Epinephrine inhibits exogenous glucose utilization in exercising horses

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Geor, Raymond J., Kenneth W. Hinchcliff, Laura J. McCutcheon, and Richard A. Sams. Epinephrine inhibits exogenous glucose utilization in exercising horses. J Appl Physiol 88: 1777–1790, 2000.—This study examined the effects of preexercise glucose administration, with and without epinephrine infusion, on carbohydrate metabolism in exercising horses. Six horses completed 60 min of treadmill exercise at 55 ± 1% maximum O2 uptake in 1 h after oral administration of glucose (2 g/kg; G trial); 2 h after oral glucose and with an intravenous infusion of epinephrine (0.2 µmol·kg−1·min−1; GE trial) during exercise, and 3 h after water only (F trial). Glucose administration (G and GE) caused hyperinsulinemia and hyperglycemia (−8 mM). In GE, plasma epinephrine concentrations were three- to fourfold higher than in the other trials. Compared with F, the glucose rate of appearance was ~50% and ~33% higher in G and GE, respectively, during exercise. The glucose rate of disappearance was ~100% higher in G than in F, but epinephrine infusion completely inhibited the increase in glucose uptake associated with glucose administration. Muscle glycogen utilization was higher in GE (349 ± 44 mmol/kg dry muscle [dm]) than in F (218 ± 28 mmol/kg dm) and G (201 ± 35 mmol/kg dm). We conclude that 1) preexercise glucose augments utilization of plasma glucose in horses during moderate-intensity exercise but does not alter muscle glycogen usage and 2) increased circulating epinephrine inhibits the increase in glucose rate of disappearance associated with preexercise glucose administration and increases reliance on muscle glycogen for energy transduction.

stable isotopes; glucose kinetics; catecholamines; muscle glycogen; insulin

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supply associated with an intravenous infusion of glucose attenuates the increase in plasma epinephrine in horses, during exercise at 35% of maximum oxygen uptake ($V_{O2max}$) (16). Thus there is evidence that β-adrenergic stimulation constrains glucose uptake in horses during exercise. Moreover, on the basis of plasma epinephrine concentrations, an increase in carbohydrate availability reduces β-adrenergic stimulation during exercise. Taken together, these findings suggest that, under conditions of increased carbohydrate availability, attenuation of the sympathoadrenal response may contribute to an increase in whole body glucose disposal. To date, however, this hypothesis has not been tested.

The present studies were, therefore, undertaken to determine the effects of glucose supply (pre-exercise oral glucose administration vs. water placebo) and adrenergic mechanisms (pre-exercise glucose administration with and without an intravenous infusion of epinephrine during exercise) on tracer-determined whole body glucose uptake in horses during moderate-intensity exercise. It was hypothesized that pre-exercise glucose administration would attenuate the sympathoadrenal response to exercise and increase utilization of blood glucose, evidenced by an increase in glucose rate of disappearance ($R_g$). Conversely, the increase in β-adrenergic stimulation associated with epinephrine infusion would reverse the effects of pre-exercise glucose administration such that whole body glucose disposal would be similar to that measured in control (a 24-h fast before exercise) trials. Because β-adrenergic stimulation can also affect muscle glycogenolysis, a further objective was to determine the effects of these interventions on net 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.

Horses. The subjects were six horses (2 Standardbred and 4 Thoroughbred; all geldings), 3–9 yr old, body mass 408–527 kg (471 ± 25 kg, mean ± SD). The horses were conditioned and undertaking regular treadmill exercise (5 days/wk) for 2 mo before the study. Four days per week, horses exercised for 30–45 min at 55% of $V_{O2max}$, and on the fifth day a protocol of moderate- (20 min at 55% $V_{O2max}$) and higher (10 min at 75% $V_{O2max}$) intensity exercise was completed. During the conditioning and experimental periods, the horses were housed indoors and fed a diet of timothy grass and alfalfa hay and a pelleted concentrate ration, and they had access to a salt and mineral block. 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 ($V_O$) 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 4° 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, $V_O$ was measured every 10 s during the exercise test. $V_{O2max}$ was defined as the value at which $V_O$ reached a plateau, despite further increases in speed. A plateau was defined as a change in $V_O$ 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 55% of $V_{O2max}$ was calculated for each horse.

Experimental design. The effects of pre-exercise glucose administration and of epinephrine infusion on carbohydrate metabolism during moderate-intensity exercise were examined in a three-way crossover study. All horses undertook 60 min of treadmill exercise at a workload equivalent to 55% $V_{O2max}$ in each of three experimental conditions: 1) 1 h after administration of a 20% glucose solution (2 g/kg body wt; G trial); 2) 1 h after administration of a 20% glucose solution and with an intravenous infusion of epinephrine (0.2 µmol·kg⁻¹·min⁻¹), commencing at the onset of exercise (GE trial); and 3) 1 h after administration of water only (F trial). For each horse, the administered fluid volumes (glucose solution or water) were the same (~8–9 liters). Trials were separated by 7 days, and the order of the trials was randomized but balanced among treatments.

Experimental protocol. The horses were fasted for 24 h before each experiment and were confined to their stalls during this time period. Body weight was measured on entry to the laboratory. After aseptic preparation and local anesthesia of the overlying skin, catheters (14 gauge, 5 1/4 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 (0.248 ± 0.007 µmol·kg⁻¹·min⁻¹) infusion of [6,6-²H]glucose (99% enriched; Cambridge Isotopes, Cambridge, MA) in 0.9% saline was then initiated with the use of a calibrated infusion pump (PHD 2000; Harvard Apparatus, South Natick, MA). After a 90-min equilibration period, during which the horses stood in stocks, the glucose or water treatments were administered by nasogastric gavage. Five minutes before exercise, a sample of middle gluteal muscle was obtained by percutaneous biopsy (see Sampling procedures). Thereafter, the horses were positioned on the treadmill (4° incline), and a loose-fitting face mask for measurement of respiratory gas exchange was applied. A thermocouple (model T-180; Physitemp Instruments, Clifton, NJ) attached to a thermometer (BAT-10, Physitemp Instruments) was inserted 20–25 cm beyond the anal sphincter for measurement of temperature within the rectum during exercise. The horses completed a 5-min warm-up (3 m/s treadmill belt speed) followed by 60 min of running at a speed calculated to elicit 55% $V_{O2max}$. The rate of ([6,6-²H]glucose infusion was tripled (0.747 ± 0.02 µmol·kg⁻¹·min⁻¹) during the warm-up. In the GE trial, an epinephrine solution (12.5 mg of epinephrine diluted in 500 ml of 0.9% saline for a concentration of 25 µg/ml) was infused (0.2 µmol·kg⁻¹·min⁻¹) by a second pump (volumetric infusion pump, model Vet/IV 2.2, Heska), whereas an equivalent volume of 0.9% saline was administered during the F and G trials. The epinephrine or saline solutions were infused via a three-way stopcock to allow simultaneous administration of the tracer and epinephrine or saline. The epinephrine and saline infusions were commenced at the onset of exercise (after completion of the warm-up). 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; values for room temperature and relative humidity during the experiments were 22.4 ± 0.9°C and 58 ± 5%
Respiratory gas exchange measurements. VO₂, carbon dioxide production (VCO₂), and respiratory exchange ratio (RER) were measured with an open-circuit calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH) as previously described (24). Flow through the system was ~1,500 l/min STP when the horse was stationary and 9,000 l/min during running. Data for expired O₂ (electrochemical cell, Columbus Instruments) and CO₂ (single-beam nondispersive infrared sensor, Columbus Instruments) concentrations were measured continuously and were reported at 10-s intervals. The gas-analysis system was calibrated before the start of each exercise test by using gas mixtures with O₂ and CO₂ concentrations that spanned the measurement range. The overall accuracy of the system was verified repeatedly by the nitrogen dilution method (10). Discrepancy between simulated VO₂ produced by nitrogen dilution and the value measured by the system was ±3% at nitrogen flow rates equivalent to a VO₂ of 54 l/min (~140 ml·kg⁻¹·min⁻¹ for a 385-kg horse). Standard equations were used to calculate VCO₂ and VCO₂ and R values were calculated by dividing VCO₂ by VO₂.

Rectal temperature. Temperature within the rectum (T re) was measured at rest before the start of exercise and at 10-min intervals during the exercise trial. The thermocouple had a response time of ~1°C/s and was calibrated in a heated water bath with a precision thermometer (Fisher Scientific, Mississauga, Ontario).

Sampling procedures. Blood samples were obtained at −75, −60, −45, −30, and −15 min before exercise and at 0, 5, 10, 20, 30, 40, 50, and 60 min of exercise (where the “−75” sample was collected 75 min after the start of isotope infusion and the water or glucose solutions were administered immediately after collection of the “−60” sample). Blood samples were divided (6-ml aliquots) into four different tubes for subsequent analysis. Two aliquots of each sample were placed in evacuated tubes containing EDTA. These samples were later analyzed for plasma isotopic enrichment, hemocrit, plasma total protein, nonesterified fatty acid (NEFA), and glycerol concentrations. A 5-ml aliquot was placed in a tube containing sodium fluoride-potassium oxalate for subsequent determination of plasma glucose and lactate concentrations. The final aliquot was placed in a tube containing no additive and was later analyzed for serum insulin concentration. Additional blood samples for subsequent measurement of epinephrine and norepinephrine concentrations were obtained at 0, 10, 20, 40, and 60 min of exercise and were placed in test tubes that held 120 µl of a solution containing 0.24 M EGTA-reduced glutathione. 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 via the needle biopsy technique (40). Muscle biopsies were obtained 5 min before commencement of exercise and in 3 min of cessation of exercise. Muscle samples were immediately placed in liquid nitrogen and stored at −80°C until analysis. In the G and GE trials, urine was collected during the 30-min postexercise period. Urine volume was measured, and an aliquot was saved for subsequent measurement of glucose concentrations.

Plasma isotopic enrichment. Plasma samples (0.7 ml) were deproteinized by adding 1.2 ml of 0.3 N Zn(SO)₄ and 1.2 ml of 0.3 N Ba(OH)₂. Each tube was then vortexed and incubated in the ice bath for 20 min. After centrifugation (3,000 rpm for 20 min at 4°C), the supernatant was harvested, and the water was removed from the tubes by vacuum centrifugation (Savant Instruments, Farmingham, NY). The pentacetate derivative of [6,6-²H]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, the reaction mixture was partitioned by sequential addition of 1.5 ml 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 5989A mass spectrometer equipped with a 30-m × 0.25-mm B-5 capillary column (J & W Scientific, Folsom, CA) and with the use of 1-µl injections. The initial oven temperature was set at 110°C and was gradually increased by 35°C/min until it reached a final temperature of 255°C. The transfer line was set at 250°C, the detector temperature was set at 200°C, and the quadrupole temperature was set at 115°C. Ions were formed by electron impact ionization (70 eV), and molecular ions were monitored. Ions with mass-to-charge ratios (m/z) of 200 (m + 0), 201 (m + 1), and 202 (m + 2) were selectively monitored, i.e., m/z 200 and m/z 201 correspond to the unlabeled and labeled ions, respectively. The tracer-to-tracer ratio was calculated directly from measured ion abundance ratios and was equal to R - R♭, where R and R♭ represent the measured tracer and tracee ion abundance ratios for enriched and unenriched (background or preinfusion) samples, respectively. Correlation was made for the contribution of singly labeled molecules (m/z 201) to the apparent enrichment at m/z 202 (61). 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 (F, G, and GE) were analyzed during the same analytic session.

Plasma biochemical analyses. Plasma glucose and lactate concentrations were measured by the glucose oxidase and lactate oxidase methods, respectively, with an automated analyzer (Yellow Springs Instruments, Yellow Springs, OH). Urine glucose concentration was also measured by use of the glucose oxidase autoanalyzer. 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 an enzymatic spectrophotometric method [tri-glycerides kit 337A (without the triglyceride hydrolysis step); Sigma Chemical]. Intra- and interassay coefficients of variation for these biochemical methods were <1.0% and 2.5%, respectively. Hemocrit was measured by the microhemocrit technique. Plasma total protein was measured by refractometry (Cambridge Instruments, Buffalo, NY). All samples were analyzed in duplicate.

Muscle glycogen and lactate. 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. One portion was extracted according to the general procedures of Harris et al. (23), and duplicate extracts were analyzed for lactate content by enzymatic fluorometric methods (41). Muscle glycogen concentrations (as glucosyl units) were determined on a second freeze-dried aliquot, which was extracted and analyzed according to the procedure of Passoneau and Lauderdale (48). Intra-assay (12 replicates of a single sample) and interassay (6 replicates) coefficients of variation for the muscle glycogen assay were 2.5% and 4.1%, respectively.

Calculations of glucose kinetics. Glucose rate of appearance (Ra) and Rd (tissue uptake) at rest were calculated by using the steady-state tracer dilution equation (61)

\[
Ra = \frac{F - V_d \cdot C_m}{1 + E} \cdot \frac{dE}{dt}
\]

where F is the infusion rate of the isotope (in mmol·kg\(^{-1} \cdot \text{min}^{-1}\)); IE\(_{1}\) and IE\(_{2}\) are the stable isotopic enrichment of the infused and plasma, respectively; and -1 accounts for the tracer’s contribution to the turnover rate of the substrate (61). The rate of infusion was calculated by multiplying the concentration of glucose in the infused solution by the concentration of glucose in the plasma, respectively; and

\[
Rd = \frac{dC_m}{dt} \cdot \frac{(1 + E) - C_m}{(1 + E)^2} \cdot \frac{dE}{dt}
\]

where \(V_d\) is the effective volume of distribution, E is the plasma isotopic enrichment, \(C_m\) is the measured plasma concentration of the tracer, 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. However, our interpretation of the data presented herein is not affected by the assumed value for \(V_d\) because the difference in 

\(R_a\) did not exceed 10% when values were calculated using values for \(V_d\) ranging from 40 to 210 ml/kg.

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

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

The glucose kinetics were calculated between two different blood-collection time points (t\(_1\) and t\(_2\)). However, these data are presented as t\(_2\). For example, the \(R_a\) at t = 20 min during exercise is actually the \(R_a\) between 10 and 20 min.

Rates of energy expenditure and whole body substrate oxidation. Total energy expenditure (TEE) and absolute rates of CHO\(_{ox}\) and lipid oxidation were calculated by using the following equations (12)

\[
\text{TEE (kcal/min)} = 3.9 \cdot \frac{V_{CO_2}}{RER} - 1.11 \cdot \frac{V_{O_2}}{2}
\]

\[
\text{CHO}_{ox} (\text{g/min}) = 4.585 \cdot \frac{V_{CO_2}}{2} - 3.2255 \cdot \frac{V_{O_2}}{2}
\]

Lipid oxidation (g/min) = 1.7012 \(\frac{V_{O_2}}{2} - 1.6946 \cdot \frac{V_{CO_2}}{2}\)

where \(V_{O_2}\) and \(V_{CO_2}\) are in liters per minute and it was assumed that protein oxidation made negligible contribution to \(V_{O_2}\) and \(V_{CO_2}\). The calculated values were based on respiratory gas exchange values averaged over 5-min intervals for the first 10 min of exercise and averaged over 10-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 by the molecular weight of palmitate (259) and the horse’s body weight.

Studies in dogs (62) and in humans (35) have demonstrated that increases in glucose uptake by working muscle account for most of the increment in whole body \(R_a\) during exercise. Furthermore, ~90–95% of glucose \(R_a\) is oxidized during exercise (33). Thus glucose \(R_a\) provides a reasonable approximation of blood glucose oxidation. Muscle glycogen (plus lactate) oxidation was calculated as the difference between total \(CHO_{ox}\) and glucose \(R_a\). 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 \(R_a\)), other carbohydrate sources (muscle glycogen and lactate), and lipid to TEE during the 30–60 min period of exercise were estimated by using standard caloric equivalents.

Statistical analyses. Unless otherwise stated, data are presented as means ± SE. The data were analyzed as a three-way crossover design by use of a two-way repeated-measures ANOVA [repeated measures on treatment (i.e., F, G, or GE) and time factors]. As the data for muscle glycogen did not exhibit homogeneous variances, these data were subject to logarithmic transformation before ANOVA. Data for net muscle glycogen utilization and the absolute and relative contributions by different substrates to TEE during the 30–60 min period of exercise were analyzed by a one-way repeated-measures ANOVA (repeated measures on the treatment factor). Percent data (relative contributions by different substrates) were subject to arcsine transformation before ANOVA. The null hypothesis was rejected at p = 0.05 for the main effects (treatment and time) and p = 0.10 for the interaction. Significant differences identified by ANOVA were isolated using the Student-Newman-Keuls post hoc test. The Sigmastat 2.0 software package (Jandel Scientific, San Rafael, CA) was used for statistical computations.

RESULTS

Individual values for \(V_{O_2 max}\), ranged from 127 to 168 ml·kg\(^{-1} \cdot \text{min}^{-1}\) (mean 141 ± 6.8 ml·kg\(^{-1} \cdot \text{min}^{-1}\)). Mean running speed during the exercise protocol was 5.5 ± 0.2 m/s (range 5.2–6.0 m/s). The mean \(V_{O_2}\) measured during the 60 min of exercise corresponded to a relative workload of 55 ± 1.0% of \(V_{O_2 max}\) (range 53–57%). \(T_{re}\) was not different throughout exercise in F compared with G. However, \(T_{re}\) was significantly lower in GE than in G and in F between 30 and 60 min of exercise (Table 1). Preexercise body weight was similar among the three trials (F: 493 ± 15; G: 495 ± 17; GE: 496 ± 15 kg). Mean values for loss of body weight during exercise were higher in GE (18.8 ± 1.2 kg) than in F (15.5 ± 1.2 kg) and G (15.3 ± 1.3 kg). However, this difference did

\[
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not reach statistical significance (P = 0.15; power at α = 0.05:0.21).

Plasma glucose concentration. At rest and before administration of glucose or water, plasma glucose concentrations were similar among the three trials (Fig. 1). During the 60-min period after administration of the oral glucose load, plasma glucose concentration increased during both G and GE (8.2 ± 0.4 and 8.3 ± 0.3 mM, respectively; P < 0.05 vs. F) but remained unchanged in F (5.1 ± 0.1 mM). During exercise in F, plasma glucose concentration increased steadily to reach a peak of 10.3 ± 0.5 mM. In G, plasma glucose decreased from preexercise values during the first 20 min of exercise but subsequently increased, and, between 20 and 60 min of exercise, plasma glucose concentration was similar in F and G. During GE, epinephrine infusion resulted in marked hyperglycemia during exercise, with peak values of 17.2 ± 0.3 mM. Plasma glucose concentrations were higher (P < 0.05) throughout exercise in GE than in F. Similarly, plasma glucose was higher (P < 0.05) in GE than in G between 20 and 60 min of exercise (Fig. 1).

Serum insulin. As expected, oral glucose administration in G and in GE resulted in a substantial increase (time × treatment, P < 0.0001) in serum IRI (Fig. 2). During exercise, serum IRI decreased in all trials but remained elevated (P < 0.05) in the G trial compared with F and GE for the first 30 min of exercise. No differences in serum IRI concentration existed among trials between 40 and 60 min of exercise.

Urine glucose. Five horses in the G trial urinated during the early postexercise period, whereas urine

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Table 1. Hematocrit, plasma total protein and lactate concentrations, and rectal temperature before and during 60 min of exercise at 55% of VO₂max.

Fig. 1. Plasma glucose concentration during rest and 60 min of exercise at 55% of maximum oxygen uptake (VO₂max) when fasted (F), administered glucose 60 min before exercise (G), or administered glucose 60 min before exercise and infused with epinephrine during exercise (GE). Values are means ± SE; n = 6. *Mean glucose concentration during G and GE significantly greater than during F trial, P < 0.05. #Mean plasma glucose concentration during GE significantly greater than during F and G, P < 0.05.

Fig. 2. Serum immunoreactive insulin (IRI) concentration during rest and 60 min of exercise at 55% of VO₂max. Values are means ± SE; n = 6. *Mean IRI concentration during G and GE significantly greater than during F trial, P < 0.05. #Mean IRI concentration during G significantly greater than during F and GE, P < 0.05.
was obtained from all six horses in the GE trial. On average, urine was collected 15–20 min after exercise. The mean volume of urine collected in G and GE was $1.9 \pm 0.1$ and $2.1 \pm 0.2$ liters, respectively. Urine glucose concentration was significantly higher in GE ($47.5 \pm 14.5$ mM; range 9.5–121.4 mM) than in G (mean 5.7 ± 2.6 mM; range 2.1–8.5 mM).

Plasma catecholamines. Preexercise epinephrine and norepinephrine concentrations did not differ among trials (Fig. 3, A and B). Epinephrine infusion (GE) resulted in plasma concentrations three- to fourfold higher ($P < 0.0001$) than those in F and G (Fig. 3A). On the other hand, plasma norepinephrine concentrations were lower ($P < 0.001$) in GE than in F and G throughout exercise (Fig. 3B). After 60 min of exercise, plasma epinephrine was significantly lower in G than in F (Fig. 3A).

Hematocrit, plasma total protein, NEFA, glycerol, and lactate. Administration of glucose or water was not associated with changes in hematocrit and plasma total protein concentration ($t = -75$ min vs. $t = 0$ min in Table 1). In all trials, hematocrit and plasma total protein increased significantly early during exercise, but no differences were detected among trials.

Resting plasma NEFA concentrations were between 0.45 and 0.68 mM (Fig. 4B). Glucose administration (G and GE) resulted in significant decreases in plasma NEFA. During the F trial, plasma NEFA concentration decreased during the first 5 min of exercise and thereafter gradually increased to reach a peak of $0.97 \pm 0.10$ mM after 60 min of exercise (Fig. 4B). Plasma NEFA concentrations also increased gradually during exercise in G. However, plasma NEFA was significantly lower in G than in F throughout exercise. In contrast, epinephrine infusion (GE) resulted in plasma NEFA concentrations that were similar to those measured in F, but significantly higher compared with G. In all trials, plasma glycerol concentrations increased during exercise (Fig. 4A). However, throughout exercise plasma glycerol was higher ($P < 0.05$) in GE than in G and F. Plasma glycerol concentrations were lower ($P < 0.05$) in G than in F between 20 and 60 min of exercise.

Epinephrine infusion greatly intensified ($P < 0.0001$, time × treatment effect) the increase in plasma lactate concentration early in exercise (Table 1). In the G and F trials, plasma lactate concentrations rose gradually, reaching a peak at the end of exercise of $7.0 \pm 0.9$ and $6.1 \pm 0.7$ mM, respectively. In GE, however, plasma lactate increased rapidly from $0.7 \pm 0.1$ mM at rest to $6.4 \pm 0.7$ mM after 10 min of exercise, with values of $\sim 8–9$ mM for the remainder of exercise. Plasma lactate
concentration was significantly higher in GE than in F and G between 5 and 60 min of exercise.

Gas exchange and whole body CHO oxidation and fat oxidation. There was a small but significant (P < 0.001) increase in VO$_2$ during exercise, but VO$_2$ was similar among the three trials (Table 2). There was a significant (P < 0.0001) treatment × time interaction for RER. In GE, RER was greater than 1.0 for the first 15 min of exercise and was significantly higher compared with G and F during this time period. RER was higher (P < 0.05) in GE than in F for the remainder of exercise. RER was also higher in G than in F at most time points during the exercise trial. However, RER was not different between 20 and 60 min of exercise in GE compared with G (Table 2).

Whole body rates of CHO oxidation and fat oxidation are presented in Table 2. As RER was greater than 1.0 during the first 15 min of exercise in GE, rates of whole body substrate oxidation rates were restricted to the 20- to 60-min period. Total CHO oxidation decreased during exercise in F but remained stable in G. Total CHO oxidation was higher in G and GE than in F between 20 and 60 min of exercise (Tables 2 and 3). Conversely, fat oxidation was suppressed (P < 0.05, all time points) in G and GE compared with F. After 60 min of exercise, fat oxidation rates were 48 ± 24, 32 ± 24, and 29 ± 4 µmol·kg$^{-1}$·min$^{-1}$ for F, G, and GE, respectively (Table 2).

Glucose kinetics. Before oral administration of glucose, plasma isotopic enrichment (Fig. 5), glucose Ra (Fig. 6A), and glucose Rd (Fig. 6B) were similar among the three trials. In F, an isotopic steady state was maintained throughout the preexercise period, but isotopic enrichment decreased during exercise despite the threefold increase in tracer infusion rate. During both G and GE, isotopic enrichment decreased after oral glucose administration and was lower (P < 0.05) throughout exercise compared with that in F (Fig. 5).

At rest after oral glucose administration, the mean glucose Ra and Rd during both G and GE were two- to threefold greater than during F (P < 0.05, Fig. 6, A and B). Throughout exercise, the glucose Ra during G remained ~70–80% higher (P < 0.05) than during F. Whereas glucose Ra reached a plateau of ~42–44 µmol·kg$^{-1}$·min$^{-1}$ between 30 and 60 min of exercise in F, the Ra increased throughout exercise in G to a peak of 76.4 ± 2.1 µmol·kg$^{-1}$·min$^{-1}$ at 60 min. Glucose Ra during GE was intermediate to that during G (P < 0.05 between 20 and 60 min) and F (P < 0.05 between 30 and 50 min; Fig. 6A).

During exercise, there was a significant time × trial interaction (P < 0.0001) for glucose Rd. Whereas glucose Rd increased with the onset of exercise in all trials, the increase was much larger in G (Fig. 6B). In F, glucose Rd increased gradually and reached a plateau of ~32–34 µmol·kg$^{-1}$·min$^{-1}$ during the last 20 min of exercise. After the early sharp increase, glucose Rd decreased between 10 and 30 min of exercise in G but thereafter remained steady at ~55 µmol·kg$^{-1}$·min$^{-1}$ (P < 0.05 vs. F at all time points during exercise). Epinephrine infusion almost completely inhibited the augmentation in glucose Rd associated with preexercise glucose administration. With the exception of the 5-min time point, glucose Rd in GE was not different from that in F. Glucose MCR demonstrated a similar pattern (Fig. 7). MCR was approximately twofold higher (P < 0.05) in G than in F and GE throughout exercise, whereas there were no differences between F and GE for MCR.

Muscle glycogen and lactate. Preexercise muscle glycogen concentration was similar among the three trials. However, postexercise muscle glycogen concentration was lower (P < 0.04) in GE than in F and G (Fig. 8A). Therefore, net muscle glycogen utilization was higher (P < 0.03) in GE (349 ± 44 mmol/kg dry muscle) compared with F (218 ± 28 mmol/kg dry muscle) and G.

### Table 2. Oxygen uptake, respiratory exchange ratio, and calculated rates of carbohydrate and fat oxidation during 60 min of exercise at 55 ± 1% of VO$_2$max

<table>
<thead>
<tr>
<th>Time, min</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_2$, ml·kg$^{-1}$·min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>68 ± 2</td>
<td>70 ± 2</td>
<td>71 ± 2</td>
<td>73 ± 3</td>
<td>74 ± 2</td>
<td>75 ± 2</td>
<td>75 ± 1</td>
</tr>
<tr>
<td>G</td>
<td>68 ± 3</td>
<td>70 ± 2</td>
<td>71 ± 2</td>
<td>73 ± 2</td>
<td>74 ± 3</td>
<td>75 ± 3</td>
<td>76 ± 3</td>
</tr>
<tr>
<td>GE</td>
<td>69 ± 5</td>
<td>71 ± 3</td>
<td>72 ± 3</td>
<td>74 ± 4</td>
<td>76 ± 4</td>
<td>77 ± 3</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>F</td>
<td>0.95 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.92 ± 0.01</td>
<td>0.91 ± 0.01</td>
<td>0.91 ± 0.01</td>
<td>0.89 ± 0.01</td>
</tr>
<tr>
<td>G</td>
<td>0.97 ± 0.01</td>
<td>0.98 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.93 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>GE</td>
<td>1.04 ± 0.01</td>
<td>1.03 ± 0.01</td>
<td>0.99 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.96 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>CHO oxidation, µmol·kg$^{-1}$·min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>405 ± 14</td>
<td>421 ± 14</td>
<td>413 ± 18</td>
<td>394 ± 21</td>
<td>390 ± 17</td>
<td>370 ± 15</td>
<td>357 ± 18</td>
</tr>
<tr>
<td>G</td>
<td>431 ± 18</td>
<td>469 ± 14</td>
<td>470 ± 17</td>
<td>445 ± 18</td>
<td>451 ± 19</td>
<td>435 ± 21</td>
<td>425 ± 23</td>
</tr>
<tr>
<td>Fat oxidation, µmol·kg$^{-1}$·min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>22 ± 3</td>
<td>21 ± 4</td>
<td>30 ± 2</td>
<td>36 ± 3</td>
<td>39 ± 3</td>
<td>42 ± 3</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>G</td>
<td>17 ± 3</td>
<td>12 ± 2</td>
<td>13 ± 2</td>
<td>20 ± 2</td>
<td>24 ± 3</td>
<td>29 ± 3</td>
<td>34 ± 2</td>
</tr>
<tr>
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<td>29 ± 4</td>
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<td>29 ± 4</td>
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</tr>
</tbody>
</table>

Values are means ± SE for 6 horses. VO$_2$, oxygen uptake; RER, respiratory exchange ratio; CHO, carbohydrate. Rates of carbohydrate and fat oxidation were calculated from respiratory gas exchange data, averaged over the preceding time interval. *P < 0.05, GE vs. F; †P < 0.05, G vs. F; ‡P < 0.05, GE vs. G.
(201 ± 35 mmol/kg dry muscle). There was no difference in net muscle glycogen utilization in F compared with G. Similarly, the exercise-associated increase in muscle lactate concentration was similar in the F and G trials (Fig. 8B). However, end-exercise muscle lactate was higher (P < 0.05) in GE than in F and G.

The calculated rates of muscle glycogen oxidation (total CHOox - glucose Rd) during the final 30 min of exercise corroborated the muscle biopsy data (Table 3). Muscle glycogen oxidation was significantly greater in
Table 3. Total carbohydrate oxidation, rate of glucose disappearance, and muscle glycogen (and lactate) oxidation during exercise at 55 ± 1% of \( \dot{V}O_{2\text{max}} \)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Total CHO Oxidation, ( \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} )</th>
<th>Glucose Rate of Disappearance, ( \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} )</th>
<th>Glycogen Oxidation, ( \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>390 ± 17</td>
<td>32 ± 2</td>
<td>359 ± 21</td>
</tr>
<tr>
<td>40-50</td>
<td>370 ± 15</td>
<td>31 ± 2</td>
<td>342 ± 18</td>
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<tr>
<td>50-60</td>
<td>357 ± 18</td>
<td>33 ± 3</td>
<td>325 ± 17</td>
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<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>451 ± 19†</td>
<td>54 ± 2†</td>
<td>398 ± 23</td>
</tr>
<tr>
<td>40-50</td>
<td>435 ± 21†</td>
<td>54 ± 3†</td>
<td>382 ± 20</td>
</tr>
<tr>
<td>50-60</td>
<td>425 ± 23†</td>
<td>58 ± 3†</td>
<td>365 ± 22†</td>
</tr>
<tr>
<td>Glucose + Epinephrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40</td>
<td>470 ± 27*</td>
<td>30 ± 3*</td>
<td>438 ± 25*†</td>
</tr>
<tr>
<td>40-50</td>
<td>453 ± 23*</td>
<td>29 ± 4*</td>
<td>423 ± 25*‡</td>
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<tr>
<td>50-60</td>
<td>427 ± 21*</td>
<td>34 ± 3*</td>
<td>396 ± 21*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 horses. Muscle glycogen (plus lactate) oxidation was estimated from the difference between total carbohydrate oxidation and rate of glucose disappearance. † \( P < 0.05 \), G vs. F; * \( P < 0.05 \), GE vs. F; ‡ \( P < 0.05 \), G vs. F; * † \( P < 0.05 \), GE vs. F.

GE than in F (all time points) and G (30- to 40-min and 40- to 50-min periods).

Overall pattern of substrate utilization. Figure 9 depicts the estimated relative caloric contribution from fat, muscle glycogen (plus lactate), and blood glucose during the final 30-min period of exercise. The total rate of energy expenditure was similar among the three trials (342 ± 18, 346 ± 21, and 350 ± 22 cal·kg\(^{-1} \)·min\(^{-1} \) for F, G, and GE, respectively). In F, the relative energy expenditure from fat, blood glucose, and muscle glycogen was 32 ± 2, 5.7 ± 0.5, and 62.3 ± 2%, respectively (Fig. 9). The contribution by fat to total energy was ~33% lower in G (22.5 ± 2%) and GE (20 ± 3%) compared with F. Thus the contribution by all sources of carbohydrate (blood glucose, muscle glycogen, lactate) to energy use was significantly higher in G (77 ± 3%) and GE (80 ± 2%) compared with F (68 ± 2%). In GE there was a shift to greater use of muscle glycogen (74.6 ± 3%), whereas the contribution by blood glucose (5.5 ± 1%) was similar to that during F. In contrast, the relative contribution of blood glucose during G (12.5 ± 2%) was significantly greater than in F and GE, whereas the contribution from muscle glycogen (65 ± 2%) was lower (\( P < 0.05 \)) compared with GE but not different from F (Fig. 9).

**DISCUSSION**

The present study examined the interactive effects of increased blood glucose availability (preexercise glucose administration) and increased circulating epinephrine (preexercise glucose administration and infusion of epinephrine during exercise) on carbohydrate metabolism in horses during moderate-intensity exercise. The main findings were 1) a more than twofold increase in whole body glucose uptake during exercise after oral glucose administration compared with exercise after a 24-h fast; 2) almost complete inhibition of the increase in glucose \( R_d \) associated with preexercise glucose administration produced by an infusion of epinephrine; 3) an increase in net intramuscular glycogen use and lactate accumulation when epinephrine was infused during exercise; and 4) augmentation of \( \dot{V}O_{2\text{max}} \) in both trials preceded by oral administration of glucose.

**Glucose kinetics.** As expected, preexercise administration of glucose resulted in marked hyperglycemia (Fig. 1) and hyperinsulinemia (Fig. 2). The increase in plasma glucose concentration was accompanied by increments in glucose \( R_a \) and \( R_d \). Similarly, during exercise, glucose \( R_a \) was significantly higher in the trials preceded by glucose administration compared with the fasting condition. In humans, ingestion of glucose 30–50 min before exercise results in an increase in glucose \( R_a \) during exercise compared with placebo trials (1, 44). However, preexercise glucose ingestion markedly suppresses hepatic glucose production during exercise, and the increase in glucose \( R_a \) reflects ongoing intestinal uptake of glucose (44). In the present study, we did not measure the contribution of gut-derived \( R_a \) (intestinal uptake) to the total glucose \( R_a \). Nonetheless, it is probable that continued uptake of glucose from the intestinal tract contributed to the higher glucose \( R_a \) in the trials preceded by oral glucose administration. However, systemic glucose availability during exercise was lower in GE than in G (Fig. 6A). The reason for this difference cannot be determined from our data.

Consistent with findings in humans (44), the hyperglycemia and hyperinsulinemia associated with preexercise glucose administration was accompanied by a large increase in glucose \( R_d \) early in exercise (G trial; Fig. 6B). As the increment in glucose \( R_d \) in G was not matched by a similar increase in glucose \( R_a \), plasma glucose concentration decreased during the first 20 min of exercise (Fig. 1). In marked contrast, epinephrine infusion (GE) inhibited the increase in glucose uptake.

**Fig. 9.** Relative caloric contributions from other carbohydrates (CHO) (muscle glycogen plus lactate), lipid, and blood glucose in 6 horses during the 30- to 60-min period of exercise. *Values significantly different between G and GE vs. F, \( P < 0.05 \). **Contribution from blood glucose greater in G than in F and GE, \( P < 0.05 \). #Contribution from other carbohydrates greater in GE than in F and G, \( P < 0.05 \).
associated with preexercise glucose administration, and plasma glucose concentrations increased progressively throughout exercise. Because values for glucose $R_d$ are derived from estimates of glucose $R_a$, part of the attenuation in glucose uptake during exercise can be attributed to the reduction in glucose $R_d$ compared with the G trial. However, even though glucose $R_d$ was $\sim 40\%$ higher in GE than in F between 30 and 60 min of exercise, glucose $R_d$ and MCR in GE were not different from F at most time points during exercise (Fig. 6B). Taken together, these results indicate that increases in circulating epinephrine exert a potent inhibitory effect on glucose disposal in horses during moderate-intensity exercise, even when glucose supply is increased by administration of glucose before exercise.

Several mechanisms may account for the effects of epinephrine infusion on glucose $R_d$ and MCR, including direct effects on glucose transport into muscle (18) and indirect effects on glucose uptake because of differences in circulating insulin (59) and NEFA concentrations (22) and alterations in the rate of intramuscular glycogenolysis (34). Plasma insulin was lower in GE than in F during the first 30 min of exercise, even though preexercise concentrations were similar (Fig. 2). The more rapid decrease in plasma insulin concentrations during exercise in GE likely reflected intensified $\beta$-adrenergic-mediated inhibition of insulin secretion associated with the increase in circulating epinephrine (14). Muscle contractions and insulin have been shown to have synergistic effects on glucose uptake by muscle in dogs (62) and humans (59) during exercise. Therefore, the higher plasma insulin concentration in G compared with F and GE may have contributed to the increase in glucose $R_d$ and MCR in the G trial. However, plasma insulin concentrations were similar among trials during the last 30 min of exercise, indicating that other mechanisms contributed to the between-trial differences in glucose disposal.

Lower plasma NEFA concentrations in G also may have contributed to the higher glucose uptake in this trial compared with GE and F. Preexercise glucose administration resulted in a significant decrease in plasma NEFA, and, although plasma NEFA increased during exercise in all trials, concentrations were significantly lower in G than in F and GE (Fig. 4B). In humans, preexercise carbohydrate ingestion inhibits lipolysis during low- to moderate-intensity exercise and thereby suppresses the increase in plasma NEFA concentration (8, 25). Similarly, in horses, preexercise consumption of a carbohydrate meal (corn grain) attenuates the increase in fatty acid concentration during exercise (36, 58). On the other hand, $\beta$-adrenergic mechanisms stimulate lipolysis during exercise (2), probably accounting for the higher plasma NEFA in GE than in G throughout exercise (Fig. 4B). In dogs, suppression of circulating NEFA concentrations by infusion of nicotinic acid, an inhibitor of lipolysis, results in increased whole body glucose disposal during exercise (5). Conversely, increased plasma NEFA has been demonstrated to attenuate glucose uptake by muscle in humans during leg exercise (22), although other investigators have not found an effect of increased plasma NEFA on glucose uptake (54). Nonetheless, it is possible that the lower circulating NEFA during exercise in G compared with GE and F may, in part, explain the increase in glucose uptake during the G trial.

The lower glucose $R_d$ and MCR during exercise in GE also may have reflected more direct effects of epinephrine on glucose transport into muscle. In vitro studies have demonstrated that epinephrine inhibits glucose uptake in rat skeletal muscle (18, 31). The mechanism of this inhibition of glucose uptake into muscle by epinephrine has not been elucidated, although it is possible that $\beta$-adrenergic stimulation decreases the intrinsic activity of GLUT-4 in muscle, perhaps by phosphorylation of these transporter proteins. However, Lee et al. (39) showed that, although epinephrine causes phosphorylation of GLUT-4 in skeletal muscle, this had no effect on glucose transport. Alternatively, the lower glucose uptake may be related to the inhibitory effects of epinephrine on glucose phosphorylation in muscle. Studies of resting (31) and contracting (17) muscle have demonstrated accelerated glycogenolysis and accumulation of intracellular glucose and glucose-6-phosphate during epinephrine stimulation. Because glucose-6-phosphate is an inhibitor of hexokinase (34), this accumulation could reduce glucose phosphorylation, thereby reducing glucose uptake into muscle. We did not measure glucose-6-phosphate concentrations in muscle biopsies from the horses in this study. However, given the higher rate of muscle glycogenolysis in the GE trial, it is possible that accumulation of glucose-6-phosphate and inhibition of hexokinase contributed to the lower glucose uptake during exercise with epinephrine infusion.

The inhibitory effect of epinephrine on glucose disposal measured in the horses in the present study is consistent with data from dog and humans. Infusion of epinephrine (0.5 $\mu$g·kg$^{-1}$·min$^{-1}$) into running dogs decreased whole body glucose uptake and resulted in marked hyperglycemia (28). Furthermore, $\beta$-blockade with propranolol abolished the effects of epinephrine on glucose uptake, implicating $\beta$-adrenergic mechanisms in the effect of epinephrine on glucose disposal (28). Intra-arterial infusion of epinephrine (0.04 $\mu$g·kg$^{-1}$·min$^{-1}$) into one leg decreased its glucose uptake by $\sim 60\%$ compared with uptake of blood-borne glucose by the contralateral leg during two-legged exercise (30). More recently, Mora-Rodriguez and Coyle (46) demonstrated that an infusion of epinephrine that results in only modest increases in plasma epinephrine concentrations (control: 0.6 $\pm$ 0.1 nM; epinephrine infusion: 1.9 $\pm$ 0.2 nM) also suppresses glucose clearance in men during exercise at 25% of peak $V_{\text{O}_2}$.

Muscle glycogen utilization. A second objective of this study was to determine the effect of preexercise glucose administration, with and without epinephrine infusion during exercise, on intramuscular glycogen utilization. In humans, the hyperglycemia and hyperinsulinemia
resulting from glucose ingestion 30 to 60 min before exercise has been associated with an increase in muscle glycogen utilization in some (7, 21), but not all (11, 20), studies. The increase in muscle glycogen usage under these circumstances has been attributed to a reduction in the supply of free fatty acids in contracting skeletal muscle, thereby increasing the reliance on muscle glycogen as a fuel source (4). There is also conflicting evidence regarding the effect of preexercise carbohydrate ingestion on muscle glycogenolysis in horses during exercise. Lawrence and colleagues (37) reported that, compared with exercise after an overnight fast, glycogen utilization was higher when horses consumed 1 kg of corn grain (a high-carbohydrate meal) 1, 3, or 5 h before exercise. However, other studies from the same laboratory utilizing similar preexercise feeding regimens did not find an increase in intramuscular glycogenolysis when a carbohydrate meal was ingested before exercise (36, 38). Similarly, in the present study, we did not detect an effect of preexercise glucose administration (G trial) on net muscle glycogen degradation during exercise (Fig. 8A).

On the other hand, net muscle glycogen utilization was \( \sim 65\% \) higher when epinephrine was infused during exercise. There were no previous reports of the effects of epinephrine infusion on muscle metabolism in horses during exercise. However, an increase in circulating epinephrine has been demonstrated to increase skeletal muscle glycogenolysis in rats (53), dogs (28), and humans (9, 17, 30) during submaximal exercise. Epinephrine activates phosphorylase kinase in muscle, thereby promoting conversion of glycogen phosphorylase from the inactive \( \alpha \) form to the more active \( \beta \) form (19). After activation, there is progressive reversal of phosphorylase back to the \( \beta \) form, and epinephrine is thought to be less important for stimulation of glycogenolysis with increasing duration of exercise. Given the markedly higher values for RER in GE compared with F and G during the first 20 min of exercise (Table 2), it is possible that the effect of epinephrine on muscle glycogenolysis was greatest early in exercise. However, the estimates of muscle glycogen oxidation, calculated from the difference between whole body CHO\(_{\text{ox}}\) and glucose R\(_{\text{d}}\), also indicated higher glycogen usage in GE than in F and G during the last 30 min of the exercise protocol (Table 3).

The exercise-associated increase in muscle lactate concentration was also enhanced by epinephrine infusion (Fig. 8B). Furthermore, plasma lactate concentration was two- to fourfold higher in GE than in F and G, particularly early in exercise (Table 1). These findings suggest that lactic acid production increased when epinephrine was infused during exercise, and they provide further evidence of the stimulatory effect of epinephrine on muscle glycogenolysis and glycolysis. Our findings are in agreement with studies in humans showing that epinephrine infusion increases lactate accumulation in muscle during submaximal exercise (9) and results in greater lactate release from working tissues (30). Furthermore, blood lactate concentrations in humans during exercise are higher with epinephrine infusion compared with control trials (9, 60).

Overall pattern of substrate utilization. The results of the present study indicate that preexercise glucose administration in horses results in increased CHO\(_{\text{ox}}\) during exercise (Table 2 and Fig. 9). During the final 30 min of exercise, CHO\(_{\text{ox}}\) was \( \sim 15\% \) higher in G than in F (Table 3), and, overall, the contribution by carbohydrate to TEE was \( \sim 10\% \) higher in G. During the same time period, rates of fat oxidation were \( \sim 30\% \) lower in G than in F (Table 2). Previous studies in humans during moderate-intensity exercise also have demonstrated increased CHO\(_{\text{ox}}\) with a concomitant reduction in fat oxidation, when carbohydrate (glucose or fructose) is ingested before exercise (8, 25). In humans, the reduction in fat oxidation is due to inhibition of lipolysis (25) and an increase in CHO\(_{\text{ox}}\) in muscle (8). Horowitz et al. (25) reported that a 10–30 µU/ml increase in plasma insulin concentration before exercise completely abolished the exercise-associated increase in lipolysis. Furthermore, this low rate of lipolysis limited the availability of free fatty acids during exercise (25). Similarly, in the present study it is possible that the higher insulin concentrations after glucose administration reduced whole body lipolysis, evidenced by the lower plasma NEFA and glycerol concentrations during exercise in G (Fig. 4).

Fat oxidation was also lower in GE compared with F (Table 2). However, this decrease in fat utilization did not appear to be due to reduced lipolysis and free fatty acid availability as plasma concentrations of NEFA and glycerol were similar in the GE and F trials (Fig. 4). On the other hand, the epinephrine-induced increases in glycogenolysis and glycolysis may have reduced fat oxidation in muscle. In humans, it has recently been reported that an increase in glycolytic flux associated with elevated carbohydrate availability directly reduced muscle fatty acid oxidation by limiting entry of long-chain fatty acids into mitochondria (8). Under these circumstances, it is proposed that there is an increase in the concentration of malonyl-CoA in muscle, with inhibition of carnitine palmitoyltransferase I, the enzyme that regulates the entry of long-chain fatty acids into mitochondria (8, 26).

The augmentation in CHO\(_{\text{ox}}\) in the G trial was explained by an increased utilization of blood-borne glucose, as muscle glycogen utilization did not differ between G and F (Fig. 8A). The relative contribution of blood glucose to TEE was more than twofold higher in G than in F (Fig. 9). Interestingly, plasma glucose concentration was similar in these two trials between 20 and 60 min of exercise, indicating that factors other than glucose availability were most important for this increase in glucose uptake. These factors could include insulin-mediated GLUT-4 translocation, lower circulating epinephrine and NEFA concentrations, and activation of oxidative enzymes within contracting muscle (32). Our estimates of the relative contribution of blood glucose to energy expenditure did not account for the loss of glucose in urine. However, in the G trial,
estimated urinary glucose excretion represented <0.5% of the total glucose $R_d$. On the other hand, the marked hyperglycemia during exercise in GE resulted in substantial loss (range: ~20–115 mM) of glucose in urine. If it is assumed that most of this glucose was excreted during exercise, up to 4–5% of the total glucose $R_d$ in GE was due to renal excretion. Therefore, the actual contribution of blood glucose to energy use was probably lower in GE than in F, further emphasizing the potent inhibitory effects of epinephrine on glucose uptake.

Another finding of the present study was the significant attenuation of the exercise-induced increase in $T_e$ when epinephrine was infused. Between 30 and 60 min of exercise, $T_e$ was ~0.7–1.0°C lower in GE than in F and G (Table 1). Furthermore, mean whole body fluid loss was higher in GE compared with the other trials, although this difference did not reach statistical significance. It is likely that this effect of epinephrine on $T_e$ was due to an increase in evaporative heat loss during exercise. In horses at rest, epinephrine infusion induces a marked sweating response, an effect mediated by β-adrenoceptors (55). Conversely, β-adrenergic blockade reduces sweating in horses during high-intensity exercise (50) and augments the increase in core temperature in ponies during submaximal exercise (55). This effect of epinephrine in horses is in marked contrast to findings in humans. Mora-Rodriguez et al. (47) have demonstrated that an infusion of epinephrine that elevated plasma concentrations approximately sixfold compared with the control condition resulted in an ~0.4°C increase in esophageal temperature. This impairment to thermoregulation with epinephrine infusion was due to cutaneous vasoconstriction, evidenced by significant reductions in forearm and skin vascular conductance (47).

The lower plasma norepinephrine during exercise in GE (Fig. 3B) was indicative of decreased sympathetic adrenergic outflow, given that plasma norepinephrine is mainly derived from the spillover of norepinephrine by sympathetic nerves (14). Given the high circulating epinephrine in GE (Fig. 3B), one possible explanation for the attenuated plasma norepinephrine response is stimulation of α2-adrenoceptors in presynaptic terminals, with a reduction in norepinephrine release and spillover into circulation. Alternatively, the marked hyperglycemia in GE (Fig. 1) may have contributed to the diminished plasma norepinephrine concentrations. In humans, sustained hyperglycemia (by glucose infusion) attenuates the plasma norepinephrine response to exercise (43, 47). The mechanism of this response has not been elucidated.

In summary, this study has demonstrated that preexercise administration of glucose augments CHO uptake and utilization of blood-borne glucose in horses during moderate-intensity exercise but does not alter muscle glycogen usage. Conversely, an infusion of epinephrine during exercise almost completely inhibits the increase in glucose $R_d$ associated with preexercise glucose administration and increases reliance on muscle glycogen for energy transduction. These findings indicate that epinephrine is a potent inhibitor of peripheral glucose uptake, even under conditions of increased glucose supply. A direct inhibition of glucose transport into muscle and/or attenuation of glucose phosphorylation in muscle probably contributed to the lower glucose uptake with epinephrine infusion.

We especially acknowledge Dr. Karel Pacak at the National Institutes of Health for performing the catecholamine analyses. The technical assistance of Hua Shen at the University of Guelph and by Leia Hill, Candace Sack, and Steve Clinard is also gratefully acknowledged. Thanks are also due to the School of Physical Activity and Educational Services, College of Education, The Ohio State University, for assistance with analysis of blood and muscle samples. This study was supported by grants from the Equine Research Funds at The Ohio State University and Purina Mills, St. Louis, MO.

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