Plasma glucose kinetics during prolonged exercise in trained humans when fed carbohydrate

DAMIENTH, ANGUS,3 MARK A. FEBBRAIO,1 AND MARK HARGREAVES2

1Department of Physiology, The University of Melbourne, Parkville, Victoria 3052; and 2Exercise, Muscle and Metabolism Unit, School of Health Sciences, Deakin University, Burwood, Victoria 3125, Australia

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Angus, Damien J., Mark A. Febbraio, and Mark Hargreaves. Plasma glucose kinetics during prolonged exercise in trained humans when fed carbohydrate. Am J Physiol Endocrinol Metab 283: E573–E577, 2002. First published May 15, 2002; 10.1152/ajpendo.00443.2001.—Nine endurance-trained men exercised on a cycle ergometer at ~68% peak $V_{\text{O}_2}$ uptake to the point of volitional fatigue [232 ± 14 (SE) min] while ingesting an 8% carbohydrate solution to determine how high glucose disposal could increase under physiological conditions. Plasma glucose kinetics were measured using a primed, continuous infusion of [6,6-2H]glucose and the appearance of ingested glucose, assessed from [3-3H]glucose that had been added to the carbohydrate drink. Plasma glucose was increased ($P < 0.05$) after 30 min of exercise but thereafter remained at the preexercise level. Glucose appearance rate ($R_a$) increased throughout exercise, reaching its peak value of $118 \pm 7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at fatigue, whereas gut $R_a$ increased continuously during exercise, peaking at $105 \pm 10 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at the point of fatigue. In contrast, liver glucose output never rose above resting levels at any time during exercise. Glucose disposal ($R_d$) increased throughout exercise, reaching a peak value of $118 \pm 7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at fatigue. If we assume 95% oxidation of glucose $R_a$, estimated exogenous glucose oxidation at fatigue was $1.36 \pm 0.08 \text{g/min}$. The results of this study demonstrate that glucose uptake increases continuously during prolonged, strenuous exercise when carbohydrate is ingested and does not appear to limit exercise performance.

DURING PROLONGED, STRENUOUS EXERCISE, muscle glycogen and blood-borne glucose are major substrates for oxidative metabolism, and fatigue often coincides with depletion of these carbohydrate reserves (3, 5, 11). The ingestion of carbohydrate delays fatigue by maintaining blood glucose availability and a high rate of carbohydrate oxidation, despite low muscle glycogen levels (3, 5), and by improving muscle energy balance (19). Having said that, fatigue is not prevented and still occurs despite adequate blood glucose availability and apparent rates of carbohydrate oxidation. Although several studies have assessed glucose kinetics during 1–2 h of strenuous exercise when trained subjects were fed carbohydrate (14, 15, 18), we are aware of only one case study in which glucose kinetics during exercise to the point of fatigue has been examined when subjects were fed carbohydrate (4). Thus our first aim was to undertake such a study in a group of well-trained subjects to determine whether peripheral glucose uptake increases continuously during prolonged, strenuous exercise.

On the basis of indirect calorimetry and muscle glycogen measurements, it has been estimated that blood glucose oxidation may be as high as ~1.7 g/min and may account for 100% of total carbohydrate oxidation during the latter stages of prolonged exercise when carbohydrate is ingested (5). In contrast, direct measurement of glucose tracer oxidation during nonexhaustive exercise suggests a maximal rate of oxidation of ~1–1.1 g/min, despite ingestion of carbohydrate at rates as high as 2 g/min (see Ref. 13 for review). Thus our second aim was to examine how high tracer-determined glucose uptake could increase in well-trained subjects under the physiological conditions of prolonged, glycogen-depleting exercise with maintenance of glycemia and insulinemia by carbohydrate ingestion.

METHODS

Subjects. Nine male endurance-trained cyclists/triathletes [33 ± 3 (SE) yr, 78 ± 3 kg] volunteered as subjects for this investigation after being informed of all possible risks and providing written consent. The study was approved by The University of Melbourne Human Research Ethics Committee. Peak pulmonary oxygen uptake ($V_{\text{O}_2 \text{peak}}$) was determined during incremental exercise to volitional fatigue on an electrically braked cycle ergometer (Lode, Groningen, The Netherlands) and averaged 4.6 ± 0.1 l/min.

Experimental protocol. In the 24 h before the trial, each subject was provided with a food parcel [15.6 MJ, 71% carbohydrate, 15% protein, 14% fat] and was asked to adhere to the diet and refrain from exercise and the intake of alcohol, tobacco, and caffeine. On the morning of the trial, subjects arrived at the laboratory in the fasted state. Subjects were weighed, and catheters were inserted into an antecubital vein of one forearm for the collection of blood samples.
samples and into the contralateral arm for tracer infusion. A primed (3.3 mmol), continuous (~50 μmol/min) infusion of [6,6-H]glucose (Cambridge Isotope Laboratories, Cambridge, MA) was commenced and maintained during a subsequent 2-h rest period. Exercise commenced with a 5-min warm-up at a power output of 200 W. To maintain a reasonable level of plasma tracer enrichment, the tracer infusion rate was increased to ~100 μmol/min at the onset of exercise. This did not appear to significantly alter glucose kinetics, because values obtained were similar to those observed in our previous study that utilized a similar exercise intensity, in which the tracer infusion rate was not increased (18). During the warm-up, subjects ingested 250 ml of an 8% (wt/vol) glucose solution labeled with 0.75 μCi [3-2H]glucose (Du Pont, Biotechnology Systems, Wilmington, DE) per gram of glucose and ingested 250 ml of the glucose beverage every 15 min thereafter. After the warm-up, the power output was increased to that eliciting an average of 68 ± 2% \( \dot{V}O_2 \text{peak} \) (223 ± 14 W), and subjects cycled at this power output until volitional fatigue. Each subject was encouraged to a similar extent by the same investigator, and fatigue was defined as the time when the subject could no longer maintain a cadence of 40 revolutions/min on the cycle ergometer.

Venous blood samples were obtained at rest, at 30-min intervals during the first 2 h of exercise, and then at 15-min intervals until fatigue for later measurement of plasma glucose, [6,6-\(^2\)H]glucose enrichment, and [3-\(^3\)H]glucose specific activity. Additional blood was obtained at rest, at 60-min intervals, and at fatigue for measurement of plasma lactate, free fatty acids (FFA), insulin, and glucagon. Every 30 min during exercise, expired gas was directed via a Hans Rudolph valve into Douglas bags, and heart rate was monitored continuously. All trials were performed at 20–22°C, with an electric fan circulating air to minimize thermal stress to the subjects.

Analytical techniques. Dried expirate was analyzed for oxygen and carbon dioxide concentrations (Applied Electrochemistry S-3/VI and CD-3A, respectively, Ametek, Pittsburgh, PA). These analyzers were calibrated using commercial gases of known composition. The volume of expired air was measured on a Parkinson-Cowan gas meter that had been calibrated against a Tissot spirometer. Conventional equations were used to determine oxygen uptake, respiratory exchange ratio (RER), and rates of substrate oxidation, with the assumption of a nonprotein RER. Plasma glucose and lactate were measured by an automated analyzer (EML 105, Radiometer, Copenhagen, Denmark); FFA were determined in a spectrophotometric assay (NEFA-C test kit, Wako Chemicals, Osaka, Japan). Plasma insulin (Instar, Stillwater, MN) and glucagon (1) were determined by radioimmunoassay. Plasma [6,6-\(^2\)H]glucose enrichment and [3-\(^3\)H]glucose specific activity were measured as described previously (18). Briefly, 50 μl of plasma were mixed with 500 μl of 0.3 M Ba(OH)\(_2\) and 500 μl of 0.3 M ZnSO\(_4\) and spun. For determination of [6,6-\(^2\)H]glucose enrichment, 80-μl aliquots of the supernatant were placed in glass vials, dehydrated, and derivatized with the use of pyridine and acetic anhydride. The derivatized glucose level was then measured with a gas chromatograph-mass spectrometer (5890 series 2 gas chromatograph, 5971 mass spectrometer detector, Hewlett-Packard, Avondale, PA). For determination of [3-\(^3\)H]glucose specific activity, 700 μl of supernatant were dried overnight, reconstituted with 0.5 ml of distilled water and 10 ml of scintillant, and after 1 h of refrigeration were counted (LS CA 3801, Beckman Instruments, Irvine, CA).

Calculations. The rates of glucose appearance (Ra) and disappearance (Rd) at rest and during exercise were calculated by using a modified, one-pool, non-steady-state model (22), with an assumption of a pool fraction of 0.65 and an estimate of the apparent glucose space as 25% of body weight. Although glucose Rd measures total glucose Rd, the liver (25) and gut are the major, if not sole, sources of the increase in endogenous glucose production during exercise when carbohydrate is ingested. Thus hepatic glucose production (HGP) was calculated as the difference between the total Rd and the appearance of ingested glucose (gut Rd; see Ref. 21). The metabolic clearance rate (MCR) of glucose was calculated by dividing the calculated glucose Rd by the prevailing plasma glucose concentration.

Statistical analysis. Data from the experimental trials were analyzed by one-way ANOVA for repeated measures, with significance set at \( P < 0.05 \). Specific differences were located using the Student-Newman-Keuls post hoc test when ANOVA revealed a significant interaction. Data are reported as means ± SE.

RESULTS

Subjects exercised for 232 ± 14 min. Pulmonary RER was maintained during the first 2 h of exercise but was lower (\( P < 0.05 \)) than the 30-min value during the latter stages of exercise and at fatigue (Table 1). Estimated carbohydrate oxidation did not change significantly during exercise, although the value at fatigue tended (\( P = 0.069 \)) to be lower than that at 30 min (Table 1). Plasma glucose increased after 30 min of exercise but thereafter remained at levels similar to those at rest (Fig. 1). Plasma [\(^3\)H]glucose enrichment was 3.6 ± 0.3% immediately before exercise, fell to 1.5 ± 0.1% after 2 h of exercise, but remained at this level until fatigue. Similarly, [\(^3\)H]glucose specific activity increased from 47 ± 4 dpm/μmol at rest to 271 ± 22 dpm/μmol after 2 h of exercise and remained at this level until fatigue. Glucose Ra increased with exercise, reaching its peak value at fatigue (118 ± 7 μmol·kg\(^{-1}\)·min\(^{-1}\) (118 ± 7 μmol·kg\(^{-1}\)·min\(^{-1}\)), Fig. 1). Likewise, gut Rd increased continuously during exercise, reaching a peak of 105 ± 10 μmol·kg\(^{-1}\)·min\(^{-1}\) at fatigue. In contrast, HGP never rose above resting values at any point during exercise (Fig. 1). Both glucose Rd and MCR increased.

Table 1. Pulmonary respiratory exchange ratio and estimated carbohydrate oxidation during exercise to fatigue at 68 ± 2% \( \dot{V}O_2 \text{peak} \)

<table>
<thead>
<tr>
<th></th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
<th>Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER</td>
<td>0.95 ± 0.01</td>
<td>0.95 ± 0.01</td>
<td>0.92 ± 0.01</td>
<td>0.92 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td>CHOox, g/min</td>
<td>3.40 ± 0.23</td>
<td>3.38 ± 0.18</td>
<td>3.03 ± 0.01</td>
<td>2.97 ± 0.18</td>
<td>2.74 ± 0.15</td>
<td>2.86 ± 0.23</td>
<td>2.65 ± 0.27</td>
</tr>
</tbody>
</table>

Subjects ingested 1 l/h of an 8% glucose solution throughout exercise. Values are means ± SE (\( n = 9 \)). RER, respiratory exchange ratio; CHOox, carbohydrate oxidation. *\( P < 0.05 \), different from 30 min value. †\( P = 0.069 \), fatigue vs. 30 min.
during exercise, peaking at 118 ± 7 μmol·kg⁻¹·min⁻¹ and 23.9 ± 1.6 ml·kg⁻¹·min⁻¹, respectively, at fatigue (Fig. 2). Plasma lactate during exercise was not different from the resting value, whereas plasma FFA decreased after 60 min of exercise but then increased to levels higher than rest at 180 min and fatigue (Table 2). Plasma insulin concentrations increased above rest (P < 0.05) after the 1st h of exercise and carbohydrate ingestion. After 2 h of exercise, however, plasma insulin concentrations were not different from resting values and then fell to below resting values at the point of fatigue (Table 2). Plasma glucagon was not different from rest during the first 3 h of exercise but was elevated at the point of fatigue (Table 2). Estimated substrate oxidation during the exercise bout is summarized in Fig. 3. With the assumption of 95% oxidation of glucose R₄ (14), total carbohydrate oxidation was partitioned into that derived from plasma glucose and that from other carbohydrate sources, primarily muscle glycogen. As exercise duration increased, there was a progressive increase in plasma glucose and fat oxidation and a reduction in the oxidation of other carbohydrates. At the point of fatigue, the contributions of fat, plasma glucose, and other carbohydrates to total energy expenditure were 31, 38, and 31%, respectively.

**DISCUSSION**

The results of the present study demonstrate that trained subjects have a high capacity for glucose uptake during prolonged strenuous exercise when fed carbohydrate. Furthermore, glucose uptake increases throughout exercise and shows no sign of leveling off, even at the point of fatigue. This implies that glucose uptake is not a limiting factor for prolonged exercise performance with carbohydrate ingestion and that the locus of fatigue under these conditions must lie elsewhere.

Carbohydrate ingestion has been shown to delay, but not prevent, fatigue during prolonged strenuous exercise (3, 5). The maintenance of blood glucose availability and glucose uptake in the present study implies that the observed fatigue cannot be due to reduced glucose supply to contracting skeletal muscle. It remains a possibility that intracellular glucose metabolism may be compromised, or that glucose cannot compensate for the declining muscle glycogen availability,
although in either case we have no data to support or refute such a suggestion. On the basis of direct measurement of vastus lateralis muscle glycogen levels, previous studies (3, 5) have suggested that the increase in exercise capacity observed with carbohydrate ingestion occurs in the absence of any net muscle glycogen degradation. In contrast, in the present study, the estimated oxidation of other carbohydrates at fatigue (~1.2 g/min), presumably muscle glycogen, still accounted for ~31% of total energy expenditure and ~45% of total carbohydrate oxidation (Fig. 3). It is possible that the estimates of muscle glycogen oxidation from indirect calorimetry and glucose R\textsubscript{d} in the present study include glycogen use in muscles, other than the vastus lateralis, that may be recruited in the latter stages of prolonged, strenuous exercise. Interestingly, with the assumption of an average muscle glycogen concentration at fatigue of 35 mmol/kg wet wt (3, 5) and an active muscle mass of 8–10 kg, there would still be sufficient muscle glycogen to sustain a carbohydrate oxidation rate of 1.2 g/min for 40–50 min. Thus, it is possible that there may be an absolute requirement for muscle glycogen, either in the type I muscle fibers or at particular locations within muscle fibers, for the maintenance of strenuous exercise. Recently, it has been demonstrated that reduced muscle glycogen availability can impair excitation-contraction coupling within skeletal muscle (2, 23) and that such an effect may be due to an energetic and/or structural requirement for muscle glycogen. Finally, we cannot rule out the possibility that fatigue was the result of impaired muscle function due to mechanisms other than carbohydrate availability and/or reduced neuromuscular activation as a consequence of changes within the central nervous system.

The peak glucose R\textsubscript{d} during exercise in the present study was 118 ± 7 µmol·kg\textsuperscript{-1}·min\textsuperscript{-1} or 1.66 ± 0.09 g/min (Fig. 2), a value not that dissimilar from the estimate of ~1.7 g/min obtained by Coyle et al. (5). This represented 55% of total carbohydrate oxidation, with the remaining contribution from other carbohydrate sources, presumably muscle glycogen and lactate. Approximately 90% of the glucose R\textsubscript{d} was derived from ingested glucose, because HGP did not rise above basal levels and remained at 16 ± 1 µmol·kg\textsuperscript{-1}·min\textsuperscript{-1} throughout exercise (Fig. 1). Thus we estimate an endogenous glucose oxidation of 1.36 ± 0.08 g/min, a value slightly higher than that which has been suggested to be “maximal” (1–1.1 g/min, see Ref. 13).

Our results clearly demonstrate a high capacity for glucose uptake in trained athletes. It has been suggested that training enhances the capacity for glucose uptake (16), and there are several mechanisms by which this might be achieved. Trained athletes are characterized by higher skeletal muscle GLUT4 protein expression, increased activities of hexokinase and oxidative enzymes, higher proportions of type I skeletal muscle fibers, and greater capillary density, all of which could account for a greater ability to take up glucose in response to both exercise and insulin stimulation (7, 8). It has also recently been suggested that enhanced insulin sensitivity increases glucose uptake during exercise (20). Although the proximal signaling mechanisms responsible for increased muscle glucose uptake in response to insulin stimulation and exercise are generally thought to be different, the above-mentioned morphological and biochemical factors are common to both pathways. These factors, together with progressive muscle glycogen depletion (9) and GLUT4 recruitment to the sarcolemma (17), maintenance of glycemia and insulinemia by carbohydrate ingestion, and a blunting of the rise in plasma FFA (10), could all contribute to the high rates of glucose uptake observed during prolonged, strenuous exercise in trained athletes.
A further finding of the present study was that liver glucose output remained at basal levels throughout exercise, even at the point of fatigue. HGP averaged 0.2 g/min, a value similar to that observed in other studies, and this demonstrates that HGP is sensitive to exogenous glucose delivery by either ingestion (14, 15, 18) or infusion (12). Although liver glucose output during exercise is subject to complex and multiple regulatory controls, it appears that classical feedback mechanisms linked to blood glucose availability dominate during prolonged strenuous exercise with carbohydrate ingestion. The appearance of ingested glucose increased throughout exercise, implying no splanchic limitation to ingested glucose bioavailability. Our feeding regimen produced an average glucose intake of 1.3 g/min during exercise, approximately the same as the estimated upper limit for gastrointestinal glucose absorption, albeit under resting conditions (6). It is possible that ingestion of a larger amount of carbohydrate could result in a splanchic limitation to ingested glucose appearance, as we have observed with a 10% glucose solution (18). In addition, it has been observed that exogenous carbohydrate oxidation reaches a maximal level despite increases in the amount of ingested carbohydrate (13, 24).

In summary, we have demonstrated that glucose uptake increases continuously during prolonged strenuous exercise and does not appear to limit exercise performance. Furthermore, trained athletes have a high capacity for glucose uptake during prolonged exercise when fed carbohydrate.

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REFERENCES