Training-induced alterations of glucose flux in men

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Training-induced alterations of glucose flux in men. J. Appl. Physiol. 82(4): 1360–1369, 1997.—We examined the hypothesis that glucose flux was directly related to relative exercise intensity both before and after a 10-wk cycle ergometer training program in 19 healthy male subjects. Two pretraining trials (45 and 65% of peak O2 consumption [VO2peak]) and two posttraining trials (same absolute and relative intensities as 65% pretraining) were performed for 90 min of rest and 1 h of cycling exercise. After training, subjects increased VO2peak by 9.4 ± 1.4%. Pretraining, the intensity effect on glucose kinetics was evident with rates of appearance (Ra), disappearance (Rd), oxidation (Rox), and metabolic clearance (7.03 ± 0.56 vs. 5.20 ± 0.28 ml·kg⁻¹·min⁻¹) of glucose being significantly greater (P < 0.05) in the 65% than the 45% VO2peak trial. When Rd was expressed as a percentage of total energy expended per minute (RdE), there was no difference between the 45 and 65% intensities. Training did reduce Ra (4.63 ± 0.25), Rd (4.65 ± 0.24), Rox (3.77 ± 0.43), and RdE (15.30 ± 0.40 to 12.85 ± 0.81) without any significant alterations in mitochondrial respiratory capacity (30). Those testing subjects at the same absolute workload after training found decreased glucose utilization, whereas those testing subjects at the same relative workloads did not observe decreased glucose uptake. Because circulating hormone levels and intramuscular factors that influence glucose flux are closely tied to relative work intensity for both untrained and trained individuals (11), endocrine factors may cause glucose flux to be more closely related to relative than absolute exercise intensity. Studies that use two-leg training and one-leg testing protocols confirm the importance of training-induced alterations in the endocrine regulation of glucose. Also, short-term training studies have demonstrated decreased glucose utilization during exercise when expressed as relative vs. absolute intensity; exercise crossover concept.

FACTORS THAT INFLUENCE the patterns of substrate utilization during exercise of different intensities are numerous, and there is controversy over the effects of exercise intensity and prior endurance training on these patterns. Some believe that training decreases blood glucose utilization and increases lipid utilization (4, 6, 14, 17), whereas others (24) have shown glucose rate of appearance (Ra) to be higher in athletes than nonathletes during hard and maximal exercise. In addition, studies that have employed a one-leg training regimen followed by a two-leg test have obtained ambiguous results regarding the effects of training on limb glucose uptake. For example, Kiens et al. (23) demonstrated that glucose uptake was only transiently lower in the trained leg and there were no differences in lactate release or muscle triglyceride use between trained and untrained legs working at a given power output. Two additional studies used one-leg training protocols but tested subjects at the same relative workloads either as determined by percentage of one-leg maximal O2 consumption (VO2max) (16) or corrected for leg muscle volume (10). Those studies showed an increase in trained leg glucose uptake that was, in part, responsible for the muscle glycogen sparing observed in the trained leg. Similar results were also found in treadmill-trained rats, in which glucose uptake was the same or higher posttraining whereas levels of muscle glycogenolysis were decreased (12, 37).

The studies mentioned above differ in their methodologies as well as in their observations regarding glucose utilization after training. For example, in the studies that reported decreased glucose utilization, whole body glucose kinetics at a given power output was measured, whereas most of the studies showing constant or increased utilization measured limb net glucose uptake. In addition, the testing protocols used by investigators differed in the workload selection. Those testing subjects at the same absolute workload after training found decreased glucose utilization, whereas those testing subjects at the same relative workloads did not observe decreased glucose uptake. Because circulating hormone levels and intramuscular factors that influence glucose flux are closely tied to relative work intensity for both untrained and trained individuals (11), endocrine factors may cause glucose flux to be more closely related to relative than absolute exercise intensity. Studies that use two-leg training and one-leg testing protocols confirm the importance of training-induced alterations in the endocrine regulation of glucose. Also, short-term training studies have demonstrated decreased glucose utilization during exercise when expressed as relative vs. absolute intensity; exercise crossover concept. To our knowledge, the effects of training on whole body glucose kinetics at given relative exercise intensities have not been systematically addressed in a longitudinal study. One cross-sectional study by Coggan et al. (5) compared highly trained and untrained subjects at 80% of peak O2 consumption (VO2peak) and found no difference in Ra but a reduction in glucose Rd in the trained subjects. However, the cross-sectional design of the study could be problematic because successful endurance athletes may have a genetic propensity to utilize greater amounts of lipid. Understanding the effects of exercise and training at given relative intensities should be considered important. Exercise prescriptions for the general population, as well as the training and competitive regimens of athletes, are geared to maximizing peak power output and endurance by maintaining the same or higher relative workloads as improvements occur. Endurance training may enhance the ability to utilize lipids during mild to moderate...
exercise, but the transition to hard exercise appears to result in a crossover to predominantly carbohydrate (CHO) utilization regardless of training status (2). The purpose of our study was to examine the effects of intensity and training on glucose kinetics in male subjects to evaluate the hypothesis that during hard exercise, blood glucose flux is not affected by training.

METHODS

Subjects. Twenty healthy, nonsmoking, sedentary male subjects between the ages of 18 and 35 yr were recruited from the University of California campus community by flyers and mailings. The subjects were recruited in two groups of 10. Each group followed the same protocol except that different tracers were infused during the isotope trials (see Tracer protocol). Subjects were considered sedentary if they had participated in <2 h of regular strenuous activity per week for at least the last year and if they had a VO\(_{2}\)peak between 35 and 45 ml·kg\(^{-1}\)·min\(^{-1}\) as determined by a continuous-progressive maximal exercise test on the cycle ergometer. To qualify for participation in the study, subjects were required to be diet and weight stable, to have a body fat percentage of <20%, and to be disease/injury free as determined by medical questionnaire and physical examination. All subjects provided informed consent, and the study protocol was approved by the University of California Committee for the Protection of Human Subjects (approval 93–12–45).

General experimental design. After an initial screening interview and screening tests, two stable-isotope infusion trials were performed on a cycle ergometer for 1 h at 45 and 65% of VO\(_{2}\)peak (45UT and 65UT, respectively). These trials were performed 1 wk apart, and the order of the work intensities was randomized. Subjects began training 2 days after their second isotope trial and continued for 10 wk. The screening tests were repeated at 5 and 10 wk of training. After training, two more isotope trials were performed. One was at the same absolute workload as the 65% trial pretraining (ABT), and the second was at a workload that elicited 65% of the new, postraining VO\(_{2}\)peak or the same relative workload (RLT). The two posttraining trials were also 1 wk apart and randomized, and endurance training was continued between the two trials.

Screening tests. Body composition was determined by both skin fold measurement and underwater weighing. VO\(_{2}\)peak was determined on an electronically braked cycle ergometer (Monarch Ergometric 829E) during a continuous, progressive protocol that increased 25 or 50 W every 3 min until voluntary cessation. Respiratory gases were analyzed (Ametek S-3A1 O\(_{2}\) and Beckman LB-2 CO\(_{2}\) analyzers) and recorded by an on-line, real-time PC-based system (model RL-H7000W, Panasonic) every minute. Each subject underwent two VO\(_{2}\)peak tests before commencement of the study to assure a true maximum effort and to allow blood sampling during one test for the determination of the lactate threshold of each subject. Three-day dietary records were kept at the beginning and every 3 wk throughout the study to monitor the subject’s diet composition and quantity of intake. Dietary analysis of these records was performed by using the Nutritionist III program (N-Squared Computing, Salem, OR).

Tracer protocol. All subjects were studied in a postabsorptive state in the morning, and dietary intake was monitored for the 24 h immediately preceding each of the four isotope trials. Dinner the night before each trial (12 h) was selected by the individual subject and repeated before each trial. Each subject was given a standardized snack (555 kcal: 17% protein, 53% CHO, 30% fat) to consume before bed, 8–10 h before the trial, and a standardized breakfast (300 kcal: 17% protein, 83% CHO) to consume at least 1–2 h before reporting to the laboratory. On the morning of the trial, an arterial catheter was placed in the radial artery for sampling, and an antecubital venous catheter was placed in the opposite arm for primed continuous infusion of the tracers for 90–120 min of rest and 1 h of exercise. The first group of subjects received [1-13C]glucose, [6,6-2H]glucose (D\(_{2}\)-glucose), and [1,1,2,3,3-\(^2\)H]glycerol (D\(_{5}\)-glycerol), whereas the second group received [1-13C]palmitate, [6,6-2H]glucose, and [1,1,2,3,3-\(^2\)H]glycerol. The glycerol and palmitate kinetics data are reported separately. After the collection of background blood and expired air samples, a priming bolus was given and the subjects rested semisupine for 90 min. For both glucose isotopes, the priming doses were 125 times the resting minute infusion rate. With the use of a Harvard Apparatus syringe pump (model 2400–01, Natick, MA), the resting infusion rate was set at 15 mL/h of the continuous-infusion cocktail, which contained 8 mg/ml each of [1-13C]glucose and [6,6-2H]glucose. Thus the resulting infusion rate was 2 mg/min for both isotopes. On initiation of exercise, the infusion rate was increased to 45 mL/h (6 mg/min) for the two pretraining isotope trials and for the 65% of the old VO\(_{2}\)peak postraining trial (same absolute workload). Because of the increased metabolic flux anticipated for the 65% of the new VO\(_{2}\)peak postraining, the exercise infusion rate was increased to 60 mL/h (8 mg/min) for this trial. We chose our infusion rates on the basis of the Steele model that emphasizes constant concentrations and isotopic enrichments to facilitate the calculation and accuracy of substrate kinetics. On the basis of previous experiments, we selected infusion rates to yield steady and comparable isotopic enrichments for all of our testing workloads. Arterial samples were taken at 0, 75, and 90 min of rest and at 5, 15, 30, 45, and 60 min of exercise. All isotopes were obtained from Cambridge Isotope Laboratories (Woburn, MA), diluted in 0.9% sterile saline, pharmaceutically tested for sterility and pyrogenicity (Univ. of California School of Pharmacy, San Francisco, CA), and on the day of the experiment, passed through a 0.2-μm Millipore filter (NalgeN, Rochester, NY).

At each of the blood sampling time points, respiratory gas exchange was determined by using the same system described above, and a sample of expired air was collected in a 10 mL-vacuum container to determine 13CO\(_{2}\) isotopic enrichment. The expired air samples were stored at room temperature until they were analyzed by using isotope ratio mass spectrometry (IRMS) by Metabolic Solutions (Acton, MA). Heart rate was recorded throughout rest and exercise by using a Quinton Q750 electrocardiogram (Seattle, WA), and blood pressure was measured at each of the sampling points by auscultation. Hematocrit was determined during the last 15 min of rest and exercise to ensure that the measurements of metabolite and hormone concentrations were not influenced by changes in plasma volume.

Blood sample collection and analysis. Blood samples for the analysis of glucose and lactate concentration and glucose isotopic enrichment were collected in 8% perchloric acid. Plasma glucose concentration was determined by using a hexokinase enzymatic kit (Sigma Chemical, St. Louis, MO), and lactate plasma concentration was determined by using the method of Gutmann and Wahlefeld (15). Glucose isotopic enrichment was measured by using gas chromatography-mass spectrometry (GCMS; GC model 5890 series II and MS model 5898A, Hewlett-Packard) of the pentaacetae derivative. In preparation for GCMS analysis, each sample was neutralized with 2 N KOH, transferred to cation (AG 50W-X8, 50–100 mesh H\(^{+}\) resin) and anion (AG 1-X8, 100–200 mesh
formate resin) exchange columns, and the glucose was eluted with deionized water. The samples were then transferred to a 2-ml gas chromatography vial and lyophilized. One hundred and fifty microliters of 2:1 solution of acetic anhydride pyridine were added to each vial, and each was heated at 60°C for 30 min. For GCMS analysis, the injector temperature was set at 200°C; the initial oven temperature was set at 110°C and was gradually increased by 35°C/min until it reached a final temperature of 255°C. The transfer line was set at 250°C, the source temperature was set at 200°C, and the quadrupole temperature was set at 116°C. The carrier gas was helium, and the splitless injection was used with a 35:l ml/min ratio. Methane was used for chemical ionization, and selected ion monitoring was used to monitor ions mass-to-charge ratios 331.20, 332.20, and 333.20 for [13C]-, [12C]-, and [6,6-2H]glucose, respectively.

Catecholamine analyses. Plasma catecholamine concentrations were determined by using high-pressure liquid chromatography (HPLC) with electrochemical detection (29). Four-milliliter aliquots of arterial blood were collected in chilled storage tubes containing 20 mg of glutathione and EDTA. After collection, the tubes were immediately centrifuged for 15 min at 2,000 g. Plasma was collected and stored at −80°C until analysis. An internal standard of 100 ng/ml of dihydroxybenzylamine and 400 ml of tris(hydroxymethyl)aminomethane (Tris) was added to all samples. HPLC-grade alumina was added, and the samples were then shaken for 10 min to achieve constant alumina plasma interaction. After catecholamine extraction, the solution was centrifuged for 2 min at 12,000 g, the supernatant was discarded and 600 µl of Tris were added to the alumina pellet. The solution was again shaken, spun, and the supernatant was disposed. This step was then repeated with 600 µl double-distilled H2O. To extract the catecholamines, 125 µl of 0.1 N perchloric acid were added to the alumina. The solution was shaken and spun as above. A 120-µl sample of eluant was injected into the HPLC column (reverse phase; BioSil C-18, Bio-Rad, Richmond, CA) and eluted with mobile phase (26 ml acetonitrile, 6.9 g NaH2PO4, 120 mg EDTA, 100 mg sodium octyl sulfate, and 100 mg sodium heptane sulfonate in 974 ml of double-distilled H2O, with pH adjusted to 4.07.) Delivery rate was constant at 1 ml/min (pump model LC-10AD, Shimadzu, Tokyo, Japan) with a potential of 0.65 V (detector model 1340-C, Bio-Rad). Computer integration was used to analyze the chromatograms.

Training protocol. Subjects were required to exercise with a personal trainer in our facility 5 days/wk for 1 h each day on the cycle ergometer. The personal trainers were current undergraduate students in, or recent graduates of, the Department of Human Biodynamics and, for the most part, were competitive or recreational athletes themselves. During the first 3 wk of training, the intensity was gradually increased from 50% of each participant’s V02peak to 75% of their V02peak. Subjects were asked to warm up for 5 min and stretch before their hour of exercise. The personal trainers used heart rate monitors and data from periodic V02peak tests to adjust workloads as the subjects improved. In addition to the supervised training, subjects were required to exercise an additional hour on the weekend in any manner they desired. Subjects were weighed daily to assure that they remained weight stable and were asked to increase their caloric intake to match their increased energy expenditure without altering their normal dietary composition.

Calculations and statistics. The glucose Ra, Rd, and metabolic clearance rate (MCR) were calculated by using equations defined by Steele and modified for use with stable isotopes (42)

\[
R_a = \frac{F - V(C_1 + C_2)/2}{(V + C_1)(t_2 - t_1)}
\]

Glucose recycling rate (mg · kg⁻¹ · min⁻¹)

\[
R_d = \frac{V(C_2 - C_1)/(t_2 - t_1)}{F - V(C_1 + C_2)/2}
\]

Glucose recycling rate (mg · kg⁻¹ · min⁻¹)

\[
\]

where F represents the isotopic infusion rate; IE1 and IE2 are the glucose isotopic enrichments of either [2H]glucose and [13C]glucose at sampling times 1 (t1) and 2 (t2), respectively; C1 and C2 are concentrations at t1 and t2; and V is the estimated volume of distribution for glucose (180 ml/kg). All values for isotopic enrichment were corrected for baseline enrichments from background blood samples taken before infusion of the isotopes.

In addition to the kinetic parameters, glucose rate of oxidation (Ra) was calculated by using the IRMS analysis of the expired air samples

Relative glucose oxidation (%)

\[
\frac{[13C]glucose(VCO2)(100)}{F \times k}
\]

Glucose oxidation rate (mg · kg⁻¹ · min⁻¹)

\[
Ra(\text{relative glucose oxidation})
\]

where [13C]CO2 is the isotopic enrichment of expired [13C]CO2; VCO2 is the volume of CO2 expired per minute; F is the isotopic infusion rate; and k is the correction factor for the retention of CO2 in body pools. The value for k was set at rest to be 0.65 and during exercise at 0.90 on the basis of previous findings (36). Finally, the contributions of glucose, total CHO, and lipids to total energy expenditure were calculated by using the respiratory exchange ratio (RER), O2 consumption values, and standard caloric equivalents for lipids and CHO's.

Data are represented as means ± SE. Calculations of steady-state glucose kinetics were made by using the last two (75, 90 min) and three (30, 45, 60 min) isotopic enrichments obtained during rest and exercise, respectively. Because there were no significant differences between resting values for the two pretraining or the two posttraining trials, the resting values were combined into one pre- and one posttraining value. Also, because the study was designed with two groups of subjects receiving slightly different isotopes, the number of subjects available for particular analyses differs throughout this report. Calculations done using the [13C]glucose isotope (e.g., Ra, recycling rate) have 10 subjects, whereas all other data, such as Ra, Rd, and metabolite concentrations, are calculated with all 19 of the subjects. To assess differences between the four isotope trials, an analysis of variance with repeated measures was used. Associations were evaluated by using Pearson-product moment coefficients. Statistical significance was set at α = 0.05.
Therefore, results were pooled. Pre- and posttraining characteristics of the 19 subjects who completed the study are listed in Table 1. The subjects were weight stable throughout the study period, although they did lose a significant amount of body fat whether measured by skin folds (11.3 ± 3.5%) or underwater weighing (9.6 ± 4.0%). VO2peak improved by 9.4 ± 1.4% over the 10-wk period, but most of the increase was attained in the first 5 wk (8.1 ± 1.5%). In contrast, time to VO2peak continued to increase steadily throughout the training period, attaining a total of 27.6 ± 3.9% improvement by the completion of the study. The workload characteristics for the four isotope trials are presented in Table 2. Because of the increase in aerobic capacity resulting from training, the posttraining trial at the same absolute workload was equivalent to 56% of the subject's new VO2peak. A training effect was observed in the mean heart rate recorded during the isotope trials at the same absolute workload. However, there was no difference in mean heart rate at the same relative workloads pre- and posttraining (Table 2).

Plasma glucose concentration and glucose kinetics. Arterial glucose concentrations fell slightly during the first 15 min of exercise; however, there was no significant difference in arterial glucose concentration among the four trials during steady-state exercise, and the concentration remained steady at ≈ 4.6 mM throughout exercise (Table 2). The glucose isotopic enrichments are shown in Fig. 1, A and B, for [6,6-2H]- and [1-13C]glucose, respectively. The enrichments for all four trials were stable during rest. The enrichments were also stable during the last 30 min of exercise for all tests except for the 65UT values, which fell slightly during exercise.

Glucose Ra increased significantly between rest and exercise for all four of the exercise conditions (Fig. 2A). Furthermore, glucose Ra was 23% higher during the 65UT trial compared with the 45UT trial, demonstrating a significant intensity effect pretraining. In addi-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>%Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25.47 ± 0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>179.76 ± 1.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.62 ± 2.03</td>
<td>78.24 ± 1.89</td>
<td>-0.39 ± 0.50</td>
</tr>
<tr>
<td>Body fat, %</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Underwater weighing</td>
<td>15.50 ± 1.04</td>
<td></td>
<td></td>
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<tr>
<td>Skin folds</td>
<td>14.45 ± 0.92</td>
<td>12.73 ± 0.83</td>
<td>-11.33 ± 3.48</td>
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<tr>
<td>VO2peak, ml·kg⁻¹·min⁻¹</td>
<td>46.50 ± 1.13</td>
<td>50.71 ± 1.27</td>
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<td>l/min</td>
<td>3.63 ± 0.09</td>
<td>3.94 ± 0.11</td>
<td>8.5 ± 1.33</td>
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<tr>
<td>Time to VO2peak, min†</td>
<td>18.67 ± 1.12</td>
<td>22.95 ± 1.13</td>
<td>27.63 ± 3.91</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 19. VO2peak, peak O2 consumption. Significantly different from pretraining values, *P < 0.05. †Workload during VO2peak test was increased every 3 min.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>45UT</th>
<th>65UT</th>
<th>ABT</th>
<th>RLT</th>
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<tr>
<td>Workload, W</td>
<td>4.1 ± 0.34</td>
<td>4.26 ± 0.17</td>
<td>90.0 ± 6.24e</td>
<td>152.2 ± 6.4</td>
<td>152.7 ± 6.4</td>
<td>176.7 ± 6.1cd,e</td>
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<td>VO2, ml·kg⁻¹·min⁻¹</td>
<td>67.7 ± 1.76</td>
<td>63.6 ± 1.67a</td>
<td>216.0 ± 0.72e</td>
<td>30.1 ± 0.75</td>
<td>29.7 ± 0.87</td>
<td>33.1 ± 0.84de</td>
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<td>Respiratory exchange ratio</td>
<td>0.56 ± 0.02</td>
<td>0.86 ± 0.01</td>
<td>128.2 ± 2.75d</td>
<td>157.8 ± 3.09</td>
<td>138.9 ± 2.55c,d</td>
<td>156.2 ± 2.56e</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>85.6 ± 0.30</td>
<td>89.6 ± 0.30a</td>
<td>0.91 ± 0.01</td>
<td>0.94 ± 0.03c</td>
<td>0.92 ± 0.01</td>
<td>0.94 ± 0.02d</td>
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<tr>
<td>%Energy from CHO</td>
<td>52.2 ± 8.83</td>
<td>53.5 ± 7.3</td>
<td>68.9 ± 3.88</td>
<td>78.2 ± 5.39</td>
<td>73.4 ± 4.72</td>
<td>78.4 ± 5.85</td>
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<tr>
<td>Arterial glucose, mM</td>
<td>4.99 ± 0.11</td>
<td>4.81 ± 0.84</td>
<td>4.62 ± 0.10b</td>
<td>4.50 ± 0.10b</td>
<td>4.60 ± 0.13</td>
<td>4.65 ± 0.08b</td>
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Values are means ± SE; n = 19 for all variables except respiratory exchange ratio and %energy from carbohydrate (CHO), n = 10. VO2, O2 consumption; 45UT, 45% pretraining trial; 65UT, 65% pretraining trial; ABT, same absolute workload as 65UT; RLT, same relative workload (65% of posttraining VO2peak). All exercise values were significantly different from rest except for arterial glucose, which were different as marked. aSignificantly different between resting conditions; bSignificantly different from rest; cSignificantly different from 45UT; dsignificantly different from 65UT; esignificantly different from ABT, P < 0.05.
Fig. 2. A: effect of exercise intensity and training on plasma glucose rate of appearance (Rg). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively; n = 19 subjects. B: effect of exercise intensity and training on plasma glucose rate of disappearance (Rd). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively; n = 19 subjects. C: effect of exercise intensity and training on plasma glucose metabolic clearance rate (MCR). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively; n = 19 subjects. D: effect of exercise intensity and training on plasma glucose Rd expressed as %total energy expenditure (RdE). Values are means ± SE last 15 and 30 min for rest and exercise, respectively; n = 10 subjects. E: effect of exercise intensity and training on rate of glucose oxidation (Rox). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively; n = 10 subjects. F: effect of exercise intensity and training on rate of glucose recycling (Rr). Values are means ± SE of last 15 and 30 min for rest and exercise, respectively; n = 10 subjects. G: effect of exercise intensity and training on arterial lactate concentration. Values are means ± SE of last 15 and 30 min for rest and exercise, respectively; n = 19 subjects.
tion, when measured at the same absolute workload pre- and posttraining, $R_a$ declined significantly (21%) after training. However, when measured at the same relative intensity, there was no difference in the values pre- and posttraining.

Glucose $R_d$ was similar to that of $R_a$. Thus there was a significant intensity effect pretraining, as well as a training effect at the same absolute workload but not the same relative workload (Fig. 2B). The similarity between our glucose $R_a$ and $R_d$ is consistent with the observed stable blood glucose concentrations and isotopic enrichments during exercise. In addition, because there was no significant difference between exercise trials in blood glucose concentration, the relationship of the MCR between the four trials was similar to $R_d$ (Fig. 2C).

Because the total energy expenditure was different at the same relative intensities (14.7 vs. 16.0 kcal/min), we corrected $R_a$ and $R_d$ values for total energy expended per minute. When glucose fluxes were expressed as percentages of total energy expenditure, the values demonstrated a training effect at the same absolute workload but not the same relative workload (Fig. 2D). Glucose $R_a$ expressed as a percentage of total energy expenditure resulted in a significant decrease during exercise compared with rest.

Glucose oxidation and RER. There was a significant increase in glucose oxidation in the transition between rest and exercise for all four exercise intensities. Also, $R_{ox}$ demonstrated an intensity effect pretraining and a significant training effect at both the same absolute and relative workloads posttraining (Fig. 2E).

RER values increased significantly in the transition between rest and exercise. Values for RER during exercise were significantly higher for the 65UT trial compared with the 45UT trial, but the posttraining and pretraining 65% values were not different (Table 2). Although there was no significant training effect, RER tended to be lower at the same absolute workload but not the same relative workload.

On the basis of the RER and average $O_2$ consumption during rest and exercise for the four isotope trials, the percent contributions of glucose, total CHO, and lipid were calculated. Even at rest, slightly more CHO than lipid was oxidized, but during exercise at 65%, both pre- and posttraining, as much as 78% of the energy used to do work came from CHO sources (Table 2, Fig. 3).

Glucose recycling rate and lactate concentrations. Glucose recycling rate, which was calculated as the difference between $R_a$ as determined from [1-13C] and [6,6-14H] tracers, gives an estimate of the recycling of carbon through gluconeogenesis from three carbon precursors, predominantly lactate. The recycling rate was significantly elevated for the 65UT trial compared with the 45UT trial (Fig. 2F). Also, like glucose $R_a$, there was a significant decrease at the same absolute workload posttraining. However, unlike $R_a$, the recycling rate was also significantly reduced in a comparison of the two relative workloads pre- and posttraining. Associated with the decline in recycling rates posttraining were reduced blood lactate concentrations at both the same absolute and relative workloads (Fig. 2G). The recycling rates were lower for a given lactate concentration as well posttraining (Fig. 4A). The recycling rate was also closely associated with norepinephrine concentrations but, again, there was less recycling and a lower lactate concentration for a given norepinephrine concentration posttraining (Fig. 4, B and C).

DISCUSSION

Results of our investigation corroborate those of previous studies showing a direct relationship between exercise intensity and blood glucose flux (24, 35). Furthermore, our results are consistent with the hypothesis that glucose $R_a$ is exponentially related to relative effort as given by percent $V_{O_2}$peak (3). Results of our study on the effects of training on glucose flux are consistent with investigations that training reduces glucose flux for exercise of a given power output (4, 7). However, our results obtained by using a longitudinal design differed from cross-sectional designs in that training did not affect glucose kinetics ($R_a$ or $R_d$) when expressed at a given relative power output (5).

One of the criticisms often presented against the use of relative workloads as a means for testing the effects of training is that the energy expenditure during exercise and thus the metabolic flux differs between conditions. However, even when glucose $R_a$ (or $R_d$) values were expressed as a percent of total energy expenditure, there was no difference in glucose flux pre- and posttraining at the same relative workload. Depicting glucose flux as a function of %energy expenditure (e.g., Fig. 2D) is appropriate because it eliminates the influence of varying metabolic flux rates when making comparisons at different absolute intensities.

There have been many defined power output studies demonstrating that both short- and long-term exercise training change the balance of substrate utilization away from CHO toward lipid utilization at given absolute workloads. Support for such a conclusion comes from
observations posttraining in the form of decreases in RER values (4), increases in total fat oxidation (28), decreases in glucose flux (4), and reductions in the rate of muscle glycogenolysis (12, 20, 37). Although those studies provide useful information, they only address part of the issue of endurance training. Some of the changes in substrate utilization observed at the same absolute workload may arise from blunted hormonal responses rather than actual peripheral adaptations. Circulating levels of hormones are similar among people of widely varied training level when tested at the same relative workload despite dramatic differences in the absolute workload (11). To our knowledge, no long-term training studies have been completed that look at endurance training at the same relative workload. In one study by Kjaer et al. (24), the glucose kinetics of trained and untrained subjects were compared at maximal workloads. No differences were observed in glucose $R_d$ between groups, although elevated glucose concentrations in the trained group resulted in lower metabolic clearance rates. Unfortunately, the non-steady-state conditions in that study leave the data open to interpretation. Another study by Coggan et al. (5) compared endurance athletes and untrained individuals for 30 min of exercise at 80% $V_{O2peak}$ and showed no difference in glucose $Ra$ but a lower glucose $R_d$ in the trained subjects. However, it is unclear whether accurate flux values can be obtained under such conditions of high-intensity, short-duration exercise during which glucose concentrations are not stable. In addition, information from such cross-sectional studies should be interpreted with caution, given that a genetic propensity for lipid utilization may have predisposed such athletes to select and be successful at such endurance activities (1).

We chose to test our subjects in a fed, postabsorptive state so that the results would be more applicable to a nonlaboratory environment. Typically, subjects ate 1–2 h before reporting to the laboratory; subject preparation took a minimum of 1 h, and rest ranged from 90 to 120 min. Thus we report data on resting subjects fed 3.5–5 h previously and exercising subjects fed 4.5–6 h before study. For this reason, our RER values appear elevated above previous studies that used subjects who had not eaten for 6–12 h before the experiment (4, 28, 34). However, our values were in the same range as those of Jones et al. (22), who reported average RER values during steady-state exercise of 0.89 and 1.01 for 36 and 70% of $V_{O2peak}$, respectively. Our attempt to control diet and, thus, liver and muscle glycogen stores, may also explain why there was no difference in RER after training at the same absolute workload. A recent study in our laboratory using a cross-sectional design illustrated that, in the fed state, trained cyclists and
untrained subjects showed no difference in RER values at workloads >20% of V\textsubscript{O2peak} (B. C. Bergman and G. A. Brooks unpublished observations). Although the pretrial breakfast that we selected was composed of only CHO and protein, we do not believe that the composition of the meal elevated the RER values. Thomas et al. (39) have shown that, although the quantity of CHOs ingested affects the fuel selection, adding fat to a diet does not increase the amount of fat utilized. In addition, the thermic effect of the meal would not be expected to last more than 3–5 h, especially given the small size of the pretest meal consumed by our subjects.

In addition to feeding our subjects, the fact that we tested our subjects at the same relative workload means that the data may be more applicable to active adults as well as to athletes in a nonlaboratory setting. As athletes and others train and improve, they are capable of performing at higher absolute as well as relative power outputs. For the elite athlete, the proportioned advantages of increased fat utilization due to training (17) may be moot. Several studies have indicated that athletes work at very high intensity levels and, therefore, rely predominantly on CHO regardless of their muscle oxidative capacity (25, 31). The reliance on CHO may be partially a result of the decline in free fatty acid availability associated with high-intensity exercise (2, 13, 21, 34) but, mainly, fuel selection is determined by the glycolytic CHO flux rate (2, 9, 41). Glycolysis in working muscle is related to epinephrine levels, recruitment of type II muscle fibers, contraction-induced glycogenolysis, redox state, and other factors, many of which are directly influenced by relative exercise intensity. In the fed state, resting individuals usually have a RER value in the range 0.83–0.86, which indicates that ~50% of the energy comes from CHO sources. In the transition from rest to exercise and with increasing intensity, the oxidation of both fat and CHO sources increases in absolute terms, but CHO increases more, resulting in a relative decrease in the %contribution from lipid sources. Lipid oxidation reaches a turning point at ~50% of maximum exercise capacity and then declines in both relative and absolute terms (3, 19).

An explanation of how training results in a decreased glucose R\textsubscript{ox} for a given power output remains to be established. Houmard et al. (18) have demonstrated increased glucose transporter isoform GLUT-4 content in trained human muscle, and it is well known that for a given power output, insulin concentration is higher after training. Furthermore, training increases hexokinase activity and decreases glucose-6-phosphatase, both of which would favor glucose uptake and phosphorylation (4, 6). All of these adaptations suggest increased capacity for glucose uptake, but after training glucose uptake is less at a given absolute workload. Likely, some other training effect, such as superior mitochondrial respiratory control, lower malonyl-CoA levels, or decreased translocation or intrinsic activity of GLUT-4, is involved (2, 26, 33). As well, changes in vascular glucose conductance related to blood flow may cause muscle glucose uptake to decrease at a given power output after training.

Our subjects improved their V\textsubscript{O2peak} by 9.4% overall, whereas other endurance training studies of comparable length have shown greater increases (7, 27, 28). However, unlike our program, which was designed to increase endurance capacity by progressively increasing the workload, others have used interval-training regimens. Although interval training is effective in increasing V\textsubscript{O2max} (8), endurance training, as employed by us, results in peripheral adaptations such as increases in mitochondrial content (8, 9). Also, the capacity to increase V\textsubscript{O2max} is dependent on both the initial starting value and genetic potential (1). The subjects who were recruited for our study were untrained but active young men and thus may not have been able to increase their maximum capacity to the same extent as their endurance capacity. Furthermore, short-term training studies that recorded a reduction in glucose R\textsubscript{ox} and an increase in whole body fat oxidation with no concomitant changes in V\textsubscript{O2peak} provide evidence that large changes in maximum aerobic capacity are not critical to alter substrate selection (30). The effectiveness of our endurance training program can be seen in the decrement of kinetic and metabolite parameters after training. For example, at the same absolute workload, glucose flux and oxidation declined by 21 and 29%, respectively. There were also substantial decreases in lactate concentration (45%) and glucose recycling rate (62%) at the same absolute workload. Our endurance training program was also sufficient to cause significant decreases in glucose R\textsubscript{ox}, recycling rate, and lactate concentration at the same relative workload (18, 50, and 18% respectively).

Training reduced glucose R\textsubscript{ox} significantly despite similar values for R\textsubscript{d} at the same relative workload. It is possible that pretraining values for R\textsubscript{ox} were elevated because greater lactic acidosis caused the release of labeled bicarbonate (incorporated during the 90 min of rest). However, despite elevated blood lactate levels in our subjects at the end of exercise in the 65UT trial relative to posttraining (Fig. 2G), the lactate levels were falling in all four trials at the time of the steady-state exercise measurements. Thus it is unlikely that measured differences in glucose R\textsubscript{ox} were caused by lactic acidemia and the release of stored $^{13}$CO\textsubscript{2}. However, in a study by Jones et al. (22), the authors reported RER values of 1.0 or greater during steady-state exercise at an intensity of 70% of V\textsubscript{O2peak} and suggested that the high levels of lactate were sufficient to force the release of excess CO\textsubscript{2} despite the fall in lactate concentrations during the end of exercise. However, because the lactate values of Jones et al. were substantially higher than our 65UT values in the present study (9.94 vs. 3.3 mM, respectively), it remains unclear whether lactic acidemia is influencing our R\textsubscript{ox} calculations. An alternative conclusion is that training increased the nonoxidative disposal of glucose. However, the increase in nonoxidative glucose disposal after training is not a result of elevated glucose recycling because our values for recycling were reduced
posttraining. Therefore, training resulted in pathways of glucose disposal that were not measured by our study design. We speculate that the increased nonoxidative disposal of glucose may be related to greater cycling of glucose through glycogen in trained muscle (J. Azevedo et al., unpublished observations). Possibly, the altered hormone profile after training may allow a greater proportion of the glycosyl units cycling through the futile cycle to remain in the form of glycogen. It is also possible that in trained subjects, nonactive muscle groups are more likely to direct glucose to glycogen than in the untrained subject.

The recycling rate of $^{13}$C was significantly reduced posttraining by 62 and 50% at the same absolute and relative workloads, respectively. This reduction in recycling rate is in contrast to several training studies performed in rats that found that gluconeogenesis from lactate was actually enhanced posttraining (12, 38, 40). The results of this study indicate that there was less lactate present and less recycling for a given concentration of norepinephrine. These data suggest a reduction in signaling and, perhaps, a downregulation of adrenergic receptors in response to endurance training. Also, Fig. 4A indicates that there was less recycling for a given concentration of lactate, suggesting that the dearance of lactate for the purpose of gluconeogenesis was reduced after training. It has been established previously that lower circulating levels of lactate after training in humans are a result of increased dearance and oxidation rather than a reduction in production (27, 32). If, in our study, more lactate was disposed of through oxidation, the results of increased nonoxidative glucose disposal, lower lactate concentration, decreased glucose recycling, and similar RER values after training may be explained.

Summary and conclusions. The results of this study suggest that glucose flux is geared to relative exercise intensity both before and after training. After training, we observed a decrease in glucose flux ($R_g$, $R_d$, MCR) at the same absolute workload but not the same relative workload. Even when $R_g$ and $R_d$ were adjusted for total energy expenditure, there was no significant difference pre- and posttraining at the same relative workload. $R_{ox}$ was lower at the same absolute workload, but it was also significantly reduced at the same relative workload. Similarly, the glucose recycling rate was reduced at both workloads posttraining and was lower for a given concentration of lactate posttraining.

In the transition from moderate- to hard-intensity exercise, a crossover from lipid to CHO dependency occurs (2, 3, 19). Even after training, endogenous CHO energy sources are the predominant fuels for muscle exercise (2, 19). The data from this investigation suggest that 1) glucose use is directly related to exercise intensity; 2) training decreases glucose flux for a given power output; 3) when expressed as relative exercise intensity, training does not affect blood glucose flux; 4) training alters the pathways of glucose disposal; and 5) crossover to CHO dependence occurs during hard exercise, regardless of training state.

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