Substrate metabolism when subjects are fed carbohydrate during exercise

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Horowitz, Jeffrey F., Ricardo Mora-Rodriguez, Lauri O. Byerley, and Edward F. Coyle. Substrate metabolism when subjects are fed carbohydrate during exercise. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E828–E835, 1999.—This study determined the effect of carbohydrate ingestion during exercise on the lipolytic rate, glucose disappearance from plasma (Rgluc), and fat oxidation. Six moderately trained men cycled for 2 h on four separate occasions. During two trials, they were fed a high-glycemic carbohydrate meal during exercise at 30 min (0.8 g/kg), 60 min (0.4 g/kg), and 90 min (0.4 g/kg); once during low-intensity exercise (25% peak oxygen consumption (Vo2peak)) and once during moderate-intensity exercise (68% Vo2peak). During two additional trials, the subjects remained fasted (12–14 h) throughout exercise at each intensity. After 55 min of low-intensity exercise in fed subjects, hyperglycemia (30% increase) and a threefold elevation in plasma insulin concentration (P < 0.05) were associated with a 22% suppression of lipolysis compared with when subjects were fasted (5.2 ± 0.5 vs. 6.7 ± 1.2 µmol·kg−1·min−1, P < 0.05), but fat oxidation was not different from fasted levels at this time. Fat oxidation when subjects were fed carbohydrate was not reduced below fasting levels until 80–90 min of exercise, and lipolysis was in excess of fat oxidation at this time. The reduction in fat oxidation corresponded in time with the increase in Rgluc. During moderate-intensity exercise, the very small elevation in plasma insulin concentration (~3 µU/ml; P < 0.05) during the second hour of exercise when subjects were fed vs. when they were fasted slightly attenuated lipolysis (P < 0.05) but did not increase Rgluc or suppress fat oxidation. These findings indicate that despite a suppression of lipolysis after carbohydrate ingestion during exercise, the lipolytic rate remained in excess and thus did not limit fat oxidation. Under these conditions, a reduction in fat oxidation was associated in time with an increase in glucose uptake.

insulin; lipolysis; glucose uptake; exercise intensity; glycogen; stable isotopes

FAT AND CARBOHYDRATE are the two primary substrates oxidized by skeletal muscle during exercise (17). Carbohydrate ingestion during exercise can modify the relative contribution of these substrates to total energy production (1). However, the influence of carbohydrate ingestion on substrate oxidation depends on the intensity of exercise (24). Carbohydrate ingestion during low-intensity exercise (25–45% peak oxygen consumption (Vo2peak)) reduces fat oxidation ~40% below fasted levels (1, 15). In contrast, carbohydrate ingestion during moderate-intensity exercise (65–75% Vo2peak) does not reduce fat oxidation during the first 120 min of exercise (5, 6).

The differential effects of carbohydrate ingestion during low and moderate-intensity exercise may be related to differences in insulin response. During low-intensity exercise, carbohydrate ingestion increases plasma insulin concentration two- to threefold above fasting levels (1, 15) and increases glucose uptake by skeletal muscle (1). Furthermore, the increase in plasma insulin concentration is associated with a reduction in plasma free fatty acid (FFA) concentration (1, 15, 24) and probably a suppression of triglyceride hydrolysis (i.e., lipolysis; Ref. 3). These events favor an increase in carbohydrate oxidation and a decrease in fat oxidation (1). Unlike low-intensity exercise, the insulin response to carbohydrate ingestion during moderate-intensity exercise is almost completely suppressed (5, 12). This may explain why carbohydrate ingestion during moderate-intensity exercise does not affect fat oxidation, carbohydrate oxidation, muscle glycogen utilization (5, 12), or presumably blood glucose oxidation during the first 2 h of exercise at moderate intensity (2). Interestingly, carbohydrate ingestion during moderate-intensity exercise does not reduce fat oxidation despite a significant suppression in plasma FFA and glycerol concentrations (5, 6).

Because there is only a very small pool of unesterified fatty acids within the human body (10–40 µmol/kg), the rate of fat oxidation cannot exceed the rate of lipolysis for more than a few minutes during exercise. Therefore, a low lipolytic rate can limit fat oxidation by reducing the amount of FFA available for oxidation. In a recent study, we reported that carbohydrate ingestion 1 h before exercise (45% Vo2peak) and a subsequent modest elevation in plasma insulin concentration (10–30 µU/ml) suppressed lipolysis sufficiently to reduce fat oxidation during exercise (13). However, when exercise is initiated while subjects are fasted, lipolysis increases rapidly to relatively high levels and exceeds fat oxidation by as much as 50% (25); thus lipolysis does not limit fat oxidation in this condition. The principal aim of this study was to determine whether an elevation in plasma insulin concentration during exercise, after lipolysis has been increased to relatively high rates while subjects are fasted, will allow the lipolytic rate to remain in excess and therefore not limit fat oxidation.

It seems that in addition to suppressing lipolysis, preexercise carbohydrate ingestion and the resultant increase in plasma insulin concentration also can suppress fat oxidation during exercise by a phenomenon specific to the exercising muscle (8, 13), whereby in-
creased glycolytic flux may directly reduce fat oxidation within muscle (8, 10, 26). An additional aim of this study was to determine whether an increase in glucose uptake and glycolytic flux after carbohydrate ingestion during exercise is associated with a reduction in fat oxidation. We measured the rate of glycerol appearance in plasma (R_{aglycerol}, an index of whole body lipolysis) and the rate of glucose disappearance from plasma (R_{dglc}) after carbohydrate ingestion during exercise. Carbohydrate was ingested during low-intensity exercise (25% V_{O2peak}) to elicit a modest elevation in plasma insulin concentration (10–20 µU/ml) and during moderate-intensity exercise (68% V_{O2peak}) to elicit a very small insulin response (<5 µU/ml).

**METHODS**

**Subjects.** Six moderately trained males participated in this experiment. Their V_{O2peak}, blood lactate threshold (V_{Lact}) at lactate threshold), body weight, and age were 4.5 ± 0.7 l/min (60.3 ± 4.9 ml·kg⁻¹·min⁻¹), 74.3 ± 3.1% V_{O2peak}, 75.1 ± 7.3 kg, and 25 ± 2 yr, respectively. Subjects were informed of the possible risks, and each signed a consent form approved by the Internal Review Board of the University of Texas at Austin.

**Experimental design.** On four separate occasions, subjects arrived at the laboratory in the morning after an overnight fast (12 h) and cycled continuously for 2 h. On two occasions, subjects ingested a high-glycemic sports bar (glycemic index = 99 ± 4; Gator Bar; Quaker Oats, Barrington, IL) at 30 min (0.8 g/kg body wt), 60 min (0.4 g/kg), and 90 min (0.4 g/kg) during either low-intensity exercise trial 1; 25% V_{O2peak} (Low-Fed)) to elicit a modest elevation in plasma insulin concentration (10–20 µU/ml) or moderate-intensity exercise trial 2; 5% below lactate threshold; 68% V_{O2peak} (Mod-Fed) to elicit a very small insulin response (<5 µU/ml).

**Isotope infusion.** On the arrival of the subjects at the laboratory, Teflon catheters were inserted into veins of both forearms (one for isotope infusion and the other for blood sampling). A heating pad was affixed to the hand and forearm of the sampling arm. A blood sample was then withdrawn for determination of background isotopic enrichment. This was followed by a standardized meal consumed, containing 1 ml 0.3 N Na(OH)₂ and 0.7 ml 0.3 N Zn(SO)₄. Each tube was then vortexed and incubated in an ice bath for 20 min. After centrifugation (3,000 rpm for 15 min at 4°C), the supernatant was placed into separate tubes for glucose (0.5 ml) and glycerol (1.5 ml) analysis and the water was removed from the tubes via vacuum centrifugation (Savant Instruments, Farmingdale, NY). The aldolitrile acetate derivative of glucose was prepared by adding 3 µl of hydroxolamine-hydrochloride solution (20 mg/ml in pyridine) to the dried sample. After a 30-min incubation at 100°C, 75 µl of acetic anhydride (Supleco, Bellefonte, PA) were then added and the samples remained incubating for an additional hour. Finally, the samples were evaporated under N₂. Before injection into the GC-MS, the samples were reconstituted with ethyl acetate. The Tris-trimethylsilyl derivative of glycerol was prepared by reconstituting the dried sample with 30 µl of a trimethylsilyl solution (Tri-Sil; Pierce, Rockford, IL).

**Preliminary testing and diet.** V_{O2peak} was determined while subjects were cycling an ergometer (Monark, model 819; Varberg, Sweden) with a continuous protocol that lasted 7–10 min. Blood lactate threshold was determined on a separate occasion as previously described (6). Subjects consumed the same meals during the day before each experimental trial (~500 g carbohydrate). In the evening before each day of testing, a standardized meal was consumed, containing ~200 g of carbohydrate. Subjects performed a standardized exercise-training bout 2 days before each experiment, and they did not exercise again until the experimental trial.

**Measurement of gas exchange.** Inspired air volume was measured with a Parkinson-Cowan CD4 dry gas meter (Rayfield Equipment, Waitsfield, VT), as subjects inhaled through a two-way Daniel’s valve. The expired gases were continuously sampled from a mixing chamber and analyzed for oxygen (model SA3, Applied Electrochemistry; Ametek, Pittsburgh, PA) and carbon dioxide (Beckman, model LB-2; Schiller Park, IL). These instruments were interfaced to a computer for calculations of the rate of oxygen consumption (VO₂) and respiratory exchange ratio.

**Calculations.** The R_{aglycerol} was measured to quantify whole body lipolysis. The accuracy of this calculation requires that one mole of glycerol appears in Blood after the hydrolysis of every triglyceride. It has recently been suggested that R_{aglycerol} may underestimate whole body lipolysis because of phosphorylation of glycerol within the muscle by glycerol kinase and by incomplete glyceride hydrolysis, forming di- and monoglycerides (18). Both of these possibilities would prevent glycerol from appearing in the systemic circulation after lipolysis. To the contrary, others have indicated that neither of these phenomena occur in skeletal muscle or in adipose tissue.
and that glycerol enters the circulation (19), providing an accurate index of lipolysis.

\[ R_{\text{aglycerol}} = \frac{F - V_d(C/(1 + E))(dE/dt)}{E} \]

\[ R_d = R_a - \frac{V_d(dC/dt)(1 + E) - C(dE/dt)}{(1 + E)^2} \]

where \( F \) is isotope infusion rate, \( V_d \) is volume of distribution (estimated to be 230 ml/kg for glycerol and 100 ml/kg for glucose), C is plasma concentration of the tracee, E is tracer isotopic enrichment, and dE/dt and dC/dt are maximum rates of change of enrichment and concentration, respectively, with respect to time. Fat (i.e., triglyceride) and carbohydrate oxidations were calculated from \( V_{\text{O}_2} \) respiratory exchange ratio (nonprotein respiratory quotient), measured from expired air during the 20- to 30-, 50- to 60-, 80- to 90-, and 105- to 120-min periods of exercise (20). Muscle glycogen oxidation was calculated as the difference between total carbohydrate oxidation and \( R_{\text{Glc}} \), as calculated in previous studies (25). The validity of this calculation requires that \( R_{\text{Glc}} \) represents blood glucose oxidation. It has been reported recently that \( \sim 90\% \) of \( R_{\text{Glc}} \) is directly oxidized (4). Thus \( R_{\text{Glc}} \) does provide a reasonable representation of blood glucose oxidation.

Statistical analysis. A two-way ANOVA (treatment by time) for repeated measures with Tukey's post hoc analysis was used to determine significant differences among trials. Planned comparisons for mean values of insulin and glucose were evaluated with paired Student's t-test with a Bonferroni correction factor (\( P < 0.05 \)).

RESULTS

Low-intensity exercise. During Low-Fast, plasma glucose and insulin concentrations remained near basal levels throughout exercise (4.6–5.1 mM and 6–8 µU/ml, respectively; Fig. 1). Thirty minutes after the first carbohydrate ingestion during Low-Fed (60 min of exercise), plasma glucose concentration increased \( >30\% \) and plasma insulin concentration increased nearly threefold (9.1 ± 1.3 vs. 26 ± 4.9 µU/ml for 30 and 60 min, respectively; \( P < 0.05 \)). Both plasma glucose and insulin concentrations remained significantly elevated (\( P < 0.05 \)) during the second hour of Low-Fed vs. Low-Fast (Fig. 1).

\( R_{\text{aglycerol}} \) (i.e., index of whole body lipolysis) increased \( \sim 80\% \) above preexercise levels during the first 30 min of exercise (Fig. 2A). During Low-Fast, \( R_{\text{aglycerol}} \) increased an additional \( 50\% \) during the 30- to 120-min period. Conversely, carbohydrate ingestion attenuated any further rise in \( R_{\text{aglycerol}} \) during the 30- to 120-min period of exercise. However, the absolute rate of lipolysis did not decline below the 30-min value when subjects were fed carbohydrate, and it remained \( >5 \) µmol·kg\(^{-1}\)·min\(^{-1}\) throughout exercise (Fig. 2A).

Similar to \( R_{\text{aglycerol}} \), plasma FFA concentration increased twofold above basal levels by the end of exercise when subjects were fasted and carbohydrate ingestion attenuated this increase (Fig. 2B). As a result, during the second hour of exercise, \( R_{\text{aglycerol}} \) and plasma FFA concentrations were lower (\( P < 0.05 \)) during Low-Fed vs. Low-Fast. Despite a \( >20\% \) suppression in \( R_{\text{aglycerol}} \) and plasma FFA concentration during the 50- to 60-min period of Low-Fed vs. Low-Fast, fat oxidation was identical when subjects were fed and fasted at this time (5.3 ± 0.7 vs. 5.3 ± 0.8 µmol·kg\(^{-1}\)·min\(^{-1}\), respectively). Fat oxidation was not different among trials until the 80- to 90-min period of exercise (Fig. 3), and lipolysis was \( >25\% \) in excess of fat oxidation at this time (5.1 ± 0.7 vs. 4.0 ± 0.4 µmol·kg\(^{-1}\)·min\(^{-1}\), respectively; \( P < 0.05 \)).

Similar to plasma glucose and insulin concentrations, \( R_{\text{Glc}} \) and \( R_{\text{AGlc}} \) remained relatively unchanged from resting levels throughout exercise when subjects were fasted (Fig. 4). During Low-Fed, \( R_{\text{Glc}} \) doubled during the 30 min after the first carbohydrate ingestion (from 12.8 ± 0.7 to 24.9 ± 2.8 µmol·kg\(^{-1}\)·min\(^{-1}\) at 25 and 55 min, respectively; \( P < 0.05 \)) and remained about twofold greater (\( P < 0.05 \)) than Low-Fast throughout the second hour of exercise (Fig. 4A). Furthermore, plasma glucose concentration increased during the 30- to 60-min period of Low-Fed (Fig. 1). However, \( R_{\text{AGlc}} \) did
not increase until 70 min (nonsignificant) and was not significantly (P, 0.05) elevated above Low-Fast until the 80- to 90-min period of exercise (Fig. 4B), the same time that fat oxidation was first significantly reduced (Fig. 3). Figure 5 compares the time course of the
increase in $R_{dGlc}$ during Low-Fed vs. Low-Fast with the reduction in fat oxidation during exercise (both converted to cal·kg$^{-1}$·min$^{-1}$). It is evident that both the time course and the magnitude of the increase in $R_{dGlc}$ were closely matched to the reduction in fat oxidation.

After carbohydrate ingestion, carbohydrate oxidation increased, and thus fat oxidation decreased, in parallel with the increase in $R_{dGlc}$ (Table 1). As a result, the calculated rate of muscle glycogen oxidation was not different between Low-Fed and Low-Fast at any time (Table 1).

**DISCUSSION**

The principal finding of the present study was that despite a >50% lower lipolytic rate and plasma FFA concentration throughout the final 1 h of exercise when subjects were fed carbohydrate during low-intensity exercise compared with when they were fasted, fat oxidation was not reduced below Low-Fast levels until 80–90 min of exercise. At this time, lipolysis was 25% greater ($P < 0.05$) than fat oxidation and thus was not a limiting factor. Similarly, when subjects were fed carbohydrate during moderate-intensity exercise, lipolysis and plasma FFA concentration were reduced 20–25% during the final 1 h of exercise. However, lipolysis remained in excess of fat oxidation, and fat oxidation was not reduced below Mod-Fast levels at any time.

### Table 1. Carbohydrate oxidation, $R_{dGlc}$, and muscle glycogen oxidation rate in subjects during exercise at low and moderate oxygen consumption when fasted or fed carbohydrate

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Values are means ± SE in units of µmol·kg$^{-1}$·min$^{-1}$. *Significantly greater than when fasted, $P < 0.05$. †Significantly different from 20- to 30-min value, $P < 0.05$. $R_{dGlc}$, rate of glucose disappearance; $V_{O2peak}$, peak oxygen consumption; Low, 25% $V_{O2peak}$; Mod, 68% $V_{O2peak}$; Fast, fasted; Fed, fed carbohydrate at 30, 60, and 90 min; CHO, carbohydrate.
Clearly, when whole body lipolysis is suppressed below the ability of the muscle to oxidize fatty acids, fat oxidation is limited by a low lipolytic rate. For example, we have recently reported that elevating plasma insulin concentration to 10–30 µU/ml before exercise prevented lipolysis from increasing above 4 µmol·kg\(^{-1}\)·min\(^{-1}\) during exercise at 45% \(\dot{V}_O^2_{\text{peak}}\) and that this low rate of lipolysis limited fat oxidation during exercise (13). In the present study, however, despite a suppression of both lipolysis and fat oxidation when subjects were fed carbohydrate during low-intensity exercise, lipolysis was maintained above 5 µmol·kg\(^{-1}\)·min\(^{-1}\). Therefore, after carbohydrate ingestion during low-intensity exercise, lipolysis was not suppressed to the low levels we observed during exercise after a preexercise meal (13) and lipolysis remained in excess of fat oxidation (13). In the present study, however, despite a suppression of both lipolysis and fat oxidation when subjects were fed carbohydrate during low-intensity exercise, lipolysis was maintained above 5 µmol·kg\(^{-1}\)·min\(^{-1}\). Therefore, after carbohydrate ingestion during low-intensity exercise, lipolysis was not suppressed to the low levels we observed during exercise after a preexercise meal (13) and lipolysis remained in excess of fat oxidation. It is unlikely that a high rate of intracellular reesterification limited FFA availability sufficiently to reduce fat oxidation in the present study, because the rate of intracellular reesterification is very low during exercise (32) and has been shown to be unaffected by an elevation in plasma insulin concentration in vivo (3). Our findings imply that more fatty acids were liberated via lipolysis than were oxidized, and thus the suppression of lipolysis after carbohydrate ingestion during exercise apparently did not limit fat oxidation. Maintenance of a relatively high lipolytic rate in the present study was likely due to the fact that exercise was initiated while subjects were fasted and was maintained for 30 min before ingestion of carbohydrate. Lipolysis increased dramatically with the onset of exercise when subjects were fasted (25). In the present study, lipolysis increased ~80% during the first 30 min of low-intensity exercise, before carbohydrate ingestion. Thereafter, when the subjects were fed, lipolysis remained stable, whereas it increased when they were fasted.

Interestingly, as shown presently, as well as by others (5, 6), fat oxidation does not decline after carbohydrate ingestion during moderate-intensity exercise, when plasma insulin concentration was only slightly elevated (~3 µU/ml), despite a 40–50% lower plasma FFA concentration. A decline in plasma FFA concentration is indicative of a reduction in adipose tissue lipolysis. The present observation that an ~25% lower lipolytic rate when subjects were fed carbohydrate resulted in a 30–40% lower plasma FFA concentration without reducing total fat oxidation suggests that the suppression of lipolysis occurred predominantly in the adipocyte. Although the use of intramuscular triglycer-
and insulin concentrations are increased sufficiently to reduce fat oxidation. However, the relative reduction in plasma FFA oxidation after carbohydrate ingestion was >60% greater than the reduction in IMTG oxidation (8). These observations imply that IMTG may be less sensitive to the antilipolytic effect of insulin compared with triglycerides in adipose tissue. It seems that this relative insensitivity of IMTG to the very small increase in plasma insulin concentration observed after carbohydrate ingestion during moderate-intensity exercise (−3 µU/ml) may have prevented the reduction in lipolysis from limiting fat oxidation during exercise by allowing whole body lipolysis to exceed fat oxidation.

It has been demonstrated that when lipolysis was suppressed sufficiently to reduce fat oxidation, muscle glycogen oxidation was increased to maintain energy production during exercise (11, 13). However, it is well-known that carbohydrate ingestion during continuous exercise at moderate to high intensities (>65% VO_2peak) does not affect the change in muscle glycogen concentration during exercise (5, 12). Our findings confirm that the calculated rate of muscle glycogen oxidation is not altered by carbohydrate ingestion during exercise. Because the reduction in lipolysis from carbohydrate ingestion does not impair fat oxidation or increase muscle glycogenolysis during moderate- to high-intensity exercise, the ingested carbohydrate provides extra energy in the form of blood glucose, leading to maintained carbohydrate oxidation and enhanced performance.

The inability of carbohydrate ingestion to reduce muscle glycogen oxidation during moderate-intensity exercise may be explained by the relative insensitivity of IMTG lipolysis to insulin (allowing lipolysis to exceed fat oxidation) and by the fact that carbohydrate ingestion at this intensity failed to significantly increase blood glucose uptake during the 30- to 120-min period. During low-intensity exercise, however, the hyperglycemia and hyperinsulinemia and elevation in R_{Glc} glucose after carbohydrate ingestion were compensated by a reduction in fat oxidation. In a previous study from our laboratory, hyperglycemia and hyperinsulinemia (>10 mM and ~20 µU/ml, respectively) evoked by intravenous glucose infusion during moderate-intensity exercise (73% VO_2peak) increased carbohydrate oxidation (and reduced fat oxidation) without affecting muscle glycogen utilization, despite very high rates of glucose disposal (120–200 mmol·kg^{-1}·min^{-1}; Ref. 7). These observations indicate that when plasma glucose and insulin concentrations are increased sufficiently to increase R_{Glc} regardless of intensity (i.e., low or moderate), blood glucose oxidation increases with a compensatory reduction in fat oxidation, rather than a decline in muscle glycogen oxidation.

Presently, we found that the reduction in fat oxidation during low-intensity exercise elicited by hyperglycemia and hyperinsulinemia coincided in time with the increase in R_{Glc} (Fig. 4). R_{Glc} provides a reasonable representation of blood glucose uptake and oxidation in exercising muscle (4). Although this association between an increase in R_{Glc} and a decline in fat oxidation does not directly imply a causal relationship, an increase in blood glucose uptake and oxidation during exercise may have a direct effect on muscle, reducing its ability to oxidize fat. This supports the notion that carbohydrate metabolism in general, and increased glycolytic flux from blood glucose in this case, may regulate the relative contribution of fat and carbohydrate to total energy production during exercise.

It has recently been reported that acute hyperglycemia and hyperinsulinemia and a subsequent increase in glycolytic flux directly reduced muscle fatty acid oxidation by impairing long-chain fatty acid entry into the mitochondria (8, 27). The proposed mechanism for this impairment is that an increase in glycolytic flux increases the concentration of malonyl-CoA concentration in muscle (10), which, in turn, inhibits the enzyme that regulates the entry of long-chain fatty acids into the mitochondria (i.e., carnitine palmitoyltransferase I). Thus malonyl-CoA "senses" the availability of carbohydrate (26) and modifies the ability of the muscle to oxidize fat (i.e., via carnitine palmitoyltransferase I inhibition). This indicates that carbohydrate metabolism in muscle, as reflected by glycolytic flux, is the primary factor regulating fat oxidation under conditions where both FFA and carbohydrate are available to the muscle for oxidation. This mechanism has been documented in rat heart and skeletal muscle, whereas evidence of this mechanism in human muscle remains less clear (23).

In summary, carbohydrate ingestion and the resultant insulin response during exercise suppressed lipolysis. However, the lipolytic rate remained in excess of fat oxidation after carbohydrate ingestion during both low- and moderate-intensity exercise, indicating that this suppression in lipolysis was not responsible for reducing fat oxidation. A reduction in fat oxidation occurred at the time of an increase in R_{Glc} and glycolytic flux. Therefore, carbohydrate ingestion during both low- and moderate-intensity exercise did not suppress lipolysis sufficiently to reduce fat oxidation. However, under conditions where lipolysis is in excess of fat oxidation, carbohydrate ingestion and the resultant insulin response may regulate carbohydrate oxidation by increasing blood glucose uptake and, by doing so, fat oxidation may be reduced by a phenomenon specific to the exercising muscle.

We greatly appreciate the technical support of Dr. Andrew Coggan and Michael Sullivan. We additionally appreciate the assistance from Paul Below, Melissa Domenick, Pete Flatten, Ricardo Fritzsche, Wes Erwood, Matt Oseto, Tom Switzer, Trace Adams, and the participants of this study.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-46017 (R. R. Wolfe and E. F. Coyle), Mars Inc., and the Gatorade Sports Science Institute.
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