Influence of muscle glycogen content on metabolic regulation

SANDRA M. WELTAN, ANDREW N. BOSCH, STEVEN C. DENNIS, AND TIMOTHY D. NOAKES
Department of Physiology, University of Cape Town Medical School, Observatory 7925, South Africa

Weltan, Sandra M., Andrew N. Bosch, Steven C. Dennis, and Timothy D. Noakes. Influence of muscle glycogen content on metabolic regulation. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E72–E82, 1998.—Euglycemia was maintained in 13 subjects with low muscle glycogen [low glycogen, euglycemic (LGE), n = 8; low glycogen, euglycemic, hyperinsulinemic (LGEI), n = 5] and 6 subjects with normal muscle glycogen (NGE), whereas hyperglycemia was maintained in 8 low muscle glycogen subjects (LGH). All subjects cycled for 145 min at 70% of maximal oxygen uptake during the infusions. Insulin was infused in LGEI at 0.2 mU·kg⁻¹·min⁻¹. During exercise, respiratory exchange ratio (RER) was lower and norepinephrine higher in LGE than in NGE. In LGEI and LGH, RER at the start of exercise was the same as in LGE but did not decrease as in LGE. Free fatty acids (FFA) were higher and plasma insulin concentrations lower in LGE than NGE, LGEI, or LGH over the first 45 min of exercise. Rate of glucose infusion (Ri) and rate of glucose oxidation (Rox) were higher in LGH and LGEI than in NGE or LGE, and Ri matched Rox in all groups except LGH, in which Ri was greater than Rox. Muscle glycogen disappearance was greater in NGE than LGE, LGEI, or LGH, but the latter three groups did not differ. In conclusion, this study showed that low muscle glycogen content results in a decrease in RER, an increase in FFA and norepinephrine both at rest and during exercise, and does not affect Rox when euglycemia is maintained by infusion of glucose alone. Rox was increased only during insulin and hyperglycemia.

A DECREASE IN CARBOHYDRATE (CHO) oxidation, as indicated by a decreased respiratory exchange ratio (RER) after a low-CHO diet (8, 10, 13), has been described at rest (8, 13) and during exercise at various intensities (8, 10, 13). In addition, a low-CHO diet or fasting before exercise has been associated with a decrease in plasma insulin (8, 10, 12) and lactate (10) concentrations and an increase in plasma norepinephrine (8, 12), epinephrine (8, 12), glucagon (8, 12), growth hormone (GH); see Ref. 8), cortisol (8), glycerol (8, 13), ketone (8, 12), and free fatty acid (FFA); see Refs. 8, 10, 12, 13) concentrations. However, it is not clear whether the effects seen in these studies on substrate metabolism and hormonal responses were due to muscle or liver glycogen depletion or, except in cases in which glucose was infused during exercise (8, 12), due to a general “CHO deficiency,” i.e., a shortage of total CHO available for oxidation. In some studies, subjects were fasted overnight (10) or became hypoglycemic despite ingestion of a small preexercise meal (13). In an attempt to exclude the effects of an overall CHO deficiency, glucose has been infused during exercise in some studies (8, 12), but, in one study (12), subjects who ingested a low-CHO diet were fasted overnight while control subjects on a normal diet ate a 60% CHO meal 3 h before exercise; in the other study (8), subjects were fasted overnight and glucose infusion was begun only after subjects had reached exhaustion.

When euglycemia is maintained by glucose infusion during exercise, glucose that disappears from the circulation is replaced by infused glucose. This should prevent any metabolic or hormonal effects as a consequence of inadequate intramuscular and intrahepatic CHO availability. Thus, if the metabolic effects of glucose infusion when hyperglycemia is maintained by insulin infusion during euglycemic exercise, the effects of intramuscular and intrahepatic CHO availability will be differentiated and will provide evidence that the metabolic effects described previously (8, 10, 12, 13) are related specifically to intramuscular CHO availability.

Because hyperinsulinemia has minimal effects on hepatic glucose uptake in the absence of hyperglycemia (4), the predominant effect of insulin infusion during exercise in which euglycemia is maintained is an increase in muscle glucose uptake (5). Thus, if the metabolic effects of glucose depletion are counteracted by insulin infusion during euglycemic exercise, the effects of intramuscular and intrahepatic CHO availability will be differentiated and will provide evidence that the metabolic effects described previously (8, 10, 12, 13) are related specifically to intramuscular CHO availability.

Finally, in subjects with normal glycogen content, maintenance of hyperglycemia results in an increase in glucose oxidation during exercise compared with maintenance of euglycemia (11). However, unlike euglycemia, the rate of glucose oxidation is less than the rate of glucose infusion when hyperglycemia is maintained during exercise (11). We therefore hypothesized that the “excess” glucose that was not oxidized in subjects with normal muscle glycogen content during hyperglycemia (11) would be oxidized when muscle glycogen is low.

Therefore, the first aim of this study was to investigate the effect of low muscle glycogen content, without concomitant fatigue, on fuel substrate utilization during prolonged, moderate-intensity exercise by maintaining euglycemia and manipulating plasma insulin concentrations to provide an alternate source of intramuscular CHO. Second, we aimed to determine whether the metabolic and hormonal effects of low muscle glycogen content could be overcome by providing an excess of oxidizable CHO by intravenous glucose infusion.
GLYCOGEN CONTENT AND METABOLIC REGULATION

METHODS

Subjects

Twenty-seven endurance-trained male cyclists participated in the study, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. Cyclists were selected who regularly trained ≥250 km/wk and who had completed a 105-km cycle race in <3.5 h. Subject characteristics are given in Table 1. As radiolabelled tracers were ingested and muscle biopsies and blood samples were taken, the procedures and risks were explained to the subjects, and their written informed consent was obtained.

The total radiation dose received by each subject was ~0.11 mSv. A radiation dose of 1 mSv/yr is accepted as safe for the public, and 20 mSv/yr is regarded as safe for occupational exposure to radioactivity (personal communication, Dr. M. Shackleton, Dept. of Medical Physics, Groote Schuur Hospital, Cape Town, South Africa).

Measurements Taken Before Manipulation of Glycogen Content

On the first day of the trial, subjects ingested a small breakfast (~1,200 kJ, 30 g CHO) 3 h before arriving at the laboratory at 9:15 AM. The subject was then weighed, and percent body fat was estimated from the sum of biceps, triceps, subscapular, and suprailiac skin-fold measurements, using standard formulas (7).

Measurement of maximal oxygen uptake. Maximal oxygen uptake (VO2max) of each subject was determined during an incremental exercise test to exhaustion. Exercise was started on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands) at a workload equivalent to 3.3 W/kg body mass and increased first by 50 W after 150 s and then by 25 W every 150 s until the pedalling frequency dropped below 50 revolutions/min (rpm). Oxygen uptake (VO2) was measured on-line using a computerized system (Oxycon Alpha, J. aeger-Mijnhart, The Netherlands), and peak power output and VO2max were recorded. This information was used to adjust the work rate in the subsequent phases of the trial so that each subject exercised at an intensity corresponding to 70% of VO2max. Because metabolic and hormonal responses appear to be related to relative rather than absolute workload (16), work rate was adjusted during the trial (if necessary) to maintain exercise at this intensity.

Manipulation of muscle glycogen content. After the VO2max test, all subjects rested for 20 min and then rode for a further 90 min at 70% of VO2max, with 5-min intervals at 90% of VO2max every 20 min to deplete muscle glycogen. After this procedure, subjects were randomly assigned to one of four groups: low glycogen, euglycemic (LGE; n = 8); normal glycogen, euglycemic (NGE; n = 6); low glycogen, euglycemic, hyperinsulinemic (LGEI; n = 5); and low glycogen, hyperglycemic (LGH; n = 8).

Subjects in the low-glycogen groups (LGE, LGEI, and LGH) ingested a low-CHO diet for the 48 h after the depletion regimen. They were given a list of food choices that would provide a daily energy intake of ~6,800 kJ (16% CHO). NGE subjects followed an unrestricted diet. All subjects were instructed to do only light training on the second day (~1 h of low-intensity cycling). The low-CHO diet in the former groups, combined with light training during this period, was designed to limit muscle glycogen resynthesis while allowing recovery from the fatiguing effects of the depletion ride. In contrast, the normal diet in the latter group combined with the light training allowed repletion of muscle glycogen content to normal.

Experimental Protocol After Manipulation of Muscle Glycogen Content

Resting measurements. Subjects were again instructed to ingest a small breakfast (~1,200 kJ, 30 g CHO) 3 h before arrival at the laboratory at 9:15 AM on the third day of the trial. Shortly after the subject’s arrival in the laboratory, an 18-gauge Teflon cannula (J eloc.J. Johnson and J. Johnson, Halfway House, South Africa) was placed into his right forearm vein and connected to a three-way stopcock (Uniflex; Mallinckrodt Medical, Hennef-Siege, Germany). No blood sample was taken at this stage in case venipuncture caused a rise in circulating catecholamine concentrations. Resting VO2 and carbon dioxide production (VCO2) were determined with the subject seated and relaxed, and a urine sample was later collected for determination of urinary ketones. Twenty minutes after cannulation, a 20-ml blood sample was obtained for measurement of concentrations of plasma glucose, lactate, insulin, glucagon, epinephrine, norepinephrine, and serum FFA and GH. All aliquots of the blood sample were placed into tubes containing potassium oxalate and sodium fluoride (glucose, lactate), lithium heparin (insulin, epinephrine, norepinephrine), and EDTA and 100 µl Aprotinin (Midran; Novo Nordisk, Johannesburg, South Africa) (glucagon) and in serum separating tubes (Beckton-Dickinson) for FFA and GH. The tubes were immediately placed on ice and, after 20 min, centrifuged at 500 g and 4°C for 20 min. The supernatant (plasma or serum) was then transferred to plastic test tubes, sealed, and stored at -20°C for later measurements of glucose, lactate, insulin, and glucagon or at -80°C for later determinations of epinephrine, norepinephrine, GH, and FFA.

Leg muscle glycogen disappearance. A muscle sample was obtained from the vastus lateralis muscle before the start of and immediately on completion of exercise using a biopsy technique described previously (1). The samples were immediately placed in liquid nitrogen for later determination of muscle glycogen concentration using conventional methods (24).

Glucose and insulin infusion. A 20-gauge Teflon cannula was placed in a left forearm vein for infusion of glucose (all groups) and insulin (LGEI) during the entire 145 min of cycle ergometer exercise at 70% of VO2max. Plasma glucose concentration was maintained at ~4.5 mmol/l (euglycemic subjects) or ~9 mmol/l (hyperlglycemic subjects) by infusing a sterile 25% mass/vol glucose solution using the euglycemic (LGE, NGE), euglycemic, hyperinsulinemic (LGEI), and hyperglycemic (LGH) glucose clamp techniques (6), modified by adding known rates of increase in blood glucose oxidation over this

Table 1. Characteristics of subjects

<table>
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<tr>
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<th>LGE</th>
<th>LGEI</th>
<th>LGH</th>
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<tr>
<td>n</td>
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<td>15 ± 1</td>
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<td>363 ± 19</td>
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<td>4.57 ± 0.15</td>
<td>4.46 ± 0.13</td>
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Values are means ± SE; n, no. of subjects; LGE, low glycogen, euglycemic; NGE, normal glycogen, euglycemic; LGEI, low glycogen, euglycemic, hyperinsulinemic; LGH, low glycogen, hyperglycemic; FFM, fat-free mass; VO2max, maximal O2 consumption. No significant differences between groups.
duration of exercise at 70% of \( \dot{V}O_2_{max} \) from previous research in this laboratory (1). In LGEI, insulin (Actrapid; Novo Nordisk) was infused in normal saline at a rate determined by a pilot study of 0.2 mIU·kg\(^{-1}\)·min\(^{-1}\). All infusions were controlled using calibrated automatic syringe pumps (Travenol Laboratories, Hooksett, NH). Blood samples were obtained at 5-min intervals to measure blood glucose concentrations using a pocket glucometer (Accutrend; Boehringer Mannheim, Mannheim, Germany). Glucose oxidation rate was measured by adding 7,400 kBq/mmol [U-\(^{14}\)C]glucose (Amersham International, Buckinghamshire, UK) tracer was added to 1.8 liters of an artificially flavored water placebo. Pilot studies showed that the plasma [U-\(^{14}\)C]glucose specific activity, \(^{14}\)CO\(_2\) specific activity, and calculated rates of glucose oxidation (described in Plasma glucose and lactate specific activity) were the same irrespective of whether the tracer was ingested or infused. The tracer was thus ingested following a set protocol to later include a study in which glucose was ingested during exercise. While warming up (5 min) before the 145-min exercise test, subjects drank a 400-ml bolus of the drink, which contained 111 kBq/l of [U-\(^{14}\)C]glucose tracer. All infusions were controlled using calibrated automatic syringe pumps (Travenol Laboratories, Hooksett, NH). Blood samples were obtained at 5-min intervals to measure blood glucose concentrations using a pocket glucometer (Accutrend; Boehringer Mannheim, Fullerton, CA). At the end of exercise, a second urine specimen was obtained to determine whether the glucose infusion had resulted in glycosuria.

Measurement of substrate oxidation and hormone concentrations during exercise. Rates of glucose oxidation were measured using a [U-\(^{14}\)C]glucose tracer. Two hundred kilobecquerels of a 7,400 kBq/mmol [U-\(^{14}\)C]glucose (Amersham International, Buckinghamshire, UK) tracer was added to 1.8 liters of an artificially flavored water placebo. Pilot studies showed that the plasma [U-\(^{14}\)C]glucose specific activity, \(^{14}\)CO\(_2\) specific activity, and calculated rates of glucose oxidation (described in Plasma glucose and lactate specific activity) were the same irrespective of whether the tracer was ingested or infused. The tracer was thus ingested following a set protocol to later include a study in which glucose was ingested during exercise. While warming up (5 min) before the 145-min exercise test, subjects drank a 400-ml bolus of the drink, which contained 111 kBq/l of [U-\(^{14}\)C]glucose tracer. Every 5 min after commencement of the exercise test, subjects ingested a further 100 ml, giving a total of 600 ml/h.

After 5 min of exercise at 70% of \( \dot{V}O_2_{max} \) and at 20-min intervals during exercise, ~20 ml of blood were drawn and processed as described for later measurements of plasma [U-\(^{14}\)C]glucose specific activity and for measurement of glucose, insulin, glucagon, epinephrine, norepinephrine, FFA, and GH.

\( \dot{V}O_2 \), \( \dot{V}CO_2 \), and \(^{14}\)CO\(_2\) measurements during exercise. At the same time as the blood samples were drawn, \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) were determined on-line using a computerized system (Oxycon Alpha, Jaege-Mijnhart, The Netherlands), and expired air was trapped for later determination of \(^{14}\)CO\(_2\) specific activity. The carbon dioxide trapping mixture has been described previously (1) and consisted of 1 ml of hyamine hydroxide in methanol (Packard), 1 ml of ethanol, and two drops of 1% phenolphthalein indicator. Expired air was bubbled through the trapping mixture until the solution became dark at which point 1 nmol of carbon dioxide had been absorbed. Liquid scintillation cocktail (10 ml of Beckman Ready Gel) was then added, and \(^{14}\)CO\(_2\) radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 1500, Downer’s Grove, IL). All \(^{14}\)C counts were automatically corrected for quenching and background radioactivity.

Laboratory Analyses

Rates of and total CHO and fat oxidation. Rates of CHO and fat oxidation were calculated from the \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) (l/min) values using the formulas

\[
\text{CHO (g/min)} = 4.55 \times \dot{V}O_2 - 3.21 \dot{V}O_2 \\
\text{Fat (g/min)} = 1.67 \dot{V}O_2 - 1.67 \dot{V}CO_2
\]

Total CHO and fat oxidation were calculated from the rate vs. time curve for 145 min of exercise.

Plasma glucose and lactate concentrations. Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyzer (Glucose analyzer 2; Beckman). Lactate concentrations were measured on the same plasma samples by spectrophotometric methods (model 35; Beckman) enzymatic assays (Lactate PAP, biMerieux, Lyon, France).

Plasma glucose and lactate specific activity. A 1-ml aliquot of each of the plasma samples used for glucose determination was deproteinized by addition of 70 µl of HClO\(_4\) (3.5 M), which also served to drive off [\(^{14}\)C]bicarbonate as \(^{14}\)CO\(_2\). The samples were then centrifuged at 4°C, and the protein-free supernatant was removed and kept cold. The precipitate was next washed by resuspension in 0.76 ml of 0.13 M HClO\(_4\) and recentrifuged, and the supernatant was added to that previously saved. The last step was then repeated. The pH of the combined supernatants was then returned to between 7.0 and 8.0 by the addition of 132 µl of 3 M K\(_2\)CO\(_3\) in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-HCl buffer. The void volume, which contained some glucose, was collected, and the remaining glucose was eluted with distilled water (3 × 1 ml). Lactate was then eluted with 2 × 1 ml 1 M CaCl\(_2\) (pH 2). Five control samples were processed and run concurrently with the test samples each time the analysis was performed and was used to confirm complete separation of glucose from carbon-containing anions.

To reduce the water-to-liquid scintillation cocktail ratio during radioactivity counting, the eluates (~7 ml) were evaporated to near dryness (~0.3 ml) at 60°C over ~20 h. One milliliter of distilled water was then added to the residue and mixed with 15 ml of Ready Gel liquid scintillation cocktail for \(^{14}\)C radioactivity determinations as described previously. Because the 1-ml aliquot of plasma used for radiation counting was from the same plasma sample as previously used for the determination of glucose concentration, the specific activity in disintegrations per minute per millimole glucose could be calculated after the small (~4%) loss of radioactivity during the preparation of the sample was determined from the control plasma samples. After scintillation counting, it was found that the disintegrations per minute of the lactate were too low to be reliable when calculating the specific activity. Thus these data were not used to calculate lactate oxidation. The rate of glucose oxidation (\( R_{\text{glc}} \)) was calculated from the formula

\[
R_{\text{glc}} = \left( \frac{^{14}\text{CO}_2 \times 6}{\text{SA}_{\text{glc}}} \right) \times \dot{V}CO_2 \times 1.35
\]

where, \(^{14}\text{CO}_2 \times 6\) is the disintegrations per minute per millimole value multiplied by 6 (6 carbon atoms/molecule [\(^{14}\text{C}\)] glucose), \( \text{SA}_{\text{glc}}\) is the plasma [\(^{14}\text{C}\)] glucose specific activity in disintegrations per minute per millimole, and 1.35 is the number of grams of glucose oxidized to produce 1 liter of carbon dioxide. Units for \( \dot{V}CO_2 \) are liters per minute. Total glucose oxidation was calculated from the area under the curve of glucose oxidation vs. time curve.

Plasma insulin and glucagon and serum GH concentrations. Insulin, glucagon, and GH were determined using radioimmunoassay techniques (Coat-A-Count Insulin and Double Antibody Glucagon, Diagnostic Products, Los Angeles, CA; and human GH radioimmunoassay, Pharmacia, Uppsala, Sweden).

Plasma catecholamine concentrations. Catecholamine concentrations were determined by means of high-performance liquid chromatography (HPLC) with electrochemical detec-
tion. To 1.5 ml of plasma in a polypropylene tube, an internal standard (10 µg/ml dehydrobenzylamine in 0.1 N HCl), 0.4 ml of 2 M Tris·HCl buffer (pH 8.1), and 10 µg of acid-washed aluminium oxide were added. The mixture was shaken for 15 min, after which the supernatant was discarded and the aluminium oxide precipitate was washed three times with 1 ml of 0.02 M Tris·HCl buffer (pH 8.1). The fluid was then carefully aspirated, 0.1 ml 1% vol/vol glacial acetic acid was added, and the samples were shaken and then centrifuged at 2,000 rpm for 15 min. The clear supernatant was transferred to clean tubes and stored at −20°C for no longer than 2 days before chromatography. The final supernatant was injected onto the HPLC column (Resolve C18-Novapak 3.9 × 150 mm, particle size 5 µl, ambient temperature; Waters 510 pump, Waters 712 Wisp injector) in a volume of 15 µl. The mobile phase included 50 mM sodium acetate, 20 mM citric acid, 3.75 mM sodium-1-octane-sulfonate, 1.0 mM di-n-butylamine, and 0.135 mM NaEDTA in 95% methanol, pH 4.3. The flow rate was 1.0 ml/min (pressure 14,000 kPa), the working potential was +0.6 V against Ag/AgCl reference electrode, 0.5 nA full-scale sensitivity, and at time constant set on 2 s (active filter switched on). The cell volume was 2.5 µl. The integration was performed using a Spectra-Physics SP4400 Chrom J et Recording Integrator using the following parameters: attenuation, 64; chart speed, 0.5 cm/min; peak width, 6; peak threshold, 2,000.

Serum FFA concentrations. Serum FFA concentrations were measured using an enzymatic colorimetric assay (Haf-microtest; Boehringer Mannheim; see Ref. 29).

Urine specimens. Urine specimens collected before and at the end of exercise were examined for the presence of ketones and glucose using urinary dipsticks (Keto-Diabur-Test 5000; Boehringer Mannheim, Lewes, East Sussex, UK).

Statistical Analyses

All results are presented as means ± SE. Statistical significance (P < 0.05) of between-group differences was assessed by a two-way analysis of variance with repeated measures over time, followed by a Tukey's honest significance test for unequal n. An unpaired t-test was used for single data. For some hormonal measurements, a Spearman correlation test was used on area under the curve measurements.

RESULTS

Subject characteristics are given in Table 1. There were no significant differences between groups in any of the parameters shown.

Preexercise

Preexercise muscle glycogen concentrations were significantly higher in NGE than in LGE, LGEI, or LGH (P < 0.05; Table 2). Resting serum FFA and plasma norepinephrine concentrations before exercise were higher (1.05 ± 0.16 vs. 0.36 ± 0.08 mmol/l and 4.71 ± 0.43 vs. 2.92 ± 0.37 nmd/l, respectively) and RER lower (0.76 ± 0.03 vs. 0.88 ± 0.03; P < 0.05; Fig. 1) in subjects with low muscle glycogen content than in subjects with normal muscle glycogen content. In contrast, no significant differences were found in plasma glucose (4.48 ± 0.12 vs. 5.22 ± 0.41 mmol/l, low muscle glycogen vs. normal muscle glycogen, respectively), lactate (1.31 ± 0.10 vs. 1.52 ± 0.16 mmol/l), insulin (4.29 ± 0.63 vs. 5.03 ± 0.37 µU/ml), glucagon (92 ± 4 vs.

Table 2. Total carbohydrate and fat oxidation for 145 min of cycling and pre- and postexercise muscle glycogen content in NGE, LGE, LGEI, and LGH subjects

<table>
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<tr>
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<th>NGE</th>
<th>LGE</th>
<th>LGEI</th>
<th>LGH</th>
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<tbody>
<tr>
<td>FFA (µU/ml)</td>
<td></td>
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<tr>
<td>Preexercise</td>
<td>76</td>
<td>12</td>
<td>126</td>
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<tr>
<td>Muscle glycogen</td>
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<td>Postexercise</td>
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Values are means ± SE. *Significantly different between NGE and LGEI (P < 0.05); ‡significantly different between LGE and LGEI (P < 0.05); †significantly different between LGE and LGH (P < 0.05).
the urine of any subject at the end of the trial. Plasma lactate concentrations after 5 min of exercise were 2.37 ± 0.44, 2.78 ± 0.78, 2.19 ± 0.25, and 1.70 ± 0.26 mmol/l and declined over the duration of exercise to 2.23 ± 0.48, 1.34 ± 0.31, 1.76 ± 0.34, and 1.56 ± 0.29 mmol/l, in LGE, NGE, LGH, and LGH, respectively. Only the decline in NGE was statistically significant (P < 0.05).

The rate of glucose infusion required to maintain blood glucose concentrations at ~5 mmol/l (LGE, NGE, and LGEI) or ~9 mmol (LGH; Fig. 5A) was significantly greater in LGH than in LGEI and, in both of these groups, was significantly higher than in LGE and NGE (P < 0.05). The glucose infusion rate did not differ between the latter two groups. In all groups, the rate of glucose infusion increased progressively over the duration of exercise, with a marked increase in LGE between 80 and 100 min of exercise. The total amount of glucose infused during the 145 min of exercise (Fig. 6) was higher in LGH than in LGEI (1,484 ± 125 vs. 956 ± 121 mmol; P < 0.05) and was higher in both of these groups than in NGE or LGE (403 ± 107 and 342 ± 69 mmol, respectively).

Rates of glucose oxidation (Fig. 5B) increased progressively over the duration of exercise in all groups, and peak rates in LGH and LGEI were 8.3 ± 0.11 and 8.4 ± 0.7 mmol/min (1.51 ± 0.13 and 1.51 ± 0.19 g/min), respectively, compared with 5.6 ± 0.9 and 4.5 ± 0.8 mmol/min (1.03 ± 0.16 and 0.82 ± 0.15 g/min) in NGE and LGE, respectively (P < 0.05). Total glucose oxidation (Fig. 6) did not differ significantly between LGE and NGE (389 ± 44 vs. 513 ± 61 mmol; 70 ± 8 vs. 92 ± 11 g), but both were lower than LGH and LGEI (840 ± 74 and 785 ± 83 mmol; 151 ± 15 and 141 ± 15 g, respectively; P < 0.05). In LGE, LGEI, and LGH, total glucose oxidation matched the total amount of glucose infused (Fig. 6) and showed a corresponding increase over the duration of exercise (Fig. 5, A and B). However, in LGH, only 66% of the total amount of glucose infused was oxidized. The contribution of glucose to total CHO oxidation increased significantly (P < 0.05) over the duration of the trial in all groups to 51 ± 10, 43 ± 7, 56 ± 8, and 58 ± 5% in NGE, LGE, LGEI, and LGH, respectively (P < 0.05 for NGE vs. LGH and LGEI).

The contribution of glucose oxidation to total energy was also significantly (P < 0.05) higher in LGH and

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**Table 3. Steady-state gas exchange data and rate of carbohydrate and fat oxidation during 145 min of cycling in NGE, LGE, LGEI, and LGH subjects**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>V̇O₂, l/min</th>
<th>CHOox, g/min</th>
<th>FAox, g/min</th>
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<td></td>
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<td>LGE§</td>
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<tr>
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<td>0.44 ± 0.12*</td>
<td>0.48 ± 0.08*</td>
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<td>25</td>
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<tr>
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<td>0.51 ± 0.05†</td>
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Values are means ± SE. CHOox, rate of carbohydrate oxidation; FAox, rate of fat oxidation. *Significantly different between NGE and LGE (P < 0.05); †significantly different between LGE and LGEI (P < 0.05); § significantly different between LGE and LGH (P < 0.05); ‡significant change over time (P < 0.05).
LGEI than in NGE or LGE, reaching peaks of 41 ± 4, 37 ± 3, 26 ± 3 and 21 ± 2%, respectively.

Muscle glycogen concentrations (Table 1) were significantly higher at the start of exercise in NGE than LGE, LGEI, or LGH. Muscle glycogen disappearance (Fig. 7) was greater (P < 0.05) in NGE than LGE, LGEI, or LGH (90 ± 11 vs. 49 ± 9, 28 ± 14, and 41 ± 4 mmol/kg wet wt, respectively), but the latter three groups did not differ significantly. There were no significant differences in muscle glycogen concentrations between groups at the end of exercise (Table 1).

Plasma insulin concentrations (Fig. 3B) were significantly (P < 0.05) higher in LGEI than in NGE, LGH, or LGE throughout exercise and in NGE were significantly higher (P < 0.05) than in LGE until 45 min. There was a significant (P < 0.05) increase in plasma insulin concentrations over the duration of exercise in LGEI and a significant (P < 0.05) decrease in LGE and NGE. In contrast, there were no significant differences among groups in plasma glucagon concentrations (Fig. 4B), which did not change significantly over the duration of exercise.

Plasma norepinephrine concentrations (Fig. 3C) increased significantly in LGE until 125 min of exercise and in NGE until 65 min of exercise and did not change significantly thereafter, whereas, in LGEI and LGH, norepinephrine increased throughout exercise (P < 0.05). Plasma norepinephrine concentrations were significantly (P < 0.05) higher in LGE than in NGE throughout exercise and were intermediate in LGEI and LGH between LGE and NGE but not significantly different from LGE and NGE. Plasma epinephrine concentrations in LGE, NGE, LGEI, and LGH at 5 min of exercise were 0.06 ± 0.001, 0.07 ± 0.01, 0.68 ± 0.07, and 0.06 ± 0.01 nmol/l, respectively (P < 0.05 for LGEI vs. LGE, LGH, and NGE) and did not change significantly from basal over the duration of exercise in LGE, LGH, and NGE. In LGEI, epinephrine remained higher throughout exercise than in LGE or NGE (P < 0.05) but did not change significantly over time. Mean serum GH concentrations were 58 ± 16, 40 ± 23, 67 ± 12, and 11.72 ± 4.27 pg/ml in LGE, NGE, LGEI, and LGH, respectively, but there were no significant differences in the area under the curve, and concentrations did not change significantly over time.

Fig. 2. RER in subjects with low glycogen, euglycemia (LGE), normal muscle glycogen, euglycemia (NGE), low glycogen, euglycemia, hyperinsulinemia (LGEI), and low glycogen, hyperinsulinemia (LGH). *Significant difference between LGE and LGEI (P < 0.05); #significant difference between LGE and LGH (P < 0.05); *significant difference between LGE and NGE; #significant decrease over time (P < 0.05).

Fig. 3. Serum [FFA] (A), plasma insulin concentration (B), and plasma norepinephrine concentrations (C) in LGE, LGEI, LGH, and NGE subjects. *Significant difference between LGE and NGE (P < 0.05); †significant difference between LGE and LGH (P < 0.05); ‡significant difference between LGE and LGEI (P < 0.05); #significant change over time (P < 0.05).
DISCUSSION

The most significant findings in this study were that the rate of glucose oxidation was not influenced by muscle glycogen levels, i.e., was not different between NGE and LGE, that lowered muscle glycogen content at the start of exercise resulted in a shift toward lipid oxidation, and that both insulin infusion and hyperglycemia counteracted the metabolic effects of low glycogen content (Fig. 8).

As found in other studies (8, 13), RER was lower, and serum FFA and plasma norepinephrine concentrations were higher at rest in subjects with low muscle glycogen content (Fig. 1). Although the effect of low muscle glycogen content on RER (13) and FFA (8) concentrations at rest have been reported previously, in these studies (8, 13), resting plasma glucose concentrations were lower in subjects with low muscle glycogen content; therefore, it cannot be said with certainty that it was the low glycogen as opposed to the low blood glucose concentration that caused the increases in RER and FFA. In the current study, however, resting plasma glucose concentrations were normal and not different between groups, probably because the subjects were ingesting ~64 g of CHO daily, 30 g of which was in a mixed meal each morning and 3 h before the trial. Thus the lower RER and increased FFA can be attributed to low muscle or liver glycogen content or both. Liver glycogen content would be low, as it has been shown to decrease to 22–55 mmol/kg wet wt after 3 days on a low-CHO diet (22). If 29% (15) of the CHO ingested in the morning meal was taken up by splanchnic tissues, with a liver mass of 1.8 kg, liver glycogen content would remain very low after the meal at 49–82 mmol/kg wet wt. In addition, only moderate liver glycogen repletion takes place with glucose infusion, even in resting subjects (23). This was shown in the study of Nilsson and Hultman (23) in which infusion of glucose at a mean rate of 5.8 mmol/min in resting subjects for 4 h resulted in an increase in liver glycogen content of only 76 mmol/kg wet wt, even though plasma glucose concen-
trations remained >10 mmol/l and rose to a peak of ±20 mmol/l after 90 min. Thus it is unlikely that much liver glycogen repletion took place during the trials. However, the low RER in subjects with low muscle glycogen was restored in LGEI and LGH to that of NGE subjects as a result of insulin and glucose infusion, respectively (Fig. 2). Because insulin in the absence of hyperglycemia (LGEI subjects) increases muscle (37) but not liver glucose uptake (4), the higher RER in LGEI and LGH than in LGE would most likely be the result of an increase in glucose oxidation (Fig. 5B) as a consequence of an increase in muscle glucose uptake (5, 37).

The significant increase in rate of glucose oxidation over the duration of exercise in all groups, but particularly the similarity in increase between LGE and NGE (Fig. 5B), is in agreement with two recent studies in which there was no difference in glucose uptake between subjects with low or normal glycogen content (10) and no difference in glucose oxidation between subjects with high or normal glycogen content (1). This indicates that it is not the gradual decline in muscle glycogen content that drives the progressive increase in glucose oxidation during exercise.

When the entire exercise duration is considered, mean RER was not significantly different between NGE and LGH. However, there was a difference between these groups in the source of CHO utilized. In LGH, the contribution of glucose to total CHO oxidation rose rapidly, reaching 58 ± 5% at 105 min, but reached only 31 ± 4% in NGE at that time. The latter increased to 43 ± 7% during the last 45 min of exercise. Because glycogen content does not exert an influence on the rate of glucose oxidation, as discussed previously, the higher rate of glucose oxidation in LGH can be attributed to either the high prevailing glucose concentration, the concomitant hyperinsulinemia, or both.

The greater disappearance of muscle glycogen in NGE than in LGE, LGEI, or LGH (Fig. 7) is consistent with a number of studies that have found that higher muscle glycogen levels at the start of exercise result in a greater rate of muscle glycogen utilization during exercise (1, 10). Although there were no significant differences in muscle glycogen utilization between any of the low-glycogen groups (LGE, LGEI, and LGH), there appeared to be a tendency toward a glycogen-sparing effect with insulin infusion (LGEI).

A possible control mechanism for the fuel substrate interactions observed in this study is the "glucose-fatty acid cycle" (26), which proposes that an increase in FFA oxidation leads to an inhibition of pyruvate dehydrogenase and thereby inhibits CHO oxidation. However, when FFA were infused (31) into isolated, perfused rat hindquarters at a concentration that is saturating for FFA uptake and oxidation in this preparation, unlike the finding in the current study, FFA uptake did not differ during stimulation between muscle that was low and muscle that was high in glycogen, but glucose uptake was higher in glycogen-depleted muscle. Thus, in the isolated, perfused muscle preparation, in which neural and hormonal control is absent, FFA uptake is not stimulated, but glucose uptake is increased in glycogen-depleted muscle. Thus, by exclusion, the increased lipid oxidation in the current study was probably due to stimulation of lipolysis as a result of neural or hormonal control factors outside the muscle, which increased availability of FFA to the muscle. Similarly,
because glucose uptake is increased in isolated, glycogen-depleted muscle, the lack of increased glucose oxidation in the current study indicates neural or hormonal inhibition of glucose uptake. Therefore, under normal physiological conditions, glucose oxidation is not increased with glycogen depletion, but instead a switch takes place toward lipid oxidation due to neural or hormonal factors. High insulin concentrations, however, negate this. Teleologically, this may be a mechanism to compensate for a reduced availability of intramuscular CHO availability without predisposing to hypoglycemia.

The significant difference in plasma insulin concentrations (Fig. 3B) between LGE and NGE during exercise (but not at rest) suggests that, when euglycemia is maintained, plasma insulin concentrations are influenced by muscle glycogen content. Because the most dominant effects of insulin infusion in LGEI compared with LGE were an increase in glucose oxidation (Fig. 5B) and a decrease in FFA (Fig. 3A), muscle glucose uptake in LGE was probably limited by the lower insulin (37) and higher norepinephrine (3) concentrations (Fig. 3, A and C). This probably also occurred in NGE after the first hour of exercise when muscle glycogen concentrations of NGE would be approaching those of LGE (1).

Although the increased insulin in LGEI (Fig. 3B) decreased the norepinephrine response to glycogen depletion slightly (Fig. 3C), norepinephrine in LGEI was not significantly different from LGE. However, it was significantly different between subjects with low and normal glycogen content (LGE and NGE). Because the difference in norepinephrine persisted, with only some attenuation with insulin infusion in subjects with low muscle glycogen content, it, together with insulin, as discussed in the preceding paragraph, are the most likely controllers of the metabolic changes due to muscle glycogen depletion observed in this and other studies (13). However, the lower insulin concentrations in LGE could also be explained by the inhibition of insulin release by norepinephrine (28). Catecholamines also oppose the effects of insulin on lipid metabolism and glucose uptake (3), which would result in an increase in FFA and lipid oxidation and a lower rate of blood glucose oxidation in subjects with low muscle glycogen content than would be expected, considering the availability of glucose and the low availability of endogenous glycogen. However, a lower rate of blood glucose oxidation was not observed in LGE subjects.

The similarity in RER (Fig. 2), serum FFA, and plasma norepinephrine concentrations (Fig. 3, A and C) and insulin and glucagon concentrations (Figs. 3B and 4B) during exercise in LGH and NGE subjects suggests that these parameters are controlled in a reciprocal manner both by plasma glucose concentrations during
exercise and by muscle glycogen content at the start of exercise.

An interesting observation is that the lower RER and higher FFA and norepinephrine concentrations (Figs. 2, 3A, and 3C) during exercise in subjects with low muscle glycogen content (LGE) in this study are similar to those found in patients with muscle phosphorylase deficiency (McArdle’s disease; see Ref. 18), who have impaired utilization of muscle glycogen. Patients with McArdle’s disease have a greater than normal change in heart rate (ΔQ) for a change in VO₂ (ΔQ/ΔVO₂) during exercise, which has been linked to a deficiency of oxidizable fuel (19), since the ΔQ/ΔVO₂ decreases when the enzymatic defect is bypassed by infusing glucose (19).

Neural feedback from chemoreceptors in contracting muscle is involved in cardiovascular control (21), and, likewise, studies of McArdle’s disease strongly link the metabolic and cardiovascular defects of this disease with neural feedback from chemoreceptors in contracting muscle (18, 25, 35). Thus, given the striking similarities in the metabolic and hormonal differences between LGE subjects and McArdle’s disease patients (9, 35) and their respective controls, there are strong indications in the current study of similar direct metabolic signaling of low glycogen status from muscle, which results in an increase in norepinephrine and FFA concentrations and lipid oxidation and a decrease in insulin concentrations. However, this response can be overridden if glucose uptake into the muscle is increased by an increase in plasma glucose or insulin concentrations or both. We postulate that the most likely signaling pathway would be via group III and IV muscle afferents, since stimulation of these influences concentrations of various regulatory hormones, including insulin, in cats (33).

Because 1) muscle afferents are connected to the hypothalamic locomotor region via the nucleus reticularis gigantocellularis (27), 2) blood glucose appearance and plasma glucose and glucoregulatory hormone concentrations can all be controlled from the hypothalamus (36), and 3) there are interconnections between the various hypothalamic nuclei and between hypothalamic nuclei and the sympathetic nervous system and hypophysis (36), integration of the results of the current and previous studies (17, 25, 32, 34, 36) may explain the coordinated metabolic control via central command, neuromuscular reflexes, and humoral metabolic feedback.

The actual stimulant (or inhibitor) for the muscle afferents is not known. Glycogen itself as a signal molecule is unlikely, because patients with McArdle’s disease have adequate muscle glycogen stores but cannot utilize it, whereas LGE subjects had low muscle glycogen concentrations. Glucose 1-phosphate, glucose-1,6-bisphosphate (G-1,6-P₂), glucose 6-phosphate, fructose 6-phosphate, and glyceral 3-phosphate are all lower and fumarate and IMP higher in glycogen-depleted muscle after exercise than in muscle with normal glycogen content (30). However, of the above, only G-1,6-P₂ is low in both glycogen-depleted muscle before exercise (30) and also in muscle from patients with McArdle’s disease (38). If this molecule has an effect on group III and IV muscle afferents, the differences between LGE and NGE in RER, norepinephrine, and FFA both at rest and during exercise in this study would be explained, as would the responses reported in McArdle’s disease. G-1,6-P₂ has previously been reported to be an important molecule in metabolic control and has been shown to increase in muscle with insulin infusion (14) and to decrease with training in rats (20), an adaptation that concurs with a shift toward lipid oxidation after training (2).

In conclusion, in this study, 1) muscle glycogen depletion before exercise resulted in an increase in fat oxidation both at rest and during exercise but had no effect on the rate of oxidation of glucose when euglycemia was maintained by infusion of glucose, 2) insulin infusion in subjects with low muscle glycogen content increased glucose oxidation and decreased FFA concentrations and fat oxidation, 3) glucose oxidation was not higher in subjects with low muscle glycogen content compared with subjects with normal muscle glycogen content when euglycemia was maintained without hyperinsulinemia, and 4) the shift toward lipid metabolism both at rest and during exercise in subjects with low muscle glycogen content may be mediated by a muscle afferent pathway via norepinephrine or insulin.

We thank Gary Wilson and Judy Belonje for technical assistance and Drs. Wayne Derman and Martin Schwellnus for performing the muscle biopsies.

This study was supported by grants from the South African Sugar Association, the South African Medical Research Council, and the Nellie Atkinson and Harry Crossley Research Funds of the University of Cape Town. The insulin used in this study was donated by Novo Nordisk, South Africa.

Address for reprint requests: A. N. Bosch, Bioenergetics of Exercise Research Unit, Sports Science Institute of South Africa, Newlands 7700, South Africa.

Received 11 November 1996; accepted in final form 22 September 1997.

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