Glucose infusion attenuates endogenous glucose production and enhances glucose use of horses during exercise

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Geor, Raymond J., Kenneth W. Hinchcliff, and Richard A. Sams. Glucose infusion attenuates endogenous glucose production and enhances glucose use of horses during exercise. J Appl Physiol 88: 1765–1776, 2000.—We examined the effects of increased glucose availability on glucose kinetics and substrate utilization in horses during exercise. Six conditioned horses ran on a treadmill for 90 min at 34 ± 1% of maximum oxygen uptake. In one trial [glucose (Glu)], glucose was infused at a mean rate of 34.9 ± 1.1 µmol·kg⁻¹·min⁻¹, whereas in the other trial [control (Con)] an equivalent volume of isotonic saline was infused. Plasma glucose increased during exercise in Glu (90 min: 8.3 ± 1.7 mM) but was largely unchanged in Con (90 min: 5.1 ± 0.4 mM). In Con, hepatic glucose production (HGP) increased during exercise, reaching a peak of 38.6 ± 2.7 µmol·kg⁻¹·min⁻¹ after 90 min. Glucose infusion partially suppressed (P < 0.05) the rise in HGP (peak value 25.8 ± 3.3 µmol·kg⁻¹·min⁻¹). In Con, glucose rate of disappearance (Rδ) rose to a peak of 40.4 ± 2.9 µmol·kg⁻¹·min⁻¹ after 90 min; in Glu, augmented glucose utilization was reflected by values for glucose Rδ that were twofold higher (P < 0.001) than in Con between 30 and 90 min. Total carbohydrate oxidation was higher (P < 0.05) in Glu (187.5 ± 8.5 µmol·kg⁻¹·min⁻¹) than in Con (159.2 ± 7.3 µmol·kg⁻¹·min⁻¹), but muscle glycogen utilization was similar between trials. We conclude that an increase in glucose availability in horses during low-intensity exercise 1) only partially suppresses HGP, 2) attenuates the decrease in carbohydrate oxidation during such exercise, but 3) does not affect muscle glycogen utilization.


In horses, unlike humans and dogs, plasma glucose concentrations increase (~2–4 mM) even during moderate-intensity exercise (~35–50% VO2max) (13, 33). This finding indicates a mismatch between glucose Ra and Rδ in the horse during moderate exercise and suggests that neural feed-forward mechanisms may, in part, control liver glucose mobilization. However, because there are no reports of whole body glucose turnover in the horse during sustained exertion, no information exists concerning glucoregulatory mechanisms in this species. We hypothesized that, if feed-forward mechanisms control the glucose Ra response to exercise independent of glucose feedback, an intravenous glucose infusion would not suppress HGP under such circumstances. Therefore, the first objective of this study was to determine the effects of an intravenous glucose infusion on HGP in horses during prolonged, low-intensity (~35% VO2max) exercise.

In humans, it is well established that provision of exogenous glucose (intravenous infusion or glucose ingestion) influences metabolism during exercise (2, 8). Specifically, an increase in blood glucose availability augments glucose Ra by muscle and, during prolonged mild-to-moderate-intensity exercise (i.e., 30–70% VO2max), attenuates the decrease in carbohydrate oxidation (CHOox) and enhances endurance performance. However, most (9, 10, 17), but not all (36, 37), studies in humans have reported that net muscle glycogen utilization is not altered by carbohydrate ingestion. In horses during moderate-intensity exercise, intravenous glucose infusion (~2 g/min) prolonged running perfor-
performance by ~14% relative to the control treatment (13). Thus, similar to other species, carbohydrate administration exerts an ergogenic effect in horses during prolonged exercise. However, the effects of an increase in blood glucose availability on tracer-determined glucose uptake, muscle glycogen utilization, and CHO oxide have not been simultaneously studied in the horse. Therefore, the second objective of this study was to determine the effects of intravenous glucose infusion on glucose Rd, whole body CHO oxide, and muscle glycogen utilization in horses during 90 min of exercise at 35% $V_{O2max}$. We hypothesized that an increase in glucose availability would increase $R_d$ and attenuate the decline in $CHO_ox$ during such exercise but would not alter muscle glycogen utilization.

MATERIALS AND METHODS

All animal experiments were conducted after approval by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University and were performed in compliance with their guidelines and recommendations.

Experimental Design

The effects of increased glucose availability on glucose kinetics, whole body substrate utilization, muscle glycogen usage, and changes in specific plasma hormone and substrate concentrations during exercise were examined in a balanced randomized crossover study. On two occasions, each of six horses was studied during 90 min of treadmill exercise undertaken at a speed that elicited 35% $V_{O2max}$. In one trial (glucose (Glu)), a glucose solution (50% wt/vol) was infused at a mean (± SD) rate of 34.9 ± 1.1 µmol·kg$^{-1}$·min$^{-1}$ (3.1 ± 0.05 g/min) throughout exercise (total glucose dose ~280 g), whereas in the other trial (control (Con)) an equivalent volume of isotonic (0.9% wt/vol) saline was infused. For each horse, the two trials were separated by 7 days.

Horses

The subjects were six horses (2 Standardbred and 4 Thoroughbred; 4 geldings and 2 mares) that were 4–8 yr of age and had body mass of 428–527 kg (471 ± 32 (SD) kg). All horses were housed indoors during the experimental period; were fed a diet of Timothy grass and alfalfa hay and mixed grain; and had access to a salt and mineral block. All horses were conditioned and had undertaken treadmill exercise periodically for at least 3 mo before the study. Between experimental trials, horses received 3 days of light treadmill exercise (20 min of trotting at 4–4.5 m/s with the treadmill set at a 4° incline).

Preliminary Testing

For each horse, $V_{O2max}$ and the relationship between oxygen consumption ($V_{O2}$) and speed were determined during an incremental exercise test 1 wk before the first experiment. The incremental exercise test consisted of the horse running on a high-speed treadmill (Sato) inclined at 2° for 90 s at 4 m/s, after which the treadmill speed was increased by 1 m/s every 90 s until the horse was no longer able to maintain its position on the treadmill. $V_{O2}$ was measured every 10 s during the exercise test. $V_{O2max}$ was defined as the value at which $V_{O2}$ reached a plateau, despite further increases in speed. A plateau was defined as a change in $V_{O2}$ of <4 ml·kg$^{-1}$·min$^{-1}$ with an increase in speed. From linear regression analysis (speeds below $V_{O2max}$), the running speed that elicited 35% of $V_{O2max}$ was calculated for each horse.

Experimental Protocol

All experiments began between 0730 and 0800; food was withheld for 12 h before each experiment, and the horses were confined to their stalls for the preceding 24 h. After aseptic preparation and local anesthesia of the overlying skin, catheters (14 gauge, 5.25 in.; Angiocath, Becton Dickinson) were inserted into the right and left jugular veins for isotope infusion and blood collection, respectively. Thereafter, a blood sample was obtained for subsequent determination of background isotopic enrichment. For determination of glucose kinetics, a primed (18.0 µmol/kg), continuous (0.22 ± 0.01 µmol·kg$^{-1}$·min$^{-1}$) infusion of [6,6-$^2$H]glucose (99% enriched; Cambridge Isotopes, Cambridge, MA) in 0.9% saline was then initiated by using a calibrated infusion pump (PHD 2000, Harvard Apparatus, South Natick, MA). During a 2-h equilibration period, horses stood in stocks. After collection of blood for final baseline hormone, substrate, and glucose kinetic determinations, a sample of middle gluteal muscle was obtained by percutaneous biopsy (see Sampling Procedures). Thereafter, the horses were positioned on the treadmill (2° incline), and a loose-fitting face mask for measurement of respiratory gas exchange was applied. Subjects then began running at a speed calculated to elicit 35% $V_{O2max}$. The rate of [6,6-$^2$H]glucose infusion was doubled during the rest-to-exercise transition (0.44 ± 0.01 µmol·kg$^{-1}$·min$^{-1}$). In the Glu trial, a 50% (wt/vol) glucose solution was infused by a second pump, whereas an equivalent volume of 0.9% saline was administered during the Con trial. The glucose or saline was infused via a three-way connector to allow simultaneous administration of the tracer and glucose or saline. The glucose and saline infusions were commenced at the onset of exercise. During the exercise test, fans mounted 0.5 m in front and to the sides of the treadmill were used to maintain an air velocity of 3.5–4 m/s over the horse. Ambient conditions were similar for all trials; mean values for room temperature and relative humidity during the experiments were 22.4 ± 0.8 (SD) °C and 58 ± 6%, respectively.

Respiratory Gas Exchange Measurements

$V_{O2}$, carbon dioxide production ($V_{CO2}$), and respiratory exchange ratio (RER) were measured with an open-circuit calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH), as previously described (18). Flow through the system was ~1,500 l/min STP with the horse stationary and 9,000 l/min during running. Expired $O_2$ (electrochemical cell, Columbus Instruments) and $CO_2$ (single-beam nondispersive infrared sensor, Columbus Instruments) concentrations were measured continuously, and the data averaged were over 10-s intervals. The gas-analysis system was calibrated before the start of each exercise test by using gas mixtures with $O_2$ and $CO_2$ concentrations that spanned the measurement range. The overall accuracy of the system was verified repeatedly by the $N_2$-dilution method (15). Discrepancy between simulated $V_{O2}$ produced by $N_2$ dilution and the value measured by the system was ±3% at $N_2$ flow rates equivalent to a $V_{O2}$ of 54 l/min (~140 ml·kg$^{-1}$·min$^{-1}$ for a 385-kg horse). Standard equations were used to calculate $V_{O2}$ and $V_{CO2}$, and RER values were calculated by dividing $V_{CO2}$ by $V_{O2}$.

Sampling Procedures

Blood samples for determination of plasma isotopic enrichment and glucose concentrations, respectively, were obtained at 75, 90, and 105 min (corresponding to ~30, ~15, and 0,
respectively, on Figs. 1–3) after the start of tracer infusion and at 5, 15, 30, 45, 60, 75, and 90 min of exercise (where the 105-min sample was collected just before the onset of exercise) and placed in tubes containing EDTA and sodium fluoride-potassium oxalate. Additional blood samples were obtained at 0, 5, 15, 30, 45, 60, 75, and 90 min of exercise for subsequent measurement of hematocrit, plasma total protein, lactate, nonesterified fatty acid (NEFA), and glycerol concentrations, and at 0, 30, 60, and 90 min for measurement of glucagon, insulin, epinephrine (Epi), and norepinephrine (NE) concentrations. Blood samples (6 ml) were placed in tubes containing sodium fluoride-potassium oxalate (plasma lactate), EDTA (hematocrit, plasma total protein, NEFA, glycerol), EDTA-aprotinin [10,000 kallikrein inhibitor units/ml; TrasyloL, FBA Pharmaceuticals, New York, NY] (glucagon), 120 µl of a solution containing 0.24 M EGTA-reduced glutathione (Epi, NE), or no additive (serum insulin). Plasma or serum was obtained by centrifugation (3,000 rpm for 20 min at 4°C) within 30 min of collection and frozen at −20°C (−80°C for hormone and tracer samples) until analysis.

Muscle biopsy samples were collected percutaneously from the middle gluteal muscle by using the needle-biopsy technique. Muscle biopsies were obtained 5 min before commencement of exercise and within 3 min of cessation of exercise. Muscle samples were immediately placed in liquid nitrogen and stored at −80°C until analysis.

Analytic Techniques

Plasma isotopic enrichment. For determination of [6,6-2H]glucose enrichment, plasma samples (0.7 ml) were deproteinized by adding 1.2 ml of 0.3 N Zn(SO₄)₂ and 1.2 ml of 0.3 N Ba(OH)₃. Each tube was then vortexed and incubated in an ice bath for 20 min. After centrifugation at 3,000 rpm for 20 min at 4°C, the supernatant was harvested and placed in 13 × 100-mm screw-cap borosilicate tubes. The water was removed from the tubes by vacuum centrifugation (Savant Instruments, Farmingham, NY). The dried samples were capped and stored at 4°C until further processing. The penta-acetate derivative of glucose was prepared by adding 100 µl of a 2:1 acetic anhydride and pyridine mixture to the dried sample. After a 60-min incubation at 60°C in a dry heating block, the reaction mixture was transferred to a clean 13 × 100-mm borosilicate tube. The penta-acetate derivative was partitioned by sequential addition of 1.5 ml of double-distilled water and 0.4 ml of methylene chloride. After gentle shaking, the tubes were centrifuged at 2,000 rpm for 10 min. The upper water phase was discarded, and the remaining methylene chloride phase was evaporated under N₂. Before injection into the gas chromatograph-mass spectrometer, the samples were dissolved in 50 µl of ethyl acetate. Standards of known isotopic enrichment were prepared in an identical fashion and were analyzed with each batch of samples.

Isotopic enrichment was determined by gas chromatography-mass spectrometry on a Hewlett-Packard (Palo Alto, CA) 5989A mass spectrometer equipped with a 30-m × 0.25-mm DB-5 capillary column (J & W Scientific, Folsom, CA) and by using 1-µl injections. Ions were formed by electron-impact ionization (70 eV), and molecular ions of mass-to-charge ratios (m/z) 200 (m + 0), 201 (m + 1), and 202 (m + 2) were selectively monitored; i.e., m/z 200 and m/z 202 correspond to the unlabeled and labeled ions, respectively. The tracer-to-tracer ratio (TRR) was calculated directly from measured ion abundance ratios by the relationship $TRR = R_a - R_d$, where $R_a$ and $R_d$ represent the measured tracer and tracee ion abundance ratios for enriched and unenriched (background or preinfusion) samples, respectively. Correction was made for the contribution of singly labeled molecules (m/z 201) to the apparent enrichment at m/z 202 (41). The intra-assay coefficient of variation was 1.5 ± 0.5%, and the interassay coefficient of variation was 5.6 ± 2.1%. To control for between-day variability, all samples for a given horse (Con and Glu trials) were analyzed during the same analytic session.

Plasma biochemical analyses. Plasma glucose concentration was measured spectrophotometrically by using the hexokinase reaction with a commercial kit (Glucose-HK kit; Sigma Chemical, St. Louis, MO), and plasma lactate concentration was measured by using an automated lactate oxidase method (Sport 1500 lactate analyzer; Yellow Springs Instruments, Yellow Springs, OH). Plasma NEFA concentration was determined by using a commercial kit that employs an enzymatic colorimetric method (NEFA test kit; Wako Chemicals USA, Dallas, TX). Plasma glycerol concentration was measured by using an enzymatic spectrophotometric method [triglycerides kit 337A (without triglyceride hydrolysis step); Sigma Chemical]. Intra- and interassay coefficients of variation for these biochemical methods were <1.0 and 2.5%, respectively. Hematocrit was measured by the microhematocrit technique. Plasma total protein was measured by refractometry (Sport 1500 refractometer; Yellow Springs Instruments, Buffalo, NY). All samples were analyzed in duplicate.

Plasma hormone analyses. Plasma Epi and NE concentrations were determined by HPLC using electrochemical detection (25). Serum immunoreactive insulin (IRI) was determined in duplicate by use of a commercially available radioimmunoassay (insulin kit, Coat-a-Count Diagnostics, Los Angeles, CA) that has been validated for horse blood (31). Intra- and interassay coefficients of variation were 6.0 ± 1.5 and 11.5 ± 2.1%, respectively. Plasma immunoreactive glucagon (IRG) was determined in duplicate by use of a commercially available radioimmunoassay (glucagon kit, Coat-a-Count Diagnostics). Pooled equine plasma was used to partially validate the assay for horse plasma. Specificity for equine glucagon was demonstrated by dilutional parallelism between standard solutions and serial dilutions of endogenous glucagon in equine plasma [correlation coefficient (r) = 0.987]. Accuracy was demonstrated by adding porcine glucagon to equine plasma in concentrations from 20 to 285 pmol/l. Linear regression of the recovery curve showed an r of 0.9929. The intra-assay precision for 12 replicates (6 duplicates) of equine plasma with a mean concentration of 31 and 75 pmol/l was 7.8 and 5.2%, respectively. The interassay coefficient of variation for the same samples was 13.1 and 14.8%, respectively. For the insulin and glucagon radioimmunoassays, analysis of experimental samples was completed in a single analytic session.

Muscle glycogen. Muscle samples were weighed and subsequently freeze-dried. The freeze-dried samples were reweighed and dissected free of any blood and connective tissue. For each sample, duplicate pieces of muscle were analyzed. The samples were powdered, extracted, and analyzed for muscle glycogen concentration (as glucosyl units) according to the procedure of Passoneau and Lauderdale (29). Intra-assay (12 replicates of a single sample) and interassay (6 replicates) coefficients of variation for this assay were 2.5 and 4.1%, respectively.

Calculations of glucose kinetics. Glucose $R_a$ and $R_d$ (= tissue uptake) at rest were calculated by using the steady-state tracer dilution equation (41) $R_d = R_a - F \cdot ((IE_i/IE_p) - 1)$ where $F$ is the infusion rate of the isotope (in µmol·kg⁻¹·min⁻¹); $IE_i$ and $IE_p$ are the stable isotopic enrichment of the infused and plasma, respectively; and −1 accounts for the tracer's contribution to the turnover rate of glucose at m/z 202 (41). The intra-assay coefficient of variation was 1.5 ± 0.5%, and the interassay coefficient of variation was 5.6 ± 2.1%. To control for between-day variability, all samples for a given horse (Con and Glu trials) were analyzed during the same analytic session.
the substrate (41). The rate of infusion was calculated by multiplying the infusion pump rate by the concentration of glucose in the infusate. During exercise, \( R_a \) and \( R_d \) were calculated by using the non-steady-state equation of Steele (35). This equation is modified for use with stable isotopes as the amount of tracer infused is no longer negligible

\[
E = \frac{C_m}{V_d} \frac{dE}{dt}
\]

and

\[
R_a = R_d = \frac{dC_m}{dt} \left(1 + \frac{dE}{dt}ight) - C_m \frac{dE}{dt}
\]

where \( V_d \) is the effective volume of distribution, \( E \) is the plasma isotopic enrichment, \( C_m \) is the measured plasma concentration of the tracee, and \( dE/dt \) and \( dC_m/\)dt are maximum rates of change in enrichment and glucose concentration, respectively, as a function of time. By using this fixed, one-compartment model of Steele, it is assumed that 1) the apparent glucose space is 25% of body weight, and 2) 65% of this space represents the rapidly mixing portion of the glucose pool. Therefore, the effective \( V_d \) for glucose was assumed to be 162 ml/kg.

In the Con trial, glucose \( R_a \) was assumed to represent HGP, although a small contribution from renal glycogenolysis and gluconeogenesis is possible. In the Glu trial, the endogenous (hepatic) glucose \( R_a \) was calculated as the difference between the total glucose \( R_d \) measured by using the tracer and the known rate of glucose infusion. However, with use of stable isotope tracers, the rate of tracer (and exogenous glucose) infusion must be corrected for the naturally occurring “tracer” infused as part of the exogenous glucose. Accordingly, we measured the m/z 202/200 ion abundance of the exogenous glucose. From these measurements, the enrichment of the exogenous glucose (\( E_{exo} \)) was calculated as

\[
E_{exo}(\%) = \frac{R_{exo} - R_{end}}{1 + R_{exo} - R_{end}} \cdot 100
\]

where \( R_{exo} \) and \( R_{end} \) represent the m/z 202/200 ion abundance ratios for the exogenous glucose and endogenous tracee (background), respectively. For each Glu trial, \( E_{exo} \) was >0%, indicating that the exogenous glucose infusate was “enriched” compared with the endogenous tracee. The tracer component (\( G_{tracee} \)) of the exogenous glucose infusate (G) was calculated as

\[
G_{tracee} = G \cdot (1 - E_{exo}(\%)) / 100
\]

and the tracer component of G (\( G_{tracer} \)) was calculated as

\[
G_{tracer} = G - G_{tracee}
\]

These calculations of \( G_{tracee} \) and \( G_{tracer} \) were then used to obtain the correct infusion rates for the exogenous glucose and tracer, respectively.

The glucose metabolic clearance rate (MCR) was calculated as the \( R_d \) of glucose divided by the average glucose concentration (C) over that time period

\[
MCR (ml\cdot kg^{-1}\cdot min^{-1}) = R_d / [(C_1 + C_2)/2]
\]

Rates of energy expenditure and whole-body substrate oxidation. Total energy expenditure (TEE) and absolute rates of CHOox and lipid oxidation were calculated using the following equations (16)

\[
TEE (kcal/min) = 3.9 \cdot V\dot{CO}_2/RER - 1.11 \cdot V\dot{O}_2 \quad (1)
\]

\[
CHO_{ox} (g/min) = 4.585 \cdot V\dot{CO}_2 - 3.2255 \cdot V\dot{O}_2 \quad (2)
\]

\[
\text{Lipid oxidation (g/min)} = 1.7012 \cdot V\dot{O}_2 - 1.6946 \cdot V\dot{CO}_2 \quad (3)
\]

where \( V\dot{O}_2 \) and \( V\dot{CO}_2 \) are in liters per minute, and it was assumed that protein oxidation made negligible contribution to \( V\dot{O}_2 \) and \( V\dot{CO}_2 \) and that these equations, developed for use in humans, are also applicable to the horse. The calculated values were based on respiratory gas-exchange values averaged over the first 5 min of exercise and 15-min intervals thereafter. \( CHO_{ox} \) in grams per minute was converted to micromoles per kilogram per minute by dividing the molecular weight of glucose (180) and the horse’s body weight. Similarly, rates of fat oxidation were converted to micromoles per kilogram per minute by dividing the molecular weight of palmitate (259) and the horse’s body weight.

Total \( CHO_{ox} \) was calculated from the area under the \( CHO_{ox} \)/time curve for each subject. In addition, the area under the glucose \( R_d \)/time curve was calculated. Studies in dogs (42) and in humans (24) have demonstrated that increases in \( R_d \) by working muscle account for most of the increment in whole body \( R_d \) during exercise. Furthermore, >95% of glucose \( R_d \) is oxidized during exercise (23). Thus glucose \( R_d \) provides a reasonable approximation of blood glucose oxidation, although it is possible that some glucose is used for glycogen resynthesis during low-intensity exercise. Muscle glycogen (plus lactate) oxidation was calculated as the difference between total \( CHO_{ox} \) and glucose \( R_d \). Net muscle glycogen utilization was also calculated as the difference of the pre- and postexercise muscle glycogen concentrations.

The relative contributions by plasma glucose (glucose \( R_d \)), other carbohydrate sources (muscle glycogen and lactate), and lipid to TEE during the 0- to 30-min, 30- to 60-min and 60- to 90-min periods of exercise were estimated by using standard caloric equivalents.

Statistical analyses. Data are presented as means ± SE. The data were analyzed by using a two-way analysis of variance for repeated measures, with treatment (Con vs. Glu) and time as independent factors. Because the data for Epi and NE did not exhibit homogeneous variances, these data were subject to logarithmic transformation before ANOVA. Percent data (relative contributions by different substrates to TEE) were subject to arcsine transformation before ANOVA. The null hypothesis was rejected at \( \alpha = 0.05 \) for the main effects (treatment and time) and \( \alpha = 0.10 \) for the interaction. Significant differences identified by ANOVA were isolated by using the Student-Newman-Keuls post hoc test. Data for net muscle glycogen utilization and total \( CHO_{ox} \) were analyzed by paired t-test. The Sigmastat 2.0 software package (Jandel Scientific, San Rafael, CA) was used for statistical computations.

RESULTS

Individual values for \( V\dot{O}_2_{max} \) ranged from 121 to 164 ml·kg\(^{-1}\)·min\(^{-1}\) (mean 136.3 ± 6.1 ml·kg\(^{-1}\)·min\(^{-1}\)). Mean running speed during the exercise protocol was 4.4 ± 0.2 m/s, which corresponded to a relative work-load of 34 ± 1.0% of \( V\dot{O}_2_{max} \). \( V\dot{O}_2 \) during exercise was relatively constant and not significantly different be-
tween trials. However, whereas RER declined progressively throughout exercise in both trials, glucose infusion attenuated (interaction effect, \(P = 0.02\)) this decrease, and RER was significantly higher in Glu than in Con at 30, 75, and 90 min of exercise (Table 1).

### Table 1. Oxygen consumption, respiratory exchange ratio, and calculated rates of carbohydrate and fat oxidation during 90 min of exercise at 34 ± 1% of maximum oxygen uptake with and without intravenous glucose infusion commencing at the onset of exercise

<table>
<thead>
<tr>
<th>Time, min</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
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<tbody>
<tr>
<td>(\dot{V}O_2), ml·kg⁻¹·min⁻¹</td>
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<tr>
<td>Con</td>
<td>43.7±1.9</td>
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<td>43.3±1.2</td>
<td>44.3±1.3</td>
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<td>43.5±1.7</td>
<td>44.1±1.5</td>
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<td>44.2±1.8</td>
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<td>RER</td>
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<td>Con</td>
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<td>0.88±0.01*</td>
<td>0.86±0.01</td>
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<td>0.88±0.01*</td>
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<tr>
<td>Con</td>
<td>240.6±18.2</td>
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<td>170.2±15.4</td>
<td>141.7±16.9</td>
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<td>Glu</td>
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<td>187.1±14.3*</td>
<td>174.9±13.0*</td>
<td>161.9±15.0*</td>
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<tr>
<td>Fat oxidation, µmol·kg⁻¹·min⁻¹</td>
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</table>

Values are means ± SE for 6 horses. \(\dot{V}O_2\), oxygen uptake; RER, respiratory exchange ratio; CHO, carbohydrate; Con, control; Glu, with glucose infusion. Rates of carbohydrate and fat oxidation were calculated from respiratory gas exchange data, averaged over the preceding time interval. *Significantly different from Con, \(P < 0.05\).

Plasma Glucose Concentration and Kinetics

At rest, plasma glucose concentration and isotopic enrichment (Fig. 1, A and B) and HGP (Fig. 2B) and \(R_d\) (Fig. 3A) were similar between the two trials. In Glu, plasma glucose concentration increased progressively during exercise. Although plasma glucose tended to increase during exercise in Con, mean values were never more than ~0.9 mmol/l higher compared with
Preexercise values. Plasma glucose was significantly higher in Glu than in Con between 15 min and the end of exercise (Fig. 1A). Plasma isotopic enrichment decreased during exercise in both trials but was significantly lower in Glu than in Con between 45 and 90 min (Fig. 1B).

Total glucose $R_d$ (HGP + exogenous glucose) was 60–70% higher in Glu than in Con throughout exercise (Fig. 2A). Endogenous glucose $R_a$ (HGP) in Con increased progressively during exercise and reached a peak of $38.6 \pm 2.7 \mu mol \cdot kg^{-1} \cdot min^{-1}$ after 90 min (Fig. 2B). Glucose infusion suppressed (P < 0.05) the exercise-associated increase in HGP. However, this suppression of HGP during exercise in Glu was not complete, and values for endogenous $R_a$ increased from $8.4 \pm 0.6 \mu mol \cdot kg^{-1} \cdot min^{-1}$ at rest to a peak of $25.8 \pm 3.3 \mu mol \cdot kg^{-1} \cdot min^{-1}$ at 90 min (Fig. 2B).

In Con, the exercise-associated increase in glucose $R_d$ was biphasic; $R_d$ rose from $8.3 \pm 0.9$ to $29.1 \pm 3.1 \mu mol \cdot kg^{-1} \cdot min^{-1}$ at 45 min and further increased between 60 and 90 min to a peak of $40.4 \pm 2.9 \mu mol \cdot kg^{-1} \cdot min^{-1}$ (Fig. 3A). Glucose $R_d$ was significantly higher in Glu than in Con throughout exercise (P < 0.001). Between 30 and 90 min of exercise, glucose $R_d$ was approximately twofold higher in Glu than in Con (Fig. 3A). MCR was similar at rest in the two trials and increased to a similar extent during the initial 15 min of exercise. From 30 min until the end of exercise, MCR was significantly greater in Glu than in Con (Fig. 3B).

Plasma Hormone Concentrations

There were no differences in serum IRI and plasma IRG at rest between the two trials (Table 2). However, serum IRI was higher (main effect, P < 0.05) in Glu during exercise, whereas plasma IRG concentrations were significantly lower (interaction effect, P < 0.001) in Glu than in Con. Consequently, the insulin-to-glucagon molar ratio was significantly higher during exercise in Glu. Whereas plasma NE concentrations were similar in the two trials, glucose infusion attenuated (P < 0.05) the rise in plasma Epi. Plasma Epi concentrations were significantly lower in Glu than in Con at 90 min of exercise (Table 2).

Hematocrit, Plasma Total Protein, Lactate, Glycerol, NEFA

In both trials, hematocrit significantly increased by 5 min of exercise with no further change for the remainder of the protocol (Table 3). No differences in plasma total protein and lactate concentrations were observed between the two trials. Plasma NEFA and glycerol concentrations increased during exercise in both trials. However, plasma NEFA concentrations were lower (interaction effect, P < 0.05) in Glu than in Con between 45 and 90 min of exercise. Although mean values for plasma glycerol concentration were lower in Glu than in Con, this difference did not reach statistical significance (P = 0.19, power at $\alpha = 0.05$:0.15).

Energy Expenditure and Substrate Utilization

The total rate of energy expenditure was similar between trials, averaging $0.22 \pm 0.02 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.
Table 3. Hematocrit, plasma total protein, FFA, glycerol, and lactate concentrations during 90 min of exercise at 34 ± 1% of maximum oxygen uptake with and without intravenous glucose infusion commencing at the onset of exercise.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Hematocrit, %</th>
<th>TPP, g/dl</th>
<th>FFA, mmol/l</th>
<th>Glycerol, µmol/l</th>
<th>Lactate, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Con: 31 ± 0.5</td>
<td>Glu: 32 ± 0.7</td>
<td>Con: 6.5 ± 0.3</td>
<td>Glu: 0.42 ± 0.08</td>
<td>Glu: 75.6 ± 5.4</td>
</tr>
<tr>
<td>5</td>
<td>Con: 46 ± 1.5</td>
<td>Glu: 46 ± 1.5</td>
<td>Con: 6.9 ± 0.3</td>
<td>Glu: 0.47 ± 0.09</td>
<td>Glu: 131.3 ± 6.0</td>
</tr>
<tr>
<td>15</td>
<td>Con: 46 ± 1.5</td>
<td>Glu: 45 ± 0.9</td>
<td>Con: 6.8 ± 0.3</td>
<td>Glu: 0.63 ± 0.08</td>
<td>Glu: 162.6 ± 9.1</td>
</tr>
<tr>
<td>30</td>
<td>Con: 45 ± 1.5</td>
<td>Glu: 45 ± 0.5</td>
<td>Con: 6.8 ± 0.3</td>
<td>Glu: 0.84 ± 0.13</td>
<td>Glu: 199.8 ± 14.2</td>
</tr>
<tr>
<td>45</td>
<td>Con: 44 ± 1.5</td>
<td>Glu: 44 ± 0.5</td>
<td>Con: 6.8 ± 0.3</td>
<td>Glu: 0.62 ± 0.08</td>
<td>Glu: 246.5 ± 12.5</td>
</tr>
<tr>
<td>60</td>
<td>Con: 46 ± 1.5</td>
<td>Glu: 43 ± 0.8</td>
<td>Con: 6.9 ± 0.3</td>
<td>Glu: 0.70 ± 0.08</td>
<td>Glu: 295.8 ± 12.4</td>
</tr>
<tr>
<td>75</td>
<td>Con: 46 ± 1.6</td>
<td>Glu: 44 ± 1.1</td>
<td>Con: 7.0 ± 0.3</td>
<td>Glu: 0.75 ± 0.08</td>
<td>Glu: 353.6 ± 21.1</td>
</tr>
<tr>
<td>90</td>
<td>Con: 46 ± 1.5</td>
<td>Glu: 45 ± 1.1</td>
<td>Con: 6.8 ± 0.3</td>
<td>Glu: 0.81 ± 0.09</td>
<td>Glu: 437.1 ± 36.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 horses. TPP, total plasma protein; FFA, nonesterified fatty acids. *Significantly different from Con, P < 0.05.
different between the two trials (Con: 980 ± 45 g/90 min, Glu: 1,060 ± 80 g/90 min; Fig. 5). Net muscle glycogen utilization, calculated as the difference in muscle glycogen concentration between pre- and post-exercise biopsy samples, was also similar in the Con (164 ± 31 mmol glucosyl units/kg dry muscle) and Glu (161 ± 24 mmol glucosyl units/kg dry muscle) trials (Fig. 6).

Estimates of the absolute and relative caloric contributions from plasma glucose, other carbohydrate sources (muscle glycogen plus lactate), and fat during the 0- to 30-min, 30- to 60-min, and 60- to 90-min periods of exercise are shown in Fig. 4. The higher \( \text{CHO}_{\text{o}} \) in Glu than in Con (Table 1) reduced (\( P < 0.05 \)) the percent contribution to total energy use by fat oxidation from between 43 and 68% in the Con trial to between 35 and 55% in the Glu trial (Fig. 4B). Conversely, the contribution by plasma glucose to energy use was significantly higher in Glu than in Con. In Glu, the percent contribution by plasma glucose to TEE increased from 12 ± 1.5% during the 0- to 30-min period to 20 ± 2.2% during the 60- to 90-min period. Corresponding values for the Con trial were 6.1 ± 1.2 and 11.2 ± 2.1% (Fig. 4B). In both trials, the contribution from muscle glycogen (and lactate) to TEE decreased from ~50-52% during the 0- to 30-min period to ~20-23% during the 60- to 90-min period. The contribution from muscle glycogen was not different between trials.

**DISCUSSION**

The present study is the first in horses to determine the effects of an increase in glucose availability on the kinetics of endogenous glucose production and whole body \( R_d \) during exercise. In addition, this study is the first, to our knowledge, to quantify the effects of an increase in glucose supply on the relative contributions from different substrate sources to TEE in horses during low-intensity exercise. The principal findings were that 1) an infusion of glucose at a rate that approximated the average endogenous \( R_d \) measured in Con trials only partially suppressed hepatic glucose output during exercise; 2) the increase in glucose availability as a result of glucose infusion (~3 g/min) resulted in twofold increases in whole body glucose \( R_d \) and the estimated contribution by plasma glucose to TEE; and 3) glucose infusion did not affect muscle glycogen utilization but attenuated the decrease in \( \text{CHO}_{\text{o}} \) and increase in fat oxidation measured during exercise in Con trials.

**Critique of Methods**

In the present study, the fixed-volume, one-compartment model developed by Steele (35) was used for calculations of glucose kinetics in the non-steady state. Although this method for calculation of glucose kinetics during exercise is well established (41), it is recognized that this approach has limitations when there are large changes in isotopic enrichment (5). Specifically, the calculated values for \( R_a \) become dependent on the assumed effective \( V_d \) (i.e., the pool fraction) when substantial changes in enrichment occur. We calculated \( R_a \) by using different values for the effective \( V_d \), ranging from the smallest plausible value (i.e., plasma volume) to the largest (i.e., extracellular fluid volume). Although the effective \( V_d \) chosen did change the \( R_a \) values, differences were ~<10%. Importantly, regardless of the assumed \( V_d \) employed, our conclusions with respect to between-trial differences in glucose kinetics are the same.

When exogenous (unlabeled) glucose is infused, endogenous glucose \( R_a \) is calculated as the difference between the total glucose \( R_a \) measured by using the tracer and the known rate of glucose infusion. However, as discussed by Coggan et al. (7), with use of stable isotopically labeled tracers, errors are introduced into this calculation when the isotopomer distribution of the exogenous glucose (\( R_{\text{o}} \)) differs from that of the endogenous glucose (\( R_d \)). If these isotopomer distributions differ, the exogenous unlabeled glucose is either enriched (\( R_{\text{o}} > R_d \)) or depleted (\( R_{\text{o}} < R_d \)) compared with the endogenous tracer, and the rate of tracer infusion must be corrected to account for the effects of the exogenous glucose on the “enrichment” of the glucose pool. In the present study, for each trial in which unlabeled glucose was infused, the exogenous substrate was “enriched” compared with the m/z 202/200 ion-abundance ratio of the horse’s endogenous glucose. However, these differences were very small (~1.0–1.5%), resulting in only minor adjustments to the tracer and exogenous tracer infusion rates.

**Glucose Kinetics**

On the basis of previous studies in horses that have reported hyperglycemia during moderate exercise (13, 33), we anticipated that HGP would exceed glucose \( R_d \) during exercise in Con, resulting in an increase in plasma glucose concentrations. However, although HGP was higher than \( R_d \) during the first 60 min of exercise in Con, this difference was small, and plasma glucose
increased by only ~0.9 mmol/l (Figs. 1 and 2). This finding suggests that, similar to other species, the exercise-induced increment in glucose $R_d$ is closely matched by the endogenous $R_a$ response in horses during low-intensity exercise. We also hypothesized that the endogenous $R_a$ response would be insensitive to feedback mechanisms associated with an increase in blood glucose availability. However, when exogenous glucose was supplied at a rate that approximated the average endogenous $R_a$ response measured in the Con trial, there was a significant suppression of the exercise-induced increment in HGP (Fig. 2B). Nevertheless, this attenuation of the endogenous $R_a$ response was only partial. During the final 45 min of exercise in Glu, when plasma glucose concentration had increased to ~8–8.5 mmol/l (Fig. 1A), there was a progressive increase in HGP, and overall the endogenous $R_a$ response in Glu was 40% lower than in Con. These findings suggest that there is a component of the increase in endogenous $R_a$ that is insensitive to metabolic feedback. It is possible that regulation of the exercise-induced increment in HGP in the horse during low-intensity exercise involves both neurogenic feed-forward and metabolic feedback mechanisms.

Inasmuch as exogenous glucose only partially suppressed HGP, the results of the present study differ from those of previous studies in humans and animals. In humans and dogs during moderate-intensity exercise, an intravenous glucose infusion that matches the endogenous $R_a$ response measured during Con trials completely inhibits the exercise-associated increment in HGP (3, 21, 22). Although the reasons for this species variation cannot be determined from our data, it is possible that greater sympathoadrenal activation in the horse during low-intensity exercise plays a role in the feed-forward regulation of hepatic glucose output. After 100 min of moderate-intensity (2-fold increase in heart rate) exercise in dogs, plasma NE and Epi concentrations were approximately twofold higher than at rest (3). Similarly, in trained human athletes exercising at ~50% of $V_{O2 max}$, plasma NE and Epi were only 2- to threefold higher compared with preexercise values (12). In contrast, in the Con trial of the present study, peak values for plasma NE and Epi were five- and ninefold higher, respectively, compared with resting concentrations (Table 2). The more striking increase in plasma catecholamines in the horse likely reflects a greater degree of sympathoadrenal activation compared with responses measured in dogs and humans at similar relative exercise intensities. Increases in circulating catecholamines, and possibly direct sympathetic neural activation, can enhance hepatic glucose mobilization during exercise, although species differences exist (38). Elucidation of the role of sympathoadrenal mechanisms in stimulation of HGP in the exercising horse awaits further investigation.

Several mechanisms may have contributed to the attenuation of HGP during Glu. In particular, glucose infusion altered the plasma concentrations of hormones that, in other species, are important for glucose regulation. Serum IRI concentrations were higher in Glu, whereas the increase in plasma IRG was attenuated by glucose infusion. Therefore, the insulin-to-glucagon molar ratio was higher during exercise in Glu (Table 2). In dogs (38), changes in these pancreatic hormones, specifically an increase in plasma glucagon with a concomitant decrease in plasma insulin, play an important role in regulation of the exercise-induced increment in hepatic glycogenolysis. Assuming such changes affect hepatic glucose output in horses during exercise, the higher serum IRI and lower plasma IRG concentrations could account for a reduction in HGP. Plasma Epi concentrations also were lower in Glu than in Con during exercise (Table 2). However, this difference was small and did not reach statistical significance until the end of exercise. Nonetheless, the reduced Epi concentrations may have contributed, in part, to the decrease in hepatic glucose output. Finally, the elevated plasma glucose concentration, independent of changes in glucoregulatory hormones, may have reduced HGP directly. In rats, increases in plasma glucose directly lower hepatic glycogenolysis and HGP by decreasing glycogen phosphorylase and increasing glucokinase activities (34). However, the progressive increase in HGP during the final 45 min of exercise in Glu, when mean plasma glucose concentrations were >7.5–8.0 mmol/l (Fig. 1A), argues against a significant role for this mechanism in the present study.

Given the substantial endogenous $R_a$ response in Glu, total glucose supply (the sum of exogenous glucose and HGP; Fig. 2A) was approximately twofold higher compared with that in the Con trial. Furthermore, by a similar magnitude, whole body glucose $R_d$ was higher in Glu than Con throughout exercise (Fig. 3A). As discussed in MATERIALS AND METHODS, it is assumed that >90% of tracer-determined whole body glucose $R_d$ reflects glucose uptake by muscle. Because we did not collect urine from the horses of the present study, we cannot eliminate the possibility that at least some of the $R_d$ reflected loss of glucose in urine. However, in horses the renal threshold for glucose is 10.5–11 mmol/l (30), although it is possible that this threshold is lower during exercise. Nevertheless, the contribution of renal losses to glucose $R_d$, if any, was likely to be small.

A number of factors regulate glucose uptake by muscle, including glucose delivery to muscle, membrane glucose transport, and intracellular metabolism. Glucose delivery is a function of muscle blood flow and prevailing glucose concentrations. In exercising dogs, tracer-determined whole body and leg $R_d$ are augmented after an increase in blood glucose availability (42). Similarly, carbohydrate ingestion or intravenous glucose infusion that elevates plasma glucose concentrations has been demonstrated to increase leg (1) and whole body (10, 21, 27) glucose disposal in humans during moderate-intensity exercise. Therefore, part of the increment in glucose uptake in Glu can be attributed to an increase in blood glucose concentration. However, MCR ($R_d$/glucose concentration) was significantly higher in Glu than in Con between 30 and 90 min of exercise (Fig. 3B), suggesting that other factors
may have contributed to the increase in glucose $R_d$. Studies in humans (39) and dogs (42) have demonstrated that muscle contractions and insulin have synergistic effects on glucose $R_d$ by muscle during exercise. In addition, a suppression of circulating NEFA availability has been shown to increase whole body and limb glucose $R_d$ significantly in dogs during exercise (4).

In the present study, serum IRI was higher during exercise in Glu (Table 2), whereas glucose infusion attenuated the exercise-associated increase in free fatty acids such that plasma NEFA concentrations were significantly lower in Glu than in Con between 45 and 90 min of exercise. Therefore, it is possible that a combination of higher serum IRI and lower plasma NEFA in Glu contributed to the increase in glucose $R_d$ and MCR.

Another factor that may have contributed to the increased glucose $R_d$ and MCR in Glu was the suppression in plasma Epi concentrations (Table 2). In human subjects during low-intensity exercise ($\sim 25\% \text{Vo}_{2\text{max}}$), an infusion of Epi that results in a modest increase in plasma Epi concentration (control $\sim 0.6 \text{nmol/l}$ vs. Epi infusion $\sim 2.0 \text{nmol/l}$) is associated with an $\sim 20\%$ reduction in glucose clearance (28). Similarly, in resting human subjects, physiological increases in plasma Epi constrain glucose $R_d$ and clearance (32). Therefore, it is possible that the lower plasma Epi concentrations in Glu during the last 30 min of exercise, in part, contributed to the increase in glucose $R_d$ and MCR.

**Substrate Utilization**

An important finding of the present study was that glucose infusion attenuated the decrease in total $\text{CHO}_{ox}$ measured during exercise in the Con trial (Table 1, Fig. 5). Previous studies in humans during moderate exercise also have demonstrated increased $\text{CHO}_{ox}$ with a concomitant reduction in fat oxidation, when glucose availability is increased either by intravenous infusion (10) or carbohydrate ingestion (1,19, 20). Overall, $\text{CHO}_{ox}$ was $\sim 20\%$ higher in Glu than in Con with a similar decrease in total fat oxidation (Table 1, Fig. 4). However, muscle glycogen (plus lactate) oxidation, estimated from the difference between total $\text{CHO}_{ox}$ and total glucose $R_g$, was similar between trials. Moreover, net muscle glycogen utilization, estimated by measurement of muscle glycogen content in pre- and postexercise biopsy samples, also did not differ between Con and Glu (Fig. 6). Therefore, the increment in $\text{CHO}_{ox}$ measured during Glu can be attributed to increased uptake and utilization of blood glucose by the working tissues.

There are little published data in horses on the effects of an acute increase in glucose availability on muscle glycogen utilization during exercise. However, in accord with the findings of the present study, Farris et al. (13) reported that an intravenous glucose infusion that elevated plasma glucose concentrations to $\sim 9$--$10$ mmol/l had no effect on glycogen utilization in the middle gluteal muscle of horses during exercise at $\sim 50\% \text{Vo}_{2\text{max}}$. In humans during prolonged cycling exercise, carbohydrate feedings that maintain blood glucose at euglycemic levels do not alter net muscle glycogen utilization (9). Similarly, a glucose-infusion protocol that maintains plasma glucose concentrations at $\sim 10$ mmol/l does not affect the net rate of muscle glycogen utilization in trained men during intense exercise ($\sim 73\% \text{Vo}_{2\text{max}}$) (10). In contrast, carbohydrate ingestion by trained men during running at $70\% \text{Vo}_{2\text{max}}$ was associated with an $\sim 28\%$ reduction in net glycogen breakdown in type I fibers of vastus lateralis muscle (37).

If it is assumed that all of the glucose $R_d$ was oxidized, plasma glucose supplied $26 \pm 2\%$ of the total $\text{CHO}_{ox}$ and $16.5 \pm 2\%$ of the energy yield during exercise in Glu. In contrast, corresponding values for the contribution by plasma glucose in Con were $17 \pm 2\%$ of the total $\text{CHO}_{ox}$ and $8.5 \pm 1\%$ of the energy yield. In the Glu trial, calculations of total glucose $R_g$ indicate that $\sim 430$--$440$ g of glucose were available during the 90 min of exercise (280 $\pm$ $10$ g exogenous glucose plus $155 \pm 12$ g from endogenous production). Therefore, there was a relatively small disparity between the quantity of glucose available and the calculated amount utilized during exercise (375 $\pm$ 20 g/90 min). On the kinetic basis of the glucose distribution volume used in the kinetic calculations, $\sim 220$ mmol (40 g) of glucose would have been needed to increase the plasma glucose concentration by $\sim 3$ mmol/l (Fig. 1A).

The higher total $\text{CHO}_{ox}$ in Glu was accompanied by a significant attenuation of fat oxidation (Table 1). Furthermore, plasma NEFA concentrations were significantly lower in Glu than in Con between 45 and 90 min of exercise (Table 3). Given the higher serum IRI, it is possible that the lower NEFA concentration levels were due to a reduction in lipolysis (19). Recent studies in humans during low- and moderate-intensity exercise have shown that increased blood glucose availability suppresses fat utilization by inhibition of both fat mobilization (i.e., lipolysis in adipose tissue) and fat oxidation within muscle (11, 19). Horowitz and colleagues (19) have shown that relatively small increases in plasma insulin ($\sim 10$--$30$ $\mu$U/ml) associated with fructose or glucose ingestion reduce whole body lipolysis and fat oxidation by $\sim 40$--$50\%$ during a subsequent bout of moderate-intensity exercise (44 $\pm$ $2\%$$\text{peak Vo}_2$). Furthermore, this group has demonstrated that the increase in whole body glucose disposal associated with preexercise glucose ingestion is accompanied by inhibition of long-chain fatty acid oxidation (11). It is likely that similar mechanisms accounted for the reduction in fat oxidation measured in the Glu trial.

In summary, this study has demonstrated that an intravenous glucose infusion at a rate that approximated the average endogenous $R_a$ measured in Con trials only partially suppressed hepatic glucose output in horses during low-intensity ($\sim 35\% \text{Vo}_{2\text{max}}$) exercise. This finding suggests that, in addition to blood-borne metabolic feedback signals, feed-forward mechanisms may regulate HGP in horses during moderate exercise. The hyperglycemia and hyperinsulinemia associated with glucose infusion resulted in an approximately twofold increases in whole body glucose uptake and the estimated contribution of plasma glucose to energy

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expenditure. Glucose infusion did not affect muscle glycogen utilization. However, the increase in plasma glucose uptake and utilization attenuated the decrease in CHOox measured in the Con trial.

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