Regulation of fuel metabolism by preexercise muscle glycogen content and exercise intensity

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During submaximal steady-state exercise, the proportions of carbohydrate- and fat-based fuels oxidized at any given time depend on a number of factors, including exercise intensity (26), substrate availability (9), training status (1), and gender (30). Of these, the relative exercise intensity has been proposed to be the major determinant of skeletal muscle energy flux and therefore ultimately substrate metabolism (for review see Ref. 5). Indeed, an increase in exercise intensity results in greater contraction-induced muscle glycogenolysis and glycolysis (14) and stimulates sympathetic nervous system activity (7), leading to a greater use of carbohydrate-derived fuels.

Preexercise substrate availability has emerged as an important regulator of the patterns of fuel oxidation during exercise. Several studies have reported that the rate of glycogen utilization during exercise is directly related to starting muscle glycogen concentration (8–10, 33). Consequently, high preexercise muscle glycogen content after a carbohydrate-rich diet would favor a greater rate of glycogenolysis during subsequent exercise, whereas low preexercise muscle glycogen content after a diet low in carbohydrate would reduce rates of glycogenolysis.

Training status has also been recognized as an important factor modifying substrate use. In this regard, Kristiansen et al. (19) have reported increased glucose uptake in the glycogen-depleted state during intense exercise after 3 wk of endurance training. These workers attributed the greater contribution of blood glucose to whole body carbohydrate metabolism to a training-induced increase in GLUT-4 protein content. Consistent with these findings, studies employing highly trained subjects have reported greater rates of plasma glucose uptake when moderate-intensity exercise was commenced with low vs. high muscle glycogen content. In contrast, similar rates of glucose uptake have been observed in moderately trained individuals when moderate-intensity exercise is commenced with either low or high muscle glycogen content (3, 10, 32). Accordingly, discrepancies in the rate of glucose uptake or disposal observed between studies (3, 10, 27, 32, 34) may, in part, be explained by differences in training status. To date, however, no single study has determined the relative effects of preexercise muscle glycogen content and exercise intensity in the hierarchy of metabolic control in the same subjects.

The present investigation was designed to determine the relative effects of exercise intensity and preexercise muscle glycogen content on rates of whole body fuel oxidation. We deliberately chose to employ exercise intensities that are typically undertaken by moderately trained individuals who participate in regular physical activity to attain health benefits. We hypothesized that an increase in exercise intensity would increase carbohydrate oxidation (i.e., whole body glycogen and blood glucose oxidation) to a greater extent than an increase in preexercise muscle glycogen content.

METHODS

Subjects

Seven male subjects who were moderately trained in cycling exercise participated as subjects in this study, which was approved by the Human Research Ethics Committee of RMIT University. The subjects’ age, body mass (BM), peak power output (PPO), and peak O2 uptake (V̇O2 peak) were 33 ± 2 yr, 80.3 ± 3.6 kg, 377 ± 20 W, and 4.64 ± 0.24 l/min, respectively (values are means ± SE). As multiple
muscle biopsy and blood samples were taken, all procedures and risks were carefully explained to each subject before their written consent was obtained.

**Preliminary Testing**

All subjects performed an incremental test to volitional fatigue (i.e., maximal test) on a Lode cycle ergometer (Groningen, The Netherlands) under standard laboratory conditions (21–22°C, 40–50% relative humidity) for the determination of PPO and V\(\text{O}_2\) peak. The maximal test commenced at a workload of 100 W, which was subsequently increased by 25 W/150 s. Throughout the maximal test and during portions of the experimental trials (described subsequently), subjects breathed through a mouthpiece attached to a Quark b\(^2\) metabolic cart (COSMED, Rome, Italy). Expired gas was passed through a flowmeter, an O\(_2\) analyzer, and a CO\(_2\) analyzer that were calibrated before testing by using a 3-liter Hans-Rudolph syringe and the last successfully completed workload. The results of the maximal workload (in W) completed on the final (uncompleted) workload to calculated minute ventilation (V\(\text{E}\)), O\(_2\) uptake (V\(\text{O}_2\)), CO\(_2\) production (V\(\text{CO}_2\)), and respiratory exchange ratio (RER) from conventional equations (25).

A maximal test was terminated at the point of volitional fatigue that coincided with the inability of a subject to maintain a cadence >70 revolutions/min and/or a RER >1.15. The highest V\(\text{O}_2\) for any 60 s was taken as the subject’s V\(\text{O}_2\) peak. PPO was calculated by adding the workload (in W) completed on the final (uncompleted) workload to the last successfully completed workload. The results of the maximal test were used to determine the exercise intensity that corresponded to ~45 (134 ± 7 W) and 70% (239 ± 13 W) of each subject’s V\(\text{O}_2\) peak to be performed in the subsequently described experimental trials.

**Exercise and Dietary Control**

Subjects commenced an experimental trial with either low (LG) or high (HG) muscle glycogen concentration. Muscle glycogen content was manipulated before each trial by means of exhaustive cycle exercise and dietary intervention. The exercise-depletion protocol has been described in detail previously (20). Briefly, ~48 h before each experimental ride, subjects reported to the laboratory and performed an intermittent ride to volitional fatigue. After a 5-min warm-up, subjects commenced cycling for 2 min at 90% of PPO, followed immediately by 2 min of cycling at 50% of PPO. When subjects were unable to complete 2 min of exercise at 90% of PPO, the power output was lowered to 80, 70, and finally 60% of PPO while the same work-rest interval was maintained. Exercise was terminated when subjects could not complete 2 min of cycling at 60% of PPO. After the exercise-depletion protocol, subjects consumed either a low-carbohydrate (LG: 0.7 g/kg BM of carbohydrate, 4.4 g/kg BM of fat, 4 g/kg BM of protein) or high-carbohydrate (HG: 10 g/kg BM of carbohydrate, 1.9 g/kg BM of fat, 1.9 g/kg BM of protein) isocaloric (~235 kJ/kg BM) diet and refrained from physical exercise for the next 48 h. A dietician constructed all diets, and dietary control included the individualization of food plans for each subject relative to BM and food preferences. All food and drinks were supplied to subjects and were prepackaged to minimize the amount of food preparation required. Subjects were also supplied with a food checklist to record their daily intake.

**Experimental Protocol**

All subjects performed a random order of four 60-min experimental rides ~7 days apart. Two of the rides were undertaken at a power output that corresponded to ~45% of individual V\(\text{O}_2\) peak and the other two at a power output eliciting ~70% of V\(\text{O}_2\) peak. On the morning of an experiment, subjects reported to the laboratory between 0700 and 0800 after a 12- to 14-h overnight fast. A Teflon catheter (Baxter, Utrecht, The Netherlands) was inserted into a vein in the antecubital space of each arm for blood sampling and infusion of isotope (described subsequently). After collection of a resting venous blood sample (10 ml), the cannula was flushed with 1 ml of 0.9% saline solution to maintain patency of the vein. Then a primed (3.3 mmol) continuous (~44 μmol/min) infusion of sterile [6,6-\(^{2}\)H\(_2\)]glucose (Cambridge Isotope Laboratories, Cambridge, MA) was commenced and maintained for 90 min of rest and throughout the 60-min exercise bout. The infusate was delivered via a peristaltic pump (Gislon, Minipuls 3, Villiers Le Bel, France) that was calibrated at the start and end of each trial. Blood samples (10 ml) were obtained every 10 min during the last 30 min of the rest period.

On completion of the resting infusion period (i.e., 90 min), local anesthesia [2–3 ml of 1% Xylocaine (lignocaine)] was administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis in preparation for a resting muscle biopsy. Approximately 100 mg of muscle were removed by using a U.C.H. biopsy needle (Popper, NY) with suction applied and immediately frozen in liquid N\(_2\). At this time, a separate site on the same leg (~5 cm distal) was prepared for a second biopsy to be taken immediately after completion of 60 min of exercise. Muscle samples were stored at ~80°C until subsequent analysis.

After resting quietly for 10–15 min, subjects commenced 60 min of continuous cycling at the predetermined workload of that trial. At regular intervals throughout exercise (5, 15, 25, 35, 45, and 55 min), subjects breathed into a mouth piece attached to the previously described automated gas analyzer for a 5-min period for the estimation of instantaneous rates of whole body carbohydrate and fat oxidation. At the same time, blood samples (10 ml) were obtained for the measurement of plasma substrate concentrations and percent enrichment of [6,6-\(^{2}\)H\(_2\)]glucose.

**Analytic Procedures**

Whole body rates of carbohydrate and fat oxidation. Whole body rates of carbohydrate and fat oxidation (g/min) were estimated from V\(\text{CO}_2\) and V\(\text{O}_2\) samples collected during exercise (25) and expressed as micromoles per kilogram per minute.

Whole body glucose oxidation was calculated as the difference between total carbohydrate oxidation (estimated from RER data) and plasma glucose oxidation estimated from tracer-derived measures of plasma glucose rate of disappearance (R\(_{\text{Ra}}\)). Such a calculation is based on the assumption that the original sources of carbohydrate oxidized during exercise are muscle glycogen and blood glucose (6).

**Determination of glucose kinetics.** Duplicate 500-μl plasma samples collected at each time point were deproteinized in 500 μl of 0.3 M Ba(OH)\(_2\) and 500 μl of ZnSO\(_4\) and spun. Eighty microliters of supernate were then transferred to a limited-volume glass insert and placed in an oven to dry overnight. The samples were then derivatized with the addition of pyridine and acetic anhydride. The derivatized samples were measured using a gas chromatograph-mass spectrometer (5890 series 2 gas chromatograph, 5971 mass spectrometer detector, Hewlett Packard, Avondale, PA) by using a selected ion-monitoring mode to determine the relative abundance of the selected ions with mass-to-valence ratios of 98 and 100. Steady-state enrichments were attained after 90 min of rest, and subsequent rates of plasma glucose appearance (R\(_{\text{A}}\)) and R\(_{\text{Ra}}\) were calculated from changes in the percent enrichment in the plasma of [6,6-\(^{2}\)H\(_2\)]glucose by using a one-pool non-steady-state model (28). This model assumes a pool fraction of 0.65 and estimates the apparent glucose space as 25% of body mass. As plasma glucose R\(_{\text{A}}\) determined using [6,6-\(^{2}\)H\(_2\)]glucose, has been shown to equal the oxidation rate of plasma glucose measured with \(^{13}\text{CO}_2\) production (16), the rate of plasma glucose R\(_{\text{Ra}}\) in the present study has been used to estimate the rate of glucose oxidation.

**Blood substrates.** Five milliliters of whole blood were placed into a tube containing fluoride EDTA, mixed, and spun in a centrifuge at 4,000 revolutions/min for 8 min at 0°C. The plasma was later...
analyzed for glucose and lactate concentrations using an automated glucose and lactate analyzer (YSI 2300 STAT PLUS, Yellow Springs Instruments, Yellow Springs, OH). Catecholamine (epinephrine and norepinephrine) concentrations were measured using an enzyme immunoassay (2-Cat EIA BA-10-1500, Labor Diagnostika Nord, Nordhorn, Germany). Four milliliters of whole blood were placed into a tube containing lithium heparin, mixed, and spun in a centrifuge (as above). The remaining plasma was stored at −80°C for later analysis of plasma insulin concentration by radioimmunoassay (Phadeseph, Insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Blood (3 ml) for the determination of plasma free fatty acid (FFA) concentration was measured by an enzymatic colorimetric method (NEFA C code 279-75409, Wako, Tokyo, Japan).

Muscle analyses. One aliquot (1 mg) of freeze-dried muscle was extracted with 250 μl of 2 M HCl, incubated at 100°C for 2 h, and then neutralized with 750 μl of 0.667 M NaOH for determination of glycogen concentration via enzymatic analyses with fluorometric detection (21).

Statistical Analyses

Metabolic data from the four trials (RER, rates of carbohydrate and fat oxidation, glucose kinetics) were compared by using a three-way repeated-measures analysis of variance (ANOVA; preexercise glycogen content × exercise intensity × time). Differences in substrate utilization were determined by using a two-factor (preexercise glycogen content and exercise intensity) ANOVA. When a significant difference was found, Newman-Keuls post hoc test was used to identify where the difference occurred. A paired-samples t-test was employed to determine differences in preexercise muscle glycogen concentration after exercise-diet manipulation. All data are reported as means ± SE, and significance was accepted when P < 0.05. The statistical analyses were undertaken using Statistica software for Windows (StatSoft, version 5.1, 1997, Tulsa, OK).

RESULTS

Depletion Protocol

The mean PPO recorded during the maximal test was 377 ± 20 W (4.7 ± 0.1 W/kg). The mean work rates performed during the depletion sessions were 340 ± 18, 302 ± 16, 264 ± 14, and 226 ± 12 W, which corresponded to 90, 80, 70, and 60% of PPO, respectively. On average, subjects performed a total of 22 ± 2 work bouts at 90%, 4 ± 1 work bouts at both...
Exercise-diet manipulation was successful in elevating resting muscle glycogen content by \( \approx 300\% \) in the HG compared with LG trials (596 \( \pm \) 43 vs. 202 \( \pm \) 21 mmol/kg dry mass; \( P < 0.001 \)). As intended, there were no differences in starting muscle glycogen content within a dietary treatment (i.e., LG or HG) for either exercise intensity (i.e., 45 and 70\% of \( \dot{V}O_2 \) peak).

The effects of the exercise-diet manipulation on muscle glycogen concentration and after 1 h of either low-intensity (45\% of \( \dot{V}O_2 \) peak) or moderate-intensity (70\% of \( \dot{V}O_2 \) peak) cycling are displayed in Table 1. A main effect of both preexercise muscle glycogen content (\( P < 0.001 \)) and exercise intensity (\( P < 0.01 \)) was observed with respect to muscle glycogen utilization. Greater utilization of muscle glycogen occurred during trials with HG compared with LG and when cycling at 70\% (HG70 and LG70) compared with 45\% (HG45 and LG45) of \( \dot{V}O_2 \) peak (see Table 1). Although there was minor net resynthesis of muscle glycogen in five of seven subjects in the LG45 trial, this value was not different from preexercise levels.

\( \dot{V}O_2, \) HR, RPE, and Energy Expenditure

As intended, the average percent of \( \dot{V}O_2 \) peak sustained within each exercise intensity was similar, whereas there was a main effect of intensity between low and moderate conditions (\( P < 0.001 \)). Accordingly, the rate of energy expenditure was similar between trials performed at the same exercise intensity (LG45: 549 \( \pm \) 12 vs. HG45: 567 \( \pm \) 17 J kg\(^{-1}\) min\(^{-1}\); and LG70: 895 \( \pm \) 24 J kg\(^{-1}\) min\(^{-1}\) and HG70: 910 \( \pm \) 24 J kg\(^{-1}\) min\(^{-1}\) ) but almost twofold higher in the moderate-intensity compared with low-intensity exercise condition (\( P < 0.0001 \)).

Table 2 displays instantaneous values for \( \dot{V}O_2 \) together with corresponding HR and rating of perceived exertion (RPE). A significant main effect of preexercise muscle glycogen content (\( P < 0.01 \)) was observed for all measures, with increases in \( \dot{V}O_2, \) HR, and RPE during trials when preexercise muscle glycogen availability was low. Similarly, there was a main effect of exercise intensity on these variables (\( P < 0.0001 \)), with trials performed at \( \approx 70\% \) of \( \dot{V}O_2 \) peak resulting in higher \( \dot{V}O_2, \) HR, and RPE compared with trials in which subjects cycled at \( \approx 45\% \) of \( \dot{V}O_2 \) peak. \( \dot{V}O_2 \) did not change throughout exercise, whereas both HR and RPE increased with time (\( P < 0.0001 \)).

### Instantaneous RER and Substrate Oxidation

RER values, together with the corresponding rates of carbohydrate oxidation and fat oxidation during cycling at 45 and 70\% of \( \dot{V}O_2 \) peak with either LG or HG are displayed in Table 3. A main effect of preexercise muscle glycogen content was observed for all measures (\( P < 0.0001 \)). During HG, rates of carbohydrate oxidation were increased whereas rates of fat oxidation were decreased compared with LG (\( P < 0.0001 \)). Exercise intensity also had a main effect on RER (\( P < 0.001 \)) and on rates of carbohydrate (\( P < 0.0001 \)) and fat oxidation (\( P < 0.05 \)).
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There was a main effect of both preexercise muscle glycogen content \((P < 0.0001)\) and exercise intensity \((P < 0.0001)\) on the average RER for the 1-h exercise bouts (Fig. 1). RER was higher for HG than LG during exercise at both 45\%

\[ 0.85 \pm 0.01 \text{ vs. } 0.74 \pm 0.01; P < 0.001 \] and 70\% \((0.90 \pm 0.01 \text{ vs. } 0.79 \pm 0.01; P < 0.001)\) of \(\dot{V}_{\text{O}_2}\) peak. RER values were also elevated when cycling at 70\% compared with 45\% of \(\dot{V}_{\text{O}_2}\) peak for both LG \((P < 0.001)\) and HG \((P < 0.01)\) conditions.

Significant differences in total carbohydrate oxidation were observed between LG and HG conditions respectively, for exercise performed at 45\% \((25 \pm 2 \text{ vs. } 89 \pm 8 \text{ g/h}; P < 0.001)\) and 70\% \((87 \pm 12 \text{ vs. } 180 \pm 13 \text{ g/h}; P < 0.001)\) of \(\dot{V}_{\text{O}_2}\) peak. Accordingly, whole body muscle glycogen utilization was less during LG than HG when cycling at both 45\% \((7 \pm 1 \text{ vs. } 72 \pm 9 \text{ g/h}; P < 0.001)\) and 70\% \((65 \pm 13 \text{ vs. } 160 \pm 13 \text{ g/h}; P < 0.001)\) of \(\dot{V}_{\text{O}_2}\) peak.

Whole body fat oxidation was greater for LG compared with HG during exercise at both 45\% \((59 \pm 4 \text{ vs. } 32 \pm 4 \text{ g/h}; P < 0.001)\) and 70\% \((73 \pm 3 \text{ vs. } 34 \pm 3 \text{ g/h}; P < 0.001)\) of \(\dot{V}_{\text{O}_2}\) peak. An interaction of preexercise muscle glycogen content and exercise intensity was observed such that exercise intensity was only found to alter fat oxidation \((P < 0.05)\) when preexercise muscle glycogen was low (LG).

Figure 2 displays plasma glucose \(R_a\) and \(R_d\) during 1 h of cycling. There was a main effect of exercise intensity such that cycling at 70\% of \(\dot{V}_{\text{O}_2}\) peak resulted in greater \(R_a\) and \(R_d\) than cycling performed at 45\% of \(\dot{V}_{\text{O}_2}\) peak \((R_a; 23.2 \pm 1.5 \text{ vs. } 19.3 \pm 1.3 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}, P < 0.05; R_d; 24.1 \pm 1.7 \text{ vs. } 19.2 \pm 0.9 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}, P < 0.05)\). Glucose \(R_d\) was significantly higher after 25 min compared with preceding time points and remained elevated for the duration of the trial. \(R_d\) averaged over 1 h of exercise was 21.5 \pm 1.7 and 21.0 \pm 1.2 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) for LG and HG, respectively. Corresponding measures of glucose \(R_a\) were 22.3 \pm 2.2 and 20.7 \pm 1.7 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\). No effect of preexercise muscle glycogen content on total glucose \(R_d\) was reported (Fig. 2).

Figure 3 displays the relative contribution of whole body muscle glycogen, plasma glucose, and fat oxidation to total energy expenditure during 1 h of cycling. Differences in the relative contribution of whole body muscle glycogen oxidation to energy expenditure were found between LG and HG respectively, for exercise at both 45\% \((5 \pm 2 \text{ vs. } 45 \pm 5\%); P <
Table 4. Plasma free fatty acid, glucose, lactate, and insulin concentrations during 60 min of cycling exercise at 45 and 70% of peak O2 uptake with low and high preexercise muscle glycogen content

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45%</td>
<td>70%</td>
<td>45%</td>
<td>70%</td>
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<tr>
<td>FFA, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>0.5±0.1</td>
<td>0.4±0.1</td>
<td>0.7±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>HG</td>
<td>0.3±0.1</td>
<td>0.2±0.1</td>
<td>0.3±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.0±0.2</td>
<td>5.1±0.2</td>
<td>4.9±0.2</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>HG</td>
<td>5.0±0.1</td>
<td>4.9±0.2</td>
<td>5.0±0.1</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>1.3±0.2</td>
<td>2.4±0.2</td>
<td>1.0±0.2</td>
<td>2.0±0.2</td>
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<tr>
<td>HG</td>
<td>1.5±0.2</td>
<td>3.7±0.3</td>
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</tr>
<tr>
<td>Insulin, μU/l</td>
<td>4.9±0.5</td>
<td>4.8±0.5</td>
<td>4.7±0.4</td>
<td>3.9±0.3</td>
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<tr>
<td>LG</td>
<td>5.0±0.6</td>
<td>5.5±0.6</td>
<td>5.2±0.5</td>
<td>5.6±0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 subjects. FFA, free fatty acid. *Main effect of muscle glycogen content: LG > HG for FFA; LG < HG for glucose, lactate, P < 0.05. †Main effect of exercise intensity: 45% > 70% V̇O2peak for FFA, P < 0.05; LG > HG for lactate, P < 0.05. ‡Main effect of time: 60 min > rest for FFA, P < 0.05; 20, 40, 60 min > rest for lactate; P < 0.0001.

0.001) and 70% (25 ± 3 vs. 60 ± 3%; P < 0.001) of V̇O2peak. In addition, moderate-intensity exercise (i.e., 70% of V̇O2peak) resulted in a greater contribution of whole body muscle glycogen to energy expenditure compared with low-intensity exercise (i.e., 45% of V̇O2peak) for both LG (25 ± 3 vs. 5 ± 1%; P < 0.001) and HG (60 ± 3% vs. 45 ± 5%; P < 0.01) conditions. The contribution of plasma glucose oxidation, estimated from tracer-derived measures of glucose Rd, to total energy expenditure did not differ between the four experimental conditions (~9%). In contrast, a main effect of both preexercise muscle glycogen content (P < 0.0001) and exercise intensity (P < 0.0001) was observed on the contribution of whole body fat oxidation to energy expenditure (LG45: 84 ± 1%, HG45: 44 ± 5%, LG70: 65 ± 3%, HG70: 29 ± 3%).

Blood Metabolites

Plasma FFA, glucose, lactate, and insulin concentrations determined during exercise are displayed in Table 4. Preexercise muscle glycogen content exerted a main effect on plasma FFA (P < 0.001), glucose (P < 0.05), and lactate (P < 0.001) concentrations, with higher values of both glucose and lactate observed for HG than LG. Plasma FFA concentrations were lower during HG compared with LG conditions. Differences in plasma FFA (P < 0.05) and lactate (P < 0.01) concentrations were also observed between exercise trials of different intensities. Greater FFA concentrations but lower lactate levels were observed during low- compared with moderate-intensity exercise. However, there was an interaction between preexercise muscle glycogen content and exercise intensity for plasma FFA (P < 0.05) and lactate (P < 0.01) concentrations, such that FFA levels were only elevated during moderate-intensity exercise when preexercise muscle glycogen was low. In addition, preexercise muscle glycogen content was only observed to affect plasma lactate concentration when exercise intensity was low. A main effect of time was observed for plasma FFA (P < 0.05) and lactate (P < 0.0001) concentrations. Plasma FFA levels rose significantly above resting values during exercise, whereas plasma lactate concentration during exercise was greater than at rest.

Table 5. Plasma epinephrine and norepinephrine concentrations during 60 min of cycling exercise at 45 and 70% of peak O2 uptake with low and high preexercise muscle glycogen content

<table>
<thead>
<tr>
<th>Measured Substance</th>
<th>Rest</th>
<th>10 min</th>
<th>60 min</th>
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<tbody>
<tr>
<td></td>
<td>45%</td>
<td>70%</td>
<td>45%</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>33±8</td>
<td>32±8</td>
<td>96±41</td>
</tr>
<tr>
<td>HG</td>
<td>30±10</td>
<td>28±5</td>
<td>46±5</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LG</td>
<td>135±18</td>
<td>272±18</td>
<td>414±78</td>
</tr>
<tr>
<td>HG</td>
<td>177±58</td>
<td>277±45</td>
<td>490±131</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 subjects. *Main effect of exercise intensity: 45% < 70% V̇O2peak for epinephrine; norepinephrine, P < 0.05. †Main effect of time: rest, 10 min < 60 min, for epinephrine; rest < 10, 60 min for norepinephrine, P < 0.01.
plasma norepinephrine rose above resting levels after 10 and 60 min of exercise. In addition, post hoc comparisons revealed significantly greater concentrations of plasma epinephrine after 10 and 60 min of exercise at 70% of \( V_{\text{O2peak}} \) when preexercise muscle glycogen content was low compared with high \( (P < 0.05) \).

**DISCUSSION**

Previous studies investigating the relationship between pre-exercise muscle glycogen content and substrate metabolism during whole body submaximal exercise have typically employed protocols that involve a single intensity \( (2, 10, 34) \). This is the first investigation to systematically quantify the relative effects of preexercise muscle glycogen content and exercise intensity on fuel metabolism in the same subjects \( (\text{i.e., the same training status}) \). The scope of this discussion will be limited to investigations that have manipulated preexercise muscle glycogen concentration using chronic \( (\text{i.e., 24–48 h}) \) exercise-diet interventions. Short-term \( (\text{i.e., 1–4 h}) \) diet and/or pharmacological interventions such as carbohydrate and lipid ingestion \( (13), \) Intralipid ingestion \( (23), \) glucose infusion \( (32), \) and nicotinic acid ingestion \( (12) \) that acutely alter substrate availability will not be considered. We acknowledge that our present study design does not allow for a comparison to be made between low, moderate, and high exercise intensities and/or preexercise muscle glycogen concentrations.

The first major finding of the present study was that, during 60 min of low-intensity exercise \( (45\% \text{ of } V_{\text{O2peak}}) \), high preexercise muscle glycogen content resulted in greater rates of whole body carbohydrate oxidation than when exercising at moderate-intensity \( (70\% \text{ of } V_{\text{O2peak}}) \) with low muscle glycogen concentration. It has been assumed that energy flux, as determined by the relative intensity of exercise, is the primary factor determining the balance of substrate utilization during exercise and that energy supply is only of secondary importance \( (4, 5) \). Indeed, when preexercise muscle glycogen stores are in the normal range \( (\text{i.e., 350–500 mmol/kg dry mass}) \), whole body rates of carbohydrate oxidation, as determined from RER values, increase concomitantly with an increase in exercise intensity \( (1, 24, 26) \). In agreement with these findings, we also observed a significant main effect of exercise intensity on rates of whole body carbohydrate oxidation. However, it is important to note that the regulation of carbohydrate metabolism in skeletal muscle involves the interplay between substrate availability, the prevailing exercise intensity, and training status.

It is well established that muscle glycogen utilization increases with the relative intensity of exercise \( (17, 31) \). In addition, altering endogenous glycogen stores by exercise and diet manipulation modifies the balance of substrate oxidation during subsequent exercise. Several studies have selectively altered skeletal muscle glycogen levels and demonstrated a positive relationship between the initial concentration and the subsequent rate of glycogenolysis \( (3, 10, 27, 34) \). For example, Spencer et al. \( (27) \) found that, when preexercise muscle glycogen content was reduced from \( 725 \text{ to } 182 \text{ mmol/kg dry mass} \), muscle glycogenolysis during \( 40 \text{ min of exercise at } 75\% \text{ of } V_{\text{O2peak}} \) was \( 200\% \) lower. Alternatively, the results of other studies that have investigated the effect of preexercise muscle glycogen content on its subsequent utilization during exercise are equivocal, with some finding no difference between rates of glycogenolysis when starting glycogen levels are high \( (11, 15, 22) \). In the present study, there was greater utilization of muscle glycogen when preexercise glycogen levels were high \( (\text{Table 1}) \). As might be expected from the higher RER values and enhanced rates of glycogen breakdown, there was also a greater contribution from carbohydrate oxidation to total energy expenditure when commencing exercise with high glycogen levels.

Despite differences in preexercise muscle glycogen content and its subsequent utilization, the contribution from plasma glucose oxidation to total energy expenditure was similar \( (\sim 10\%) \) under all experimental conditions \( (\text{Fig. 2}) \). Notably, alterations in preexercise muscle glycogen content had a markedly greater influence on the relative contribution of whole body glycogen oxidation to energy metabolism under conditions of low muscle glycogen content \( (\text{i.e., } \sim 5-\text{ vs. } \sim 1.3-\text{fold increase}) \). This finding may be explained by the increased rate of glycogenolysis associated with high preexercise muscle glycogen content that consequently reduces the relative effect of exercise intensity under these conditions. In addition, increasing muscle glycogen content exerted a greater effect on carbohydrate-derived energy metabolism, specifically the oxidation of whole body glycogen, than increasing exercise intensity \( (\text{HG}45 \text{ vs. LG}70) \).

Preexercise muscle glycogen content influences catecholamine concentrations during exercise, leading to the modification of subsequent metabolic responses \( (\text{i.e., patterns of fuel oxidation}) \). In this regard, Wojtaszewski et al. \( (34) \) reported that significantly greater levels of plasma norepinephrine during exercise when commenced exercise in a glycogen-depleted state. In the present study, preexercise muscle glycogen content did not affect either resting or exercise norepinephrine concentrations. Wojtaszewski et al. reported that enhanced concentrations of norepinephrine coincided with a greater rate of leg blood flow and increased rates of plasma glucose oxidation during exercise. Indeed, such differences in circulating norepinephrine concentrations may, in part, be responsible for the enhanced cellular glucose uptake capacity observed by Wojtaszewski et al. during exercise in the glycogen-depleted state.

Alternatively, the influence of prior exercise, diet, and substrate and hormonal status on glucose uptake needs to be considered. These factors were investigated by Steensberg et al. \( (29) \) through a series of experiments involving the manipulation of muscle glycogen content via either single- or two-legged kicking exercise and subsequent dietary manipulation. Their data suggest that preexercise muscle glycogen content only influences glucose uptake during exercise when the delivery of substrates and hormones remain constant. In contrast, when the delivery of substrates and hormones is altered, the potential effect of glycogen concentration on glucose uptake is negated. However, it is important to acknowledge that the physiological state imposed by Steensberg et al. is not typical of that experienced during whole body exercise.

Several studies have demonstrated a close temporal relationship between skeletal muscle glycogen content and glucose
uptake (8, 18, 34). Wojtaszewski et al. (34) studied well-trained subjects who commenced 60-min of moderate-intensity (~70% $\dot{V}O_2$ peak) cycling in either a glycogen-depleted (~160 mmol/kg dry mass) or glycogen-loaded (~900 mmol/kg dry mass) state. They found that leg glucose uptake was approximately two-fold higher under glycogen-depleted conditions, along with higher net fatty acid uptake and increased plasma epinephrine and norepinephrine concentrations. In contrast, Hargreaves et al. (10) reported no change in the rate of tracer-derived plasma glucose oxidation in active (but untrained) men during 40-min of moderate-intensity (~70% $\dot{V}O_2$ peak) cycling when preexercise muscle glycogen content was low (~380 mmol/kg dry mass) compared with normal (~525 mmol/kg dry mass). In support of these results, Bosch et al. (3) also found no difference in muscle glucose uptake during 2-h of exercise at 70% of $\dot{V}O_2$ peak in subjects with high (~820 mmol/kg dry mass) or normal (~525 mmol/kg dry mass) preexercise glycogen concentrations.

A possible reason to explain discrepancies in the rates of plasma glucose uptake or disposal under conditions of altered muscle glycogen content are differences in the training status of subjects. When moderately trained subjects commence exercise with either low or high muscle glycogen levels, glucose $R_d$ is similar (3, 10, 32). In contrast, in well-trained subjects glucose $R_d$ values are considerably greater when exercising in a glycogen-depleted state (27, 34). In agreement with these findings (3, 10, 32), the plasma glucose $R_d$ found to be similar in the present study when starting muscle glycogen content was elevated by ~300% (~200 vs. ~600 mmol/kg dry mass) in moderately trained men during exercise at either 45 or 70% of $\dot{V}O_2$ peak. A potential mechanism contributing to differences in glucose uptake between studies is a higher training-induced GLUT-4 protein content. In support of this contention, Kristiansen et al. (19) reported a greater contribution of plasma glucose to whole body carbohydrate metabolism after 3 wk of endurance training. The present study is the first to examine the relative effects of exercise intensity and preexercise muscle glycogen content on glucose uptake in the same subjects (i.e., the same GLUT-4 protein content).

In conclusion and in contrast to our original hypothesis, an increase in exercise intensity did not increase carbohydrate oxidation (i.e., whole body glycogen and blood glucose oxidation) to a greater extent than an increase in preexercise muscle glycogen content. Rather, additional energy required to perform exercise is derived from the oxidation of fat. Furthermore, high preexercise muscle glycogen content increases carbohydrate oxidation to a greater extent than an increase in exercise intensity from 45 to 70% of $\dot{V}O_2$ peak in the same subjects, i.e., training status. The results of the present study suggest that muscle glycogen availability does not influence plasma $R_d$ during exercise in moderately trained individuals.

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