Muscle net glucose uptake and glucose kinetics after endurance training in men


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Bergman, B. C., G. E. Butterfield, E. E. Wolfel, G. D. Lopaschuk, G. A. Casazza, M. A. Horning, and G. A. Brooks. Muscle net glucose uptake and glucose kinetics after endurance training in men. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E81–E92, 1999.—We evaluated the hypotheses that alterations in glucose disposal rate (Rd) due to endurance training are the result of changed net glucose uptake by active muscle and that blood glucose is shunted to working muscle during exercise requiring high relative power output. We studied leg net glucose uptake during 1 h of cycle ergometry at two intensities before training [45 and 65% of peak rate of oxygen consumption (V˙O2peak)] and after training [65% pretraining V˙O2peak, same absolute workload (ABT), and 65% posttraining V˙O2peak, same relative workload (RLT)]. Nine male subjects (178.1 ± 2.5 cm, 81.8 ± 3.3 kg, 27.4 ± 2.0 yr) were tested before and after 9 wk of cycle ergometer training, five times a week at 75% V˙O2peak. The power output that elicited 66.0 ± 1.1% of V˙O2peak before training elicited 54.0 ± 1.7% after training. Whole body glucose Rd decreased posttraining at ABT (5.45 ± 0.31 mg·kg⁻¹·min⁻¹) at 65% pretraining to 4.36 ± 0.44 mg·kg⁻¹·min⁻¹) but not at RLT (5.94 ± 0.47 mg·kg⁻¹·min⁻¹). Net glucose uptake was attenuated posttraining at ABT (1.87 ± 0.42 mmol·min⁻¹ at 65% pretraining and 0.54 ± 0.33 mmol/min) but not at RLT (2.25 ± 0.81 mmol/min). The decrease in leg net glucose uptake at ABT was of similar magnitude as the drop in glucose Rd and thus could explain dampened glucose flux after training. Glycogen degradation also decreased posttraining at ABT but not RLT. Leg net glucose uptake accounted for 61% of blood glucose flux before training and 81% after training at the same relative (65% V˙O2peak) workload and only 38% after training at ABT. We conclude that 1) alterations in active muscle glucose uptake with training determine changes in whole body glucose kinetics; 2) muscle glucose uptake decreases for a given, moderate intensity task after training; and 3) hard exercise (65% V˙O2peak) promotes a glucose shunt from inactive tissues to active muscle.

GLUCOSE DISPOSAL during exercise is dependent on power output and muscle mass recruited (31). Chronic endurance exercise training decreases glucose flux at a given absolute power output as observed first in rats (4) and reconfirmed in men (8, 14, 25) and women (13). However, it is unclear that active muscle is responsible for decreased whole body glucose uptake during given power outputs after training (16, 35, 36). Several studies reported unchanged leg net glucose uptake in trained compared with untrained subjects during 1 h of exercise by use of mass balance in humans (16, 36) and a nonmetabolizable glucose analog in rats (35). Only one study (30) reported significantly decreased net glucose uptake throughout exercise in humans after training. Others reported decreased net glucose uptake only during the 60th min of exercise (20) or a significant decrease at 10 min, with no further difference between trained and untrained subjects for the remaining 2 h of exercise (22). Despite increased GLUT-4 content in trained muscle (31) and increased insulin action after training (9), tracer-measured glucose disposal rate (Rd) is consistently decreased throughout exercise after endurance training (8, 14, 25). Thus there are inconsistencies between results obtained by systemic tracers and limb mass balance techniques, leaving open the role of noncontracting tissues in determining glucose flux during exercise.

In the absence of a study to determine the effects of exercise intensity and endurance training on the relationships among working muscle (exercising limb) net glucose uptake and whole body glucose kinetics, we undertook a longitudinal study on nine young men. Specifically, we sought to evaluate the hypothesis that alterations in active muscle glucose uptake with training determine the changes in whole body glucose kinetics. In addition, we sought to test the hypothesis that glycemia is maintained during exercise after training by a mechanism that shunts blood glucose from “inactive tissue,” thereby sparing glucose for working muscle (3).

METHODS

Subjects

Nine healthy sedentary male subjects aged 19–33 yr were recruited from the University of California, Berkeley campus by posted notices. Subjects gave informed consent, were considered untrained if they engaged in no more than 2 h of physical activity per week for 1 yr, and had a maximum oxygen consumption (V˙O2max) of <45 ml·kg⁻¹·min⁻¹. Subjects were included in the study if they had <25% percent body fat, were nonsmokers, were diet and weight stable, had a 1-s forced expiratory volume (FEV₁) of 70% or more of vital capacity, and were injury and disease free as determined by physical examination. This study was approved by the Committee for the Protection of Human Subjects at Stanford
Two days after the second trial, subjects began training on leg random order at 65% of pretraining $\dot{V}O_2^{peak}$ (ABT) and 65% of oxygen consumption ($\dot{V}O_2^{peak}$) during leg cycle ergometry. Formed two graded exercise tests to determine the peak rate of oxygen consumption ($\dot{V}O_2^{peak}$) during leg cycle ergometry. 

Experimental Design

After interviews and preliminary screening, subjects performed two graded exercise tests to determine the peak rate of oxygen consumption ($\dot{V}O_2^{peak}$) during leg cycle ergometry. Subjects were then tested in a random order at 45 and 65% $\dot{V}O_2^{peak}$ with 1 wk between isotope trials (see Tracer Protocol). Two days after the second trial, subjects began training on leg cycle ergometers 5 days/wk for 9 wk at 75% $\dot{V}O_2^{peak}$. Midtraining stress tests and subsequent workload adjustments were performed to maintain relative training intensity at 75% $\dot{V}O_2^{peak}$. Posttraining isotope trials were also performed in a random order at 65% of pretraining $\dot{V}O_2^{peak}$ (ABT) and 65% of postraining $\dot{V}O_2^{peak}$ (RLT), corresponding to the same absolute and relative exercise intensities before and after training, respectively. Subjects continued training during the 1 wk separating the two postraining isotope trials.

Preliminary Testing

All exercise tests were performed on an electronically braked cycle ergometer (Monark Ergometric 829E). For determination of $\dot{V}O_2^{peak}$, exercise started at a power output of 50 W, which was increased by 25 or 50 W every 3 min until exhaustion. Respiratory gases were analyzed via an indirect open circuit system (Ametek S-3A1 O$_2$ and Ametek CD-3A CO$_2$ analyzers) and recorded by an on-line, real time PC-based system. During the second maximal exercise test performed in the metabolic ward at the Palo Alto Veterans Affairs Center, a catheter was placed in an antecubital vein for withdrawal of blood for lactate threshold determination as well as routine blood analysis. Body composition was determined via both skinfold measurements (18) and underwater weighing. Three-day diet records were kept to obtain baseline and relative exercise intensities before and after training. Dietary analysis was performed with the Nutritionist III software (N-Squared Computing, Salem, OR). FEV$_1$ was determined via a 9L spirometer.

Testing Protocol

The night preceding each isotope trial, subjects were admitted to the metabolic ward where they remained until testing was completed the following day. Subjects were fed a standardized dinner (1,174 kcal: 66% carbohydrate, 21% fat, 13% protein), which was replicated the night before each experimental trial. Later that evening, subjects ate a standardized snack (500 kcal: 53% carbohydrate, 31% fat, and 16% protein) before retiring. Two subjects were tested per day with morning and afternoon testing randomly assigned to each subject for the first trial and replicated for all subsequent trials. Morning procedures started at 7 AM, whereas preliminary afternoon procedures began at 1 PM. Morning subjects ate a standardized pretrial meal with a calculated low glycemic index (11) (448 kcal: 72% carbohydrate, 10% fat, 18% protein) at 6 AM, 1 h before procedures started and 4.5–5 h before exercise. Afternoon subjects ate a standardized breakfast in the morning (729 kcal: 57% carbohydrate, 33% fat, 10% protein) and the standardized pretrial meal at noon, again 1 h before procedures began and 4.5–5 h before exercise.

Catheterizations

After local lidocaine anesthesia, the femoral artery and vein of the same leg were cannulated with standard percutaneous techniques as previously described (38). A 5.1-Fr, 50-cm, Cordis arterial flush catheter was inserted 25 cm and positioned in the distal abdominal aorta via the femoral artery. A 6-Fr thermolibel venous catheter (model 93–135–6F, American Edwards Laboratories) was placed with the tip in the distal iliac vein through a venous sheath in the femoral vein 20 cm from the skin. Both catheters were sutured to the skin and further secured by an Ace bandage wrap. The external portions of each catheter were directed toward the hip for easy access during exercise. Alternate legs were used for the two trials during both pretraining and postraining testing. One subject experienced blood leaking from catheter placements during the beginning minutes of exercise at 65% pretraining and did not perform further exercise. Two different subjects did not receive a venous catheter for one of their trials. As a result, a sample size of 6–9 was used for calculations and comparisons.

Tracer Protocol

A venous catheter was placed in an antecubital vein the morning of each trial for isotope infusion during 90 min of rest and 1 h of exercise. Background blood and breath samples were collected after catheterization of the femoral artery and vein. Subjects then received a primed continuous infusion of [6,6-2H]glucose and [3-13C]lactate while resting semisupine for 90 min. Lactate kinetics are to be reported separately. The priming bolus was equal to 125 times the resting glucose infusion rate. [6,6-2H]glucose was infused via an Intelligent pump 522 (Kendall McGaw, Irvine, California) at 2 mg/min at rest, 6 mg/min during exercise at 45% pretraining $\dot{V}O_2^{peak}$ and 65% $\dot{V}O_2^{peak}$ postraining (ABT), and 8 mg/min at 65% pretraining and 65% postraining $\dot{V}O_2^{peak}$ (RLT). These increases in tracer infusion were designed to elicit similar arterial enrichments between exercise intensities during the last 30 min of exercise. The tracer protocol was successful because arterial enrichments were not significantly different between exercise intensities during the last 30 min of exercise, which were used to calculate glucose kinetics. All isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 9% sterile saline, and tested for sterility and pyrogenicity before use (University of California School of Pharmacy, San Francisco, CA).

Muscle Biopsy and Analysis

Immediately after the start of the isotope infusion, one vastus lateralis muscle was prepared for percutaneous needle biopsy. For each experimental trial, biopsies were taken from two locations separated by 1.5 cm: the distal site for preexercise sampling and the proximal site for immediate postexercise sampling. Right and left vastus lateralis muscles were alternated between trials. Biopsies taken at rest and within 10 s of exercise cessation were immediately plunged into liquid nitrogen and subsequently stored under liquid nitrogen and shipped on dry ice. Samples were analyzed for glycogen content as previously described (24).

Blood Sampling

Blood temperature was obtained from a thermistor at the end of the venous thermolibel catheter immediately before blood sampling. Arterial and venous blood samples were drawn simultaneously and anaerobically over 5 s after 75 and 90 min of rest and at 5, 15, 30, 45, and 60 min of exercise. PO$_2$, PCO$_2$, and pH were measured within 30 min of blood sampling (ABL 300, Radiometer, Copenhagen, Denmark). Blood for determination of glucose and lactate concentration and glu-
cose enrichment was immediately transferred to tubes containing 5% perchloric acid, shaken, and placed on ice. Arterial blood for hormone analysis was mixed with aprotonin, shaken, and placed on ice. After the final blood sample at the end of exercise, samples were centrifuged at 3,000 g for 10 min, and the supernatant was transferred to storage tubes and frozen at −80°C until analysis. Hematocrit measurements were performed on both arterial and venous blood with the microhematocrit method. Whole blood hemoglobin concentration was determined on each arterial and venous sample with the cyanmethemoglobin method.

Hemodynamics

Heart rate and electrocardiogram (ECG) were continuously recorded and displayed with a three-lead ECG connected to a MacLab analog-to-digital converter (ADInstruments, Castle Hill, Australia) and tracked on a Macintosh 7200/200 Power Mac computer (Apple Computer, Cupertino, CA). Arterial blood pressure was also continuously recorded and displayed with a Transpacc pressure transducer (Baxter) positioned at the level of the heart connected to the MacLab system and calibrated before every trial. Iliac venous blood flow was determined by thermodilution technique with a cardiac output computer (model 9520, American Edwards Laboratories) with a 10-ml bolus injection of sterile saline cooled to 0°C via an ice slurry (American Edwards Laboratories). Measurements were made in triplicate or quadruplicate during rest and exercise immediately after blood sampling. The validity and precautions associated with this technique have been described previously (2).

Metabolite Analyses and Isotope Enrichment

Glucose concentration was measured in duplicate with a hexokinase enzymatic kit from Sigma Chemical (St. Louis, MO). Lactate concentration was measured in duplicate with the method of Gutmann and Wahlefeld (15) with lactate dehydrogenase. Glucose isotopic enrichment was measured with the use of gas chromatography-mass spectrometry (GC model 5890 series II and MS model 5989A, Hewlett-Packard) of the pentaacetate derivative as described previously (13).

Training Protocol

All training was performed on stationary cycle ergometers 5 days/wk with workloads adjusted to elicit heart rates, which were recorded daily, corresponding to the required intensity on the basis of maximal exercise test results. Subjects were asked to exercise 1 day/wk on their own in addition to cycle ergometry training so that total training was 6 days/wk. All subjects were exercising at 75% of their VO₂peak for 1 h by the end of the second week of training. After 4 wk of training, subjects performed another maximal exercise test to quantify increases in VO₂peak and training workloads were increased accordingly to maintain relative training intensity at 75% VO₂peak. Two weeks preceding posttraining testing, subjects began interval training during the last 10 min of each 1-h workout. Interval training was added to develop recruitment patterns conducive to reaching maximal power output during posttraining evaluation. Subjects continued training throughout the 1 wk between posttraining testing with 1 day of rest before an experimental trial and 2 days of rest after an experimental trial to recover from testing procedures. Subjects were weighed daily and asked to increase energy intake to maintain weight during the training program without changing normal macronutrient composition. Three-day diet records were collected after 4 wk of training and at the end of training to ensure maintenance of baseline diet composition.

Calculations

Leg respiratory quotient. Leg respiratory quotient (RQ) was calculated from the ratio of venous-arterial CO₂ difference (v-aCO₂) and arteriovenous O₂ difference (a-vO₂).

\[ RQ = \frac{v-aCO_2}{a-vO_2} \]

Blood CO₂ content. Blood PcO₂, PaO₂, pH, and hemoglobin (Hb) were measured on both arterial and venous samples and used in the calculations by Douglas et al. (10) for determination of blood CO₂ content, estimating CO₂ solubility and apparent dissociation constant from the equations of Kelman (21).

Blood O₂ content. Blood O₂ content was calculated with hemoglobin concentration, and saturation (SO₂) was determined from an equation from Nunn (26).

\[ VO_2 \text{ of the legs. Leg } O_2 \text{ was calculated with the Fick equation as follows:} \]
\[ \text{Leg } VO_2 = 2 \times \text{one leg } Q \times a-vO_2 \]

where Q is blood flow rate.

Whole body and leg carbohydrate oxidation. Total carbohydrate oxidation for both whole body [from respiratory exchange ratio (RER)] and exercising leg (from leg RQ) were determined from stoichiometric equations (12), assuming a whole body nitrogen excretion rate of 135 g kg⁻¹ min⁻¹ (7).

Glucose kinetics. Glucose rate of appearance (Ra), glucose disposal rate (Rd), and metabolic clearance rate (MCR) were calculated with equations defined by Steele and modified for use with stable isotopes (37).

\[ Ra \text{ (mg kg}^{-1} \text{min}^{-1}) = \frac{F - V[(C_1 + C_2)/2][(E_2 - E_1)/(t_2 - t_1)]}{[(E_1 + E_2)/2]} \]

\[ Rd \text{ (mg kg}^{-1} \text{min}^{-1}) = Ra - V[(C_2 - C_1)/(t_2 - t_1)] \]

\[ MCR \text{ (ml kg}^{-1} \text{min}^{-1}) = Rd[(C_1 + C_2)/2] \]

where F represents isotope infusion rate and IE₁ and IE₂ are glucose isotopic enrichments at sampling time points 1 (t₁) and 2 (t₂), respectively. C₁ and C₂ are glucose concentrations at t₁ and t₂, respectively, and V is the estimated volume distribution of glucose (180 ml/kg). All isotopic enrichments of glucose were corrected for background enrichments from blood samples taken before isotope infusion. Calculation of steady-state glucose kinetics was performed during the last 15 min of rest (75 and 90 min) and 30 min of exercise (30, 45, and 60 min).

Net metabolite exchange. Net metabolite exchange differences were calculated from the product of leg blood flow and arteriovenous differences where arterial and venous (superscripts a and v) hematocrit (Hct) values were used to correct for changes in plasma volume.

\[ \text{Net glucose uptake (mmol/min) = 2 (one leg } Q)[(\text{glucose}_a) - (\text{Hct}_a/Hct_v)[(\text{glucose}_v)] \]

Hormones. Radiimmunassays were performed to determine plasma concentrations of insulin (INCASTAR, Stillwater, MN) and glucagon (Diagnostic Products, Los Angeles, CA). The values reported on resting and exercising subjects are those that correspond in time to when glucose flux rates were calculated.
E84 LEG NET GLUCOSE UPTAKE AND GLUCOSE R<sub>d</sub>

Statistical Analyses

Significance of differences among mean arterial glucose and lactate concentrations from the last 30 min of exercise were analyzed with a one-factor ANOVA with repeated measures. Differences between groups and changes over time in RER, RQ, inactive tissue RQ, single leg blood flow, glucose arteriovenous difference, net uptake of glucose, glucose R<sub>a</sub> and R<sub>b</sub>, glucose MCR, and arterial enrichment were determined with a repeated-measures factorial ANOVA. Post hoc comparisons were made with Fisher’s protected least significant difference test. One-hour averages of insulin and glucagon concentrations, leg and whole body carbohydrate oxidation, percentage of glucose R<sub>a</sub> accounted for by net glucose uptake, and glycogen degradation were analyzed with paired Student t-tests. Statistical significance was set at α = 0.05. All data are presented as means ± SE.

RESULTS

Subject Characteristics

Anthropometric data for subjects pre- and posttraining are shown in Table 1. Subjects were weight stable throughout the study period, although percent body fat decreased significantly as determined by skinfold measurements (~2.2%) and underwater weighing (~1.7%). V<sub>O2peak</sub> significantly increased by 14.6% as a result of the training regimen. Consequently, posttraining trials at 66.0 ± 1.1% of pretraining V<sub>O2peak</sub> (the same absolute power output as pretraining) were performed at 54.0 ± 1.7% of posttraining V<sub>O2peak</sub>.

Muscle Glycogen Concentrations

Resting muscle glycogen concentration was significantly increased 46% after training and decreased during exercise at all intensities (Table 2). Endurance training attenuated muscle glycogen degradation at the same absolute power output (P < 0.05) by 33%. There were no differences in muscle glycogen degradation at RLT posttraining.

Insulin and Glucagon Concentrations

Resting arterial insulin concentration decreased (P < 0.05) 33% after training (Fig. 1A). Insulin concentrations during the last 30 min of exercise were not significantly different from rest posttraining at ABT or RLT. Posttraining glucagon concentrations during exercise were significantly different from rest posttraining at ABT and RLT compared with 65% pretraining (Fig. 1B). Insulin-to-glucagon ratios were unchanged during rest after training and decreased during exercise (P < 0.05), regardless of exercise intensity or training state (Fig. 1C). The insulin-to-glucagon ratio decreased at 65% pretraining compared with 45% pretraining and increased after training at ABT and RLT compared with 65% pretraining.

RER, Leg, and Inactive Tissue RQ

RERs during exercise were significantly increased at 65% pretraining (0.96 ± 0.01) compared with 45% pretraining (0.93 ± 0.01; Ref. 2). When subjects were retested after training at ABT, there was a significant decrease in RER (0.93 ± 0.01 at ABT). There were no differences in RER values when subjects were tested at the same relative exercise intensity after training (0.95 ± 0.01 at RLT). Leg RQ was not significantly different at rest before or after training. During exercise, leg RQ significantly increased from 45% (0.89 ± 0.05) to 65% pretraining (0.98 ± 0.02). There was no difference in leg RQ at ABT (0.98 ± 0.03) or RLT (1.01 ± 0.02) posttraining. From the difference between leg RQ and whole body RER, an RQ for the remainder of the body was calculated. Inactive tissue RQ increased with elevated exercise intensity before training (0.89 ± 0.04 at 45% and 0.98 ± 0.04 at 65% V<sub>O2peak</sub>) but decreased after training at ABT (0.83 ± 0.07) and RLT (0.83 ± 0.06). These data have been previously reported (2).

Glucose Kinetics

Our isotope infusion protocol was successful in promoting similar stable blood glucose enrichments despite different metabolic infusion rates pre- and post-

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Table 1. Subject characteristics before and after 9 wk of leg cycle endurance training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>Difference, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27.4 ± 2.0</td>
<td>27.1 ± 1.9</td>
<td>-0.6</td>
</tr>
<tr>
<td>Height, in.</td>
<td>70.1 ± 1.3</td>
<td>69.8 ± 1.2</td>
<td>-0.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.8 ± 3.3</td>
<td>81.3 ± 3.2</td>
<td>-0.6</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19.7 ± 1.5</td>
<td>19.5 ± 1.5</td>
<td>-0.9</td>
</tr>
<tr>
<td>Skin folds</td>
<td>19.5 ± 1.5</td>
<td>19.4 ± 1.5</td>
<td>-0.9</td>
</tr>
<tr>
<td>Underwater weighing</td>
<td>3.5 ± 0.10</td>
<td>4.0 ± 0.15</td>
<td>14.6</td>
</tr>
<tr>
<td>ml·kg&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>43.5 ± 1.3</td>
<td>50.1 ± 1.6</td>
<td>15.5</td>
</tr>
<tr>
<td>%V&lt;sub&gt;O2peak&lt;/sub&gt;</td>
<td>60.9 ± 2.7</td>
<td>65.4 ± 2.6</td>
<td>4.9</td>
</tr>
<tr>
<td>W</td>
<td>161.1 ± 4.4</td>
<td>197.2 ± 6.5</td>
<td>22.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. V<sub>O2peak</sub>, peak O<sub>2</sub> consumption.

*Significantly different from pretraining values at P < 0.05.

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Table 2. One-leg muscle glycogen concentration

<table>
<thead>
<tr>
<th>Glycogen, µmol/wet wt</th>
<th>45% Pretraining</th>
<th>65% Pretraining</th>
<th>ABT Posttraining</th>
<th>RLT Posttraining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>94.2 ± 13.8</td>
<td>94.2 ± 13.8</td>
<td>152.7 ± 11.4§</td>
<td>152.7 ± 11.4§</td>
</tr>
<tr>
<td>Postexercise</td>
<td>75.9 ± 7.7</td>
<td>42.4 ± 8.9*</td>
<td>109.2 ± 23.9*</td>
<td>86.1 ± 13.6*</td>
</tr>
<tr>
<td>Δ</td>
<td>28.5 ± 11.7</td>
<td>56.2 ± 10.1†‡</td>
<td>43.6 ± 21.9†‡</td>
<td>66.6 ± 22.3†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 9. ABT, absolute workload (65% pretraining V<sub>O2peak</sub>); RLT, relative workload (65% posttraining V<sub>O2peak</sub>). *Significantly different from rest at P < 0.05. †Significantly different from 45% pretraining at P < 0.05. ‡Significantly different from 65% pretraining at P < 0.05. §Significantly different between resting conditions at P < 0.05.

46 and 69% at 45% pretraining and 65% pretraining, respectively, and 36 and 38% at ABT and RLT, respectively (Fig. 1A). Pretraining, insulin concentration significantly decreased at 65% V<sub>O2peak</sub> compared with 45% V<sub>O2peak</sub> and was unchanged between postraining exercise intensities. Resting arterial glucagon concentration decreased (P < 0.05) 22% after training (Fig. 1B). Glucagon concentration during the last 30 min of exercise did not change from rest at 45% pretraining but significantly increased 28% at 65% pretraining. Glucagon concentrations during the last 30 min of exercise were not significantly different from rest postraining at ABT or RLT. Posttraining glucagon concentrations during exercise were significantly decreased at ABT and RLT compared with 65% pretraining (Fig. 1B). Insulin-to-glucagon ratios were unchanged during rest after training and decreased during exercise (P < 0.05), regardless of exercise intensity or training state (Fig. 1C). The insulin-to-glucagon ratio decreased at 65% pretraining compared with 45% pretraining and increased after training at ABT and RLT compared with 65% pretraining.
training (Fig. 2). Resting glucose $R_a$ and $R_d$ were not changed by training, and flux rates scaled to exercise intensity both before and after training (Figs. 3, A and B). After training, whole body glucose $R_a$ and $R_d$ decreased 23 and 20%, respectively, at ABT but were not significantly different at RLT (Fig. 3, A and B). There was no effect of training on resting glucose MCR, but glucose MCR increased in the transition from rest to exercise under all conditions and scaled to exercise intensity before and after training (Fig. 3C).

**Arterial Glucose Concentration and Net Uptake by the Legs**

Rest and exercise arterial glucose concentrations were similar before and after training, regardless of exercise intensity (Fig. 4A). Glucose arteriovenous differences were not different between training states at rest or during exercise before training (Fig. 4B). However, posttraining glucose arteriovenous differences were significantly ($P < 0.05$) lower at ABT compared with RLT and 65% pretraining (Fig. 4B). Expressed as a percentage of total vascular delivery, glucose fractional extraction during exercise was not significantly different between the two pretraining exercise intensities (2.3 ± 0.82% for 45% pretraining and 3.8 ± 0.75% for 65% pretraining; Fig. 4C). After training, fractional extraction of glucose for the hour of exercise was decreased at ABT (0.91 ± 0.60%) but not RLT (3.7 ± 1.2%) compared with before training (Fig. 4C). Single leg blood flow was not different at rest between training conditions (Fig. 4B).
states, and blood flow scaled to exercise intensity, before and after training (Fig. 4D). Leg blood flow was significantly (P < 0.05) greater at ABT compared with 65% VO2peak pretraining. Leg net glucose uptake at rest was similar before (0.35 ± 0.28 mmol/min) and after training (0.26 ± 0.06 mmol/min; Fig. 4E). After training, leg net glucose uptake at ABT was significantly decreased compared with before training (1.87 ± 0.42 mmol/min at 65% pretraining and 0.54 ± 0.33 mmol/min at ABT; Fig. 4E). However, leg net glucose uptake was similar in the trained and untrained states at the equivalent percentage of maximal effort (2.25 ± 0.81 mmol/min at RLT).

Figure 5 displays the relationship between exercise intensity and the relative role played by exercising legs in removing blood glucose. At rest, leg net glucose uptake accounted for 24% of blood glucose Rd at 65% VO2peak pretraining and 19% posttraining. For a given power output, the percentage of glucose Rd accounted for most (81%) of glucose Rd. However, at RLT, our data indicate the legs tended to fall from 61% at 65% pretraining to 38% at ABT. Nonetheless, leg net glucose uptake was similar in the trained and untrained states at the equivalent percentage of maximal effort (2.25 ± 0.81 mmol/min at RLT).

Our experiments were designed to reveal the effects of exercise and training on substrate utilization. We employed experimental and nutritional controls appropriate to represent practices and conditions typical in the population at large. For these reasons, we fed subjects to be weight stable and rested them the day before experimentation. Furthermore, we fed them standardized meals with calculated low glycemic index 4.5–5.5 h before exercise studies. Those efforts produced stable blood glucose levels during exercise, suggesting that subjects commenced exercise with normal liver glycogen reserves. Thus the effects on substrate utilization we observed are attributable to exercise intensity and endurance training and are not confounded by undernutrition, liver glycogen depletion, or hypoglycemia.

DISCUSSION

Our training program was successful in promoting significant metabolic adaptations (Tables 1 and 2; Fig. 1A). During our 9-wk training program, subjects significantly increased VO2peak (15%), decreased RER at a given absolute power output (3.2%), increased lactate threshold (60.9% pretraining vs. 65.4% VO2peak postraining), decreased arterial lactate concentration at the same relative (26%) and absolute (55%) exercise intensities (2), and increased resting muscle glycogen concentration (62%).

Nutritional Controls

Our experiments were designed to reveal the effects of exercise and training on substrate utilization. We employed experimental and nutritional controls appropriate to represent practices and conditions typical in the population at large. For these reasons, we fed subjects to be weight stable and rested them the day before experimentation. Furthermore, we fed them standardized meals with calculated low glycemic index 4.5–5.5 h before exercise studies. Those efforts produced stable blood glucose levels during exercise, suggesting that subjects commenced exercise with normal liver glycogen reserves. Thus the effects on substrate utilization we observed are attributable to exercise intensity and endurance training and are not confounded by undernutrition, liver glycogen depletion, or hypoglycemia.
Limb Net Glucose Uptake

Pre- and posttraining values for leg net glucose uptake at rest (0.35 ± 0.28 mmol/min pretraining and 0.26 ± 0.06 mmol/min posttraining) were similar to literature values (1, 16, 19, 20, 22, 27, 29, 32, 36), which range from 0.15 ± 0.03 mmol/min (32) to 0.22 ± 0.03 mmol/min (1). The magnitude of pre- and posttraining exercise net glucose uptakes was also similar to literature values (1, 16, 19, 20, 27, 29). However, regarding the effects of training, our data are similar to some (20, 30) but not others (16, 33). We found decreased leg net glucose uptake for a given absolute power output after training (Figs. 4E and 6). Henriksson (16) and Saltin et al. (33), however, reported unchanged leg net glucose uptake after training during leg cycle ergometry at the same absolute intensity. Our results may differ from others due to type of training (one-legged in Saltin et al., two-legged in present study), length of training (4 wk in Saltin et al., 9 wk in present study), and nutritional state [12- to 14-h fast in Henriksson (16), 4.5- to 5.5-h fast in present study]. Our data are similar to those of Jansson and Kaijser (20) and Richter et al. (30) who reported decreased leg net glucose uptake during exercise at the same absolute intensity after training. Thus changes in leg net glucose uptake due to endurance training (i.e., decreased net glucose uptake at ABT with no change at RLT compared with pretraining) are similar to alterations in whole body glucose flux (see Whole Body Glucose Disposal . . .).

Dampened active muscle glucose uptake is often assumed to cause decreased glucose $R_d$ at ABT after
endurance training (8, 25). However, we are the first to measure net glucose uptake and glucose turnover at absolute and relative exercise intensities to provide evidence that changes in active muscle glucose uptake can explain altered whole body $R_d$. After training, working muscle glucose uptake at ABT decreased sufficiently to attenuate whole body glucose turnover (Fig. 6). Thus, even though glucose turnover is a whole body
measure, our data suggest it is appropriate to explain dampened glucose turnover by decreased working muscle glucose uptake. However, our data do not rule out the possibility that decreased inactive tissue carbohydrate oxidation may also contribute to dampened glucose $R_d$.

It is feasible to consider the possibility that attenuated glucose uptake in inactive tissues contributes to decreased whole body glucose $R_d$ during given power outputs after training. As determined from the difference between whole body RER and leg RQ, inactive tissue RQ decreased during exercise after training. However, interpretation of RQ measurements in terms of glucose sparing is limited because of the inability to determine sources of carbohydrate oxidized. For instance, after training arterial lactate concentration was lower, and so decreased lactate uptake and oxidation by inactive tissues could have explained the lower RQ. Furthermore, on the basis of observations of unchanged or slightly increased plasma insulin concentrations during exercise after training (Fig. 1A) and constant blood glucose concentration (Fig. 4A), it is unlikely that blood glucose uptake decreased in inactive tissue at ABT. More likely, decreased inactive tissue carbohydrate oxidation may be due to reduced glycogenolysis in inactive muscle (1), potentially due to dampened catecholamine concentrations (5). Regard-

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**Fig. 5.** Percentages of glucose $R_d$ accounted for by leg net glucose uptake as a function of relative exercise intensity during rest and exercise, before and after training. See legend to Fig. 1 for details. Values are means of last 30 min of exercise for glucose $R_d$ and 1 h of exercise for net glucose uptake ± SE; n = 6–8.

**Fig. 6.** Leg and whole body carbohydrate (CHO) metabolism rates determined from leg respiratory quotient (RQ), whole body respiratory exchange ratio (RER), and net glucose uptake during exercise, before and after training. Values are means ± SE; n = 6–8. RER, leg RQ, net glucose uptake, and glycogen degradation are means of entire exercise bout. Leg (A) and whole body (B) CHO oxidation (ox.) calculated from stoichiometric equations. Net glucose uptake (C) calculated as in METHODS. Glycogen degradation (Deg.; D) calculated from difference between pre- and postexercise muscle biopsies. E: glucose $R_d$. Summation of glycogen degradation and net glucose uptake after subtracting a fraction of each corresponding to net lactate release (F).
less of the explanation for a decrease in carbohydrate oxidation by inactive tissues during leg cycling after training, our data indicate that inactive tissue carbohydrate oxidation is small in comparison with total body carbohydrate oxidation during exercise. Therefore, the component of glucose $R_d$ attributable to inactive tissue is not likely to have had major effects on blood glucose kinetics (Fig. 6).

Given adaptations to training that appear to increase glucose utilization, such as increased GLUT-4 content and hexokinase activity (5, 30), an explanation for decreased leg net glucose uptake for a given power output after training is not apparent. It is possible, however, that increased GLUT-4 content after endurance training is more important during recovery after exercise, to maximize glycogen stores in preparation for the next exercise bout. Negative correlations between leg net glucose uptake and insulin concentration ($r = -0.75$) and between glucose uptake and insulin-to-glucagon ratio ($r = -0.73$) (Figs. 1A, 1C, 4C, and 6) suggest insulin is not likely to be a major determinant of leg net glucose uptake in working muscle. Because endurance training increases insulin sensitivity (9), decreased leg net glucose uptake at ABT with unchanged or slightly increased insulin concentration (Fig. 1A) suggests that insulin-independent mechanisms determine working muscle glucose uptake. Possibly, factors such as decreased GLUT-4 translocation to the sarcolemma (30), decreased malonyl-CoA concentration or carnitine palmitoyltransferase 1 affinity for malonyl-CoA (23), or increased lactate uptake (30) are more influential than circulating insulin concentration in dampening leg glucose uptake during exercise at a given power output after training.

Whole Body Glucose Disposal and Leg Net Glucose Uptake

Our results of whole body glucose turnover are similar to others who have found decreased glucose $R_a$ and $R_d$ at the same absolute (8, 14, 25) but not the same relative exercise intensity (14) after endurance exercise training. Our results are also consistent with the idea that relative exercise intensity is a main determinant of glucose turnover (6), because there were no differences in glucose turnover at the same percentage of $V_O^2_{peak}$, either before and after training.

Even though glucose $R_d$ (and net glucose uptake, as we have shown) scale to relative exercise intensity before and after training, the gain in glucose disposal is low compared with the gain in overall metabolic rate and carbohydrate oxidation during exercise. For instance, before training, exercise at 65% $V_O^2_{peak}$ elicited a sevenfold increase in $V_O^2$ and an eightfold increase in total carbohydrate oxidation compared with rest. Under this condition, glucose $R_d$ doubled. After training, exercise at 65% $V_O^2_{peak}$ elicited eight- and ninefold increases in $V_O^2$ and total carbohydrate oxidation, respectively, compared with rest. Again, glucose $R_d$ little more than doubled during exercise compared with rest. Thus it is clear that although blood glucose flux and oxidation scale to exercise intensity, both before and after training, other carbohydrates are relatively more important as energy sources.

Sparing of glucose uptake by inactive tissues and redirection of hepatic glucose production to working legs during leg cycling exercise were not apparent at moderate power outputs. Leg glucose uptake accounted for only 48% of blood glucose $R_d$ at 45% $V_O^2_{peak}$ before training and 38% at ABT after training. The decline in the percentage of glucose $R_d$ accounted for by net glucose uptake from 61% at 65% pretraining to 38% at ABT was impressive because one leg blood flow increased significantly from 5.2 ± 0.3 to 5.8 ± 0.2 l/min at ABT (Fig. 4D; Ref. 2). Thus the decline in leg net glucose uptake for a given absolute exercise power output after training (Fig. 4E) was the result of decreased glucose extraction (Fig. 4C) not altered vascular conductance.

Under some conditions, increased blood flow from insulin-stimulated nitric oxide release (34) may enhance glucose uptake. However, we observed that during exercise after training leg net glucose uptake decreased at ABT (Fig. 4E) despite significantly greater leg blood flow (Fig. 4D). Moreover, the observations of unchanged and low arterial insulin concentrations during exercise at ABT compared with 65% pretraining (Fig. 1A) are interpreted to suggest that elevated blood flow during ABT after training was not likely due to insulin-stimulated nitric oxide release. Consistent with our observations, Pendergrass et al. (28) reported that elevated blood flow from insulin-like growth factor I infusion did not increase skeletal muscle glucose uptake. Thus it appears that decreased muscle glucose uptake at a given power output after training cannot be ascribed to alterations in blood flow. Our data are consistent with an intramuscular effect of training (e.g., decreased GLUT-4 translocation) reducing muscle glucose uptake during exercise at a given, moderate intensity power output (30).

Our data support the concept of glucose shunting to active muscles at high relative power outputs both before and after training (Fig. 5). At the same relative intensity (65% $V_O^2_{peak}$), leg net glucose uptake accounted for 61% of blood glucose $R_d$ pretraining, which increased to 81% after training (Fig. 5). Posttraining, the new exercise power output during RLT was 16% (25 W) greater than during the pretraining 65% $V_O^2_{peak}$ task, and limb blood flow increased 20% to 7.0 ± 0.3 l/min, while net glucose uptake was unchanged compared with 65% pretraining. Thus, when viewed from the perspective of relative power output, after training the working limb appears to respond essentially as before training.

Glycogen Degradation

Consistent with results of others (20, 22, 33, 36), our training program resulted in attenuated muscle glycogen degradation at the same absolute power output as before training (Table 2; Fig. 6). Henriksson (16) reported average glycogen degradation rates for 1 h of exercise at 0.82 µmol·g wet wt⁻¹·min⁻¹ for untrained and 0.7 µmol·g wet wt⁻¹·min⁻¹ for trained subjects.
Our glycogen degradation data were similar at 1.09 ± 0.17 µmol·g wet wt⁻¹·min⁻¹ during 65% pretraining and 0.73 ± 0.36 µmol·g wet wt⁻¹·min⁻¹ at ABT posttraining. Attenuated glycogenolysis after endurance exercise training may be due to altered adenine nucleotide and inorganic phosphate levels related to increased respiratory control.

We do not know of any other report that compared muscle glycogen degradation rates at a given relative exercise intensity before and after training. Most investigations analyzed muscle glycogen degradation at the same absolute power output as before training. However, Hultman and Spriet (17) compiled data from many training studies and plotted glycogen degradation rates during exercise relative to work intensity performed (VO₂max). The exponential relationship suggested similar rates of glycogen degradation between subjects of different athletic histories at a given percentage of maximal effort, supporting relative exercise intensity as a critical factor determining rate of glycogen degradation (Table 2; Fig. 6) (17). Thus our investigation is the first to provide data with muscle biopsies showing that rates of glycogen degradation are equivalent in the trained and untrained state when exercise is performed at similar relative intensities.

In the present and companion report (2), we provided data on the effects of exercise and training on limb and whole body substrate utilization; those data were obtained with diverse technologies, each with inherent and unique assumptions and limitations. Results of working limb muscle glycogen degradation (Fig. 6D) and leg net glucose uptake (Fig. 6E) when summed and corrected for net lactate release (Fig. 6F) show remarkable consistency to data derived from leg RQ (Fig. 6A) and whole body RER (Fig. 6B). The aggregated data provide a consistent picture showing preferential use of carbohydrate-derived fuels at high relative power output regardless of training state.

In conclusion, whether data were obtained by pulmonary indirect calorimetry, measurements of arteriovenous differences of glucose, CO₂ and O₂, isotope tracers, or muscle biopsies, the results of the present study (Fig. 6) show that high power output exercise depends on carbohydrate-derived fuel sources regardless of training state. Thus relative exercise intensity emerges as a major predictor of substrate utilization pattern. Leg net glucose uptake, glycogenolysis, and whole body glucose R₆ decrease for a given submaximal power output after training. Because changes were accomplished in an environment of unchanged circulating insulin concentration, intracellular signals must be responsible for the training effect, rendering working muscle less sensitive to insulin during moderate intensity exercise. Results of the present study support the concept that blood glucose is shunted from inactive tissues to active muscle during hard (65% VO₂peak) exercise, regardless of training state. Additionally, our data indicate that decreased working muscle glucose uptake at a given absolute power output postraining explains attenuated whole body glucose R₆. Although blood glucose may be directed to working muscle during exercise involving recruitment of a large fraction of total body muscle mass, the gain in net glucose uptake by working muscle during hard exercise is small compared with the gain in total body carbohydrate oxidation. Thus the apparent sparing of glucose uptake by inactive tissues and the low gain in muscle glucose uptake in response to gradations in power output are viewed as the means to maintain glycemia during hard exercise, which requires high rates of carbohydrate oxidation.

We thank the subjects for participating in our study and complying with the training program. The assistance of the nursing staff and dietitians at the Geriatric Research, Education, and Clinical Center in the Palo Alto Veterans Affairs Health Care System is appreciated. We also thank David Guido for performing muscle biopsies and Jacinda Mawson for blood gas analysis. We thank the student trainers who were vital in subject training and transport. We greatly appreciate the help of Barry Braun and Shannon Dominic in blood sampling during these trials.

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-42906 and National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-19577.

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Received 22 October 1998; accepted in final form 9 March 1999.

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