake, although intensity and duration of exercise as well as length of recovery period before study were the same as used for the trained leg. The difference in response between the trained and the exercised untrained leg could not be ascribed to differences in plasma levels of counterregulatory hormones, substrates, or electrolytes during insulin infusion. Thus the effect of training on insulin-mediated glucose disposal implied a genuine adaptation to repeated exercise. A single bout of exercise has previously been shown to increase insulin action in human muscle (3, 40). However, one of the previous studies (40) was in contrast
to the present study carried out only few hours after exercise with no intervening food intake, and it is known that postexercise carbohydrate feeding and glycogen replenishment decrease insulin-stimulated glucose uptake in muscle (7, 38). The other study was carried out 24 h after exercise (3), but the finding of increased insulin action depended entirely on an increase in muscle blood flow (3), the existence of which is inconsistent with general beliefs (40).

The training-induced enhancement of insulin action in muscle was no longer significant after 6 days of detraining (Fig. 3). In contrast, whole body studies of trained humans have shown that although insulin sensitivity wanes rapidly (6, 26, 34), responsiveness does not decrease after 5 days of detraining (26, 34). At that time the effect of insulin on hepatic glucose metabolism is probably the same as in untrained subjects (24, 34). However, in line with the whole body studies a more persistent increase in maximal insulin-mediated glucose uptake has been found in fat cells after cessation of training (11).

In agreement with the supposition that one-legged training should influence fat tissue, the two legs, release of FFA and glycerol decreased similarly in the trained compared with the untrained leg in response to insulin. The net balance of tyrosine, which reflects net protein breakdown as tyrosine is neither synthesized nor catabolized by muscle, was also identical in the two legs and not influenced by insulin infusion. This supports the view that insulin does not inhibit protein degradation in human leg muscle (29). Furthermore, by analogy to our findings selective adaptations in insulin-mediated glucose metabolism with no accompanying changes in amino acid metabolism have been claimed to develop in uremia (1) and obesity (22). However, during insulin infusion a lower tyrosine release has been found in control compared with immobilized legs in humans (39). Moreover, after training an increased responsiveness to insulin of net protein degradation in rat muscle has been found in vitro (13).

During insulin infusion leg blood flow and O₂ uptake as well as systolic blood pressure and heart rate always increased in the present study. Similar data have been presented earlier and no influence of inactivity on these variables have been found (31, 39, 40).

In conclusion, physical training increases the effect of insulin on glucose uptake in human skeletal muscle. The adaptation is due to local mechanisms depending on contractile activity during training. Glycolysis is enhanced in proportion to glycogen synthesis. Insulin clearance in muscle is not influenced by training.

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