

β -Adrenergic blockade augments glucose utilization in horses during graded exercise

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Geor, Raymond J., Kenneth W. Hinchcliff, and Richard A. Sams. β -Adrenergic blockade augments glucose utilization in horses during graded exercise. *J Appl Physiol* 89: 1086–1098, 2000.—To examine the role of β -adrenergic mechanisms in the regulation of endogenous glucose (Glu) production [rate of appearance (R_a)] and utilization [rate of disappearance (R_d)] and carbohydrate (CHO) metabolism, six horses completed consecutive 30-min bouts of exercise at $\sim 30\%$ (Lo) and $\sim 60\%$ (Hi) of estimated maximum O_2 uptake with (P) and without (C) prior administration of the β -blocker propranolol (0.22 mg/kg iv). All horses completed exercise in C; exercise duration in P was 49.9 ± 1.2 (SE) min. Plasma Glu was unchanged in C during Lo but increased progressively in Hi. In P, plasma Glu rose steadily during Lo and Hi and was higher ($P < 0.05$) than in C throughout exercise. Plasma insulin declined during exercise in P but not in C; β -blockade attenuated ($P < 0.05$) the rise in plasma glucagon and free fatty acids and exaggerated the increases in epinephrine and norepinephrine. Glu R_a was 8.1 ± 0.8 and $8.4 \pm 1.0 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ at rest and 30.5 ± 3.6 and $42.8 \pm 4.1 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ at the end of Lo in C and P, respectively. During Hi, Glu R_a increased to 54.4 ± 4.4 and $73.8 \pm 4.7 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in C and P, respectively. Similarly, Glu R_d was $\sim 40\%$ higher in P than in C during Lo (27.3 ± 2.0 and $39.5 \pm 3.3 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in C and P, respectively) and Hi (37.4 ± 2.6 and $61.5 \pm 5.3 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in C and P, respectively). β -Blockade augmented CHO oxidation (CHO_{ox}) with a concomitant reduction in fat oxidation. Inasmuch as estimated muscle glycogen utilization was similar between trials, the increase in CHO_{ox} in P was due to increased use of plasma Glu. We conclude that β -blockade increases Glu R_a and R_d and CHO_{ox} in horses during exercise. The increase in Glu R_d under β -blockade suggests that β -adrenergic mechanisms restrain Glu R_d during exercise.

stable isotopes; carbohydrate oxidation; propranolol; insulin; glucagon; catecholamines

ALTHOUGH COMPLEX MECHANISMS regulate endogenous glucose production [rate of appearance (R_a)] and utilization [rate of disappearance (R_d)] during exercise, sympathoadrenergic mechanisms are thought to play an important role, particularly during heavy exertion (8, 32, 45, 53). However, the results of several recent investigations have suggested that sympathoadrenergic mechanisms do not play an important role in the

glucose R_a response, at least in humans and dogs. Attenuation of sympathetic nerve activity to the liver and adrenal medulla, by means of anesthesia of the celiac ganglion, did not affect glucose R_a in humans during exercise at $\sim 75\%$ of maximum O_2 uptake ($\dot{V}O_{2\text{max}}$) (30). Similarly, selective α - and β -adrenergic blockade of the liver did not alter the glucose R_a response during heavy exercise in dogs (11). Furthermore, physiological increases in plasma epinephrine (Epi) do not appear to play a major role in mediating the exercise-induced increase in glucose R_a (25, 26). These findings indicate that neither sympathetic liver nerve activity nor circulating Epi is a major stimulus for glucose R_a during exercise. On the other hand, adrenergic mechanisms may be important in the regulation of the exercise-induced increase in muscle glucose uptake. In humans, physiological increases in plasma Epi inhibit glucose clearance during exercise (26, 35). Furthermore, β -blockade (propranolol administration) augments glucose R_d during submaximal and maximal exercise (39, 46). Taken together, these findings support the hypothesis that β -adrenergic mechanisms regulate glucose uptake during exercise.

Horses have an extremely high capacity for aerobic metabolism, as reflected by mass-specific rates of $\dot{V}O_{2\text{max}}$ that are two- to threefold higher than those of human athletes (13). Therefore, for exercise at a given percentage of $\dot{V}O_{2\text{max}}$, metabolic rate and absolute energy requirements are two to three times higher in horses than in humans. Despite these observations, few studies have examined mechanisms for mobilization and utilization of fuel substrates in the horse during exercise. Similar to other species, hyperglycemia is a feature of moderate- and high-intensity exercise (42). In addition, β -blockade has been shown to abolish the increase in plasma glucose concentration associated with sprint exercise (48). However, inasmuch as there have been no reports of the effects of β -blockade on glucose turnover in the horse during exercise, it is not known whether the lower plasma glucose during exercise under β -blockade reflects reduced glucose R_a or augmented glucose R_d .

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In the present study, we used a graded exercise protocol to examine the effects of workload and β -adrenergic mechanisms on the kinetics of glucose R_a and R_d in horses. We hypothesized that, compared with low-intensity exercise, moderate-intensity work would result in a mismatch between glucose R_a and R_d ($R_a > R_d$), manifested as an increase in plasma glucose concentration. We further hypothesized that β -adrenergic mechanisms would underlie this restraint of glucose R_d , such that nonselective β -adrenergic blockade would mitigate the mismatch between glucose R_a and R_d and increase glucose R_d during higher-intensity exercise. Therefore, the specific objective of this study was to examine the effects of exercise intensity and β -adrenergic blockade on endogenous production and whole body uptake of glucose during consecutive 30-min bouts of exercise at ~ 30 and $\sim 60\%$ $\dot{V}O_{2\max}$ with and without prior administration of the β -blocker propranolol.

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 nonselective β -adrenergic blockade on glucose kinetics and whole body substrate utilization during graded exercise were examined in a balanced, randomized crossover study. Each of six horses was studied on two occasions during 60 min of graded exercise: the first 30-min exercise period was undertaken at $\sim 30\%$ $\dot{V}O_{2\max}$ and then the workload was increased to $\sim 60\%$ $\dot{V}O_{2\max}$. One trial was conducted under control conditions (C trial); in the other trial the exercise protocol was performed 15 min after administration of the $\beta_1 + \beta_2$ -adrenoceptor blocking agent propranolol (0.22 mg/kg iv, P trial). For all horses, there was a 1-wk interval between trials.

Horses. Six horses (3 Standardbred and 3 Thoroughbred, 4 geldings and 2 mares), 4–7 yr of age and 409–490 kg [452 ± 28 (SD) kg] body mass, were studied. All horses were housed indoors during the experimental period, fed a diet of timothy grass-alfalfa hay and mixed grain, and had access to a salt-mineral block. All horses were conditioned and undertaking regular treadmill exercise for ≥ 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, $\dot{V}O_{2\max}$ and the relationship between O_2 uptake ($\dot{V}O_2$) 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, and then 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. $\dot{V}O_2$ was measured every 10 s during the exercise test. $\dot{V}O_{2\max}$ was defined as the value at which $\dot{V}O_2$ reached a plateau, despite further increases in speed. A plateau was defined as a change in $\dot{V}O_2$ of $< 4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with an increase in speed. From linear regression analysis (with data from speeds below $\dot{V}O_{2\max}$), the running speed that elicited 30 and 60% $\dot{V}O_{2\max}$ was calculated for each horse.

The duration of β -adrenergic blockade resulting from administration of propranolol (0.22 mg/kg body wt iv) was

studied in two horses. DL-Propranolol hydrochloride (Sigma Chemical, St. Louis, MO) was prepared as a 10 mg/ml solution in sterile 0.9% saline. After measurement of resting heart rate (HR), a bolus of isoprenaline (1 $\mu\text{g/kg}$ body wt iv), a β -adrenoceptor agonist, was administered, and its effect on HR was determined. In both horses, HR increased from ~ 40 to 170–180 beats/min within 1 min of isoprenaline administration. The increase in HR was sustained for 5–7 min and was accompanied by signs of agitation and sweating. Propranolol was administered 15 min after the initial isoprenaline challenge, and the extent of β -adrenoceptor blockade was assessed by measurement of HR responses after bolus injections of isoprenaline (5, 30, and 60 min after propranolol administration). At 30 and 60 min after propranolol, there was a brief period (~ 10 s) of cardioacceleration ~ 30 s after administration of the isoprenaline. Sweating and signs of agitation were not evident. On the basis of these observations, the duration of β -adrenoceptor blockade resulting from administration of propranolol at 0.22 mg/kg body wt iv is ≥ 60 min. In accord with previous equine studies of the metabolic effects of β -blockade (36, 48), for the experimental studies this dose of propranolol was administered 15 min before exercise.

Experimental protocol. All experiments began between 0730 and 0800; food was withheld for 12 h before each experiment, and the horses had been 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 $\mu\text{mol/kg}$), continuous [0.22 ± 0.02 (SD) $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$] infusion of [6,6- ^2H]glucose (99% enriched; Cambridge Isotopes, Cambridge, MA) in 0.9% saline was then initiated using a calibrated infusion pump (model PHD 2000, Harvard Apparatus, South Natick, MA). During a 2-h equilibration period, horses stood in stocks. Fifteen minutes before commencement of the exercise protocol, propranolol (0.22 mg/kg) or an equivalent volume of 0.9% saline was administered intravenously. After collection of blood for final baseline hormone, substrate, and glucose kinetic determinations (see *Blood sample collection and analysis*), the horses were positioned on the treadmill (2° 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 (model BAT-10, Physitemp Instruments), was inserted 20–25 cm beyond the anal sphincter for measurement of temperature within the rectum (T_{re}) during exercise. The horses then began running at a speed calculated to elicit 30% $\dot{V}O_{2\max}$. The rate of [6,6- ^2H]glucose infusion was doubled at the onset of exercise ($0.44 \pm 0.03 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). After 30 min of exercise, the treadmill speed was increased to achieve a workload of 60% $\dot{V}O_{2\max}$. Exercise was continued for a further 30 min or until development of fatigue, as evidenced by an inability to keep pace with the treadmill, despite verbal encouragement. Isoprenaline (1 $\mu\text{g/kg}$ body wt iv bolus) was administered 10 min after completion of exercise to verify that β -adrenergic blockade had been maintained during the experiment. HR was recorded at 1-min intervals for 10 min after isoprenaline administration. 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; room temperature and

relative humidity during the experiments were $17.2 \pm 0.6^\circ\text{C}$ and $35 \pm 4\%$ (means \pm SE), respectively.

Respiratory gas exchange measurements. $\dot{V}\text{O}_2$, CO_2 production ($\dot{V}\text{CO}_2$), and respiratory exchange ratio (RER) were measured with an open-circuit calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH), as previously described (22). Flow through the system was $\sim 1,500$ l/min STP with the horse stationary and 9,000 l/min during running. The gas analyzers were calibrated before the start of each exercise test with 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 nitrogen dilution method (14). Discrepancy between simulated $\dot{V}\text{O}_2$ produced by nitrogen dilution and the value measured by the system was $\pm 3\%$ at nitrogen flow rates equivalent to a $\dot{V}\text{O}_2$ of 54 l/min (~ 140 ml \cdot kg $^{-1}\cdot$ min $^{-1}$ for a 385-kg horse). Standard equations were used to calculate $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$, and RER values were calculated by dividing $\dot{V}\text{CO}_2$ by $\dot{V}\text{O}_2$.

Rectal temperature. T_{re} was measured at rest before the start of exercise and at 5-min intervals during the exercise trial. The thermocouple had a response time of $\sim 1^\circ\text{C/s}$ and was calibrated in a heated water bath with a precision thermometer (Fisher Scientific, Mississauga, ON, Canada).

Blood sample collection and analysis. Blood samples for determination of plasma isotopic enrichment and glucose concentrations were obtained at -30 , -15 , 0 , 5 , 10 , 20 , 30 , 35 , 40 , 50 , and 60 min of exercise (where -30 , -15 , and 0 refer to samples taken 75 , 90 , and 105 min, respectively, after the start of tracer infusion and 0 min is the sample collected just before the onset of exercise) and placed in tubes containing EDTA and sodium fluoride-potassium oxalate. When the exercise trial was terminated because of fatigue, the final blood sample was obtained at the point of fatigue. Additional blood samples were obtained at -30 , -15 , 0 , 5 , 15 , 30 , 45 , and 60 min (or the point of fatigue) for subsequent measurement of hematocrit, plasma total protein, lactate, nonesterified fatty acid (NEFA), glycerol, glucagon, insulin, 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-aptinin [$10,000$ 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.

Plasma isotopic enrichment. Plasma $[6,6\text{-}^2\text{H}]$ glucose enrichment was determined as previously described (18). Briefly, the penta-acetate derivative of glucose was formed and then analyzed using electron impact gas chromatography-mass spectrometry (model 5889A, Hewlett-Packard, Palo Alto, CA). Molecular ions of 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 202 correspond to the unlabeled and labeled ions, respectively. The tracer-to-tracee ratio (TTR) was calculated directly from measured ion abundance ratios as follows: $\text{TTR} = R - R_0$, where R and R_0 represent the measured tracer-to-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 (59). The intra- and interassay coefficients of variation were 1.5 ± 0.5 and $5.6 \pm 2.1\%$, respectively.

Plasma biochemical analyses. Plasma glucose concentration was measured spectrophotometrically using the hexokinase reaction with a commercial kit (Glucose-HK kit; Sigma Chemical), and plasma lactate concentration was measured using an automated lactate oxidase method (Sport 1500 lactate analyzer, Yellow Springs Instruments, Yellow Springs, OH). Plasma NEFA concentration was determined using a commercial kit that employs an enzymatic colorimetric method (NEFA test kit, Wako Chemicals, 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 (Cambridge Instruments, Buffalo, NY). All samples were analyzed in duplicate.

Plasma hormone analyses. Plasma Epi and NE concentrations were determined by HPLC by use of electrochemical detection (33). Serum immunoreactive insulin (IRI) was determined in duplicate by use of a commercially available RIA (insulin kit, Coat-a-Count Diagnostics, Los Angeles, CA) that has been validated for horse blood (37). Intra- and interassay coefficients of variation were 6.0 ± 1.5 and $11.5 \pm 2.1\%$, respectively. Plasma immunoreactive glucagon (IRG) was determined in duplicate by use of a commercially available RIA (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 ($r = 0.987$). Accuracy was demonstrated by addition of porcine glucagon to equine plasma at 20 – 285 pmol/l. Linear regression of the recovery curve showed a correlation coefficient 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 RIA, analysis of experimental samples was completed in a single analytic session.

Calculations of glucose kinetics. Glucose R_a and R_d at rest were calculated using the steady-state tracer dilution equation (59)

$$R_a = R_d = F \cdot [(IE_i \cdot IE_p) - 1]$$

where F is the infusion rate of the isotope (in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), IE_i and IE_p are the stable isotopic enrichment of the infusate and plasma, respectively, and -1 accounts for the tracer's contribution to the turnover rate of the substrate (59). The rate of infusion was calculated by multiplying the infusion pump rate by the concentration of glucose in the infusate. During exercise, glucose R_a and R_d were calculated using the non-steady-state equation developed by Steele and modified for use with stable isotopes (50)

$$R_a = \frac{F - V_d \frac{C_m}{1 + E} \frac{dE}{dt}}{E}$$

and

$$R_d = R_a - V_d \frac{\frac{dC_m}{dt} (1 + E) - C_m \frac{dE}{dt}}{(1 + E)^2}$$

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. With use of 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. Glucose metabolic clearance rate (MCR) was calculated by dividing glucose R_d by the plasma glucose concentration. Glucose R_a was assumed to represent hepatic glucose production (HGP), although a small contribution from renal glycogenolysis and gluconeogenesis is possible.

Rates of energy expenditure and whole body substrate oxidation. Total energy expenditure (TEE) and absolute rates of carbohydrate (CHO) and lipid oxidation were calculated as follows (15)

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

$$\text{CHO}_{\text{ox}} \text{ (g/min)} = 4.585 \dot{V}\text{CO}_2 - 3.2255 \dot{V}\text{O}_2 \quad (2)$$

$$\text{Lipid oxidation (g/min)} = 1.7012 \dot{V}\text{O}_2 - 1.694 \dot{V}\text{CO}_2 \quad (3)$$

where $\dot{V}\text{O}_2$ is in liters per minute and it was assumed that protein oxidation made a negligible contribution to $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ (i.e., nonprotein RER). The calculated values were based on respiratory gas exchange values averaged over 5-min intervals. CHO oxidation (CHO_{ox}) in grams per minute was converted to micromoles per kilogram per minute by dividing the molecular weight of glucose (mol wt 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 (mol wt 259) and the horse's body weight. Muscle glycogen (plus lactate) oxidation was calculated as the difference between total CHO_{ox} and glucose R_d . Coggan et al. (9) reported that, in human subjects, ~90% of glucose R_d is oxidized during submaximal exercise. Therefore, glucose R_d provides a reasonable estimate of plasma glucose oxidation during exercise. Finally, the absolute and relative contributions by plasma glucose, other CHO sources (muscle glycogen and lactate), and lipid to total energy expenditure during the 20- to 30- and 35- to 45-min periods of exercise were estimated using standard caloric equivalents (4.2 kcal/g CHO, 9.0 kcal/g lipid).

Statistical analyses. Values are means \pm SE. The data for all dependent measures were analyzed using a two-way

ANOVA for repeated measures, with treatment (control vs. propranolol) and time as independent factors. Inasmuch as the data for Epi and NE did not exhibit homogeneous variances, these data were subject to logarithmic transformation before ANOVA. Percent data 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 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 $\dot{V}\text{O}_{2\text{max}}$ ranged from 123 to 159 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (mean $137.2 \pm 4.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Mean running speeds during the graded exercise protocol were 4.2 ± 0.1 and $7.3 \pm 0.2 \text{ m/s}$, which corresponded to relative workloads of 32.8 ± 1.0 and $58.8 \pm 1.5\% \dot{V}\text{O}_{2\text{max}}$. The relative workloads in the C and P trials were similar; i.e., β -blockade did not affect the $\dot{V}\text{O}_2$ -speed relationship. Duration of exercise differed between treatments. Whereas all horses completed the 60-min protocol in the C trial, none of the horses finished the P trial. Mean duration of exercise in the P trial was $49.9 \pm 1.2 \text{ min}$ (range 47–56 min). Despite the shorter duration of exercise in the P trial, end-exercise T_{re} was significantly greater in the P trial than in the C trial (Table 1). Postexercise isoprenaline administration resulted in a rapid and sustained cardio-acceleration in all horses in the C trial, whereas there was little change in HR after isoprenaline challenge in the P trial (Table 2). Subjectively, the horses appeared quieter and less motivated to run after propranolol administration.

Plasma glucose concentration and kinetics. Data for plasma glucose concentration, isotopic enrichment, and glucose R_a (HGP) and R_d are presented in Figs. 1 and 2. At rest, plasma glucose concentration was similar in the two experiments. In the C trial, plasma glucose concentration was not significantly changed from rest during the lower workload but increased progressively during exercise at the higher workload,

Table 1. Hematocrit, plasma total protein and lactate concentrations, and T_{re} during graded exercise under control conditions or after administration of propranolol

	0 min	5 min	15 min	30 min	35 min	45 min	60 min (or end)
Hematocrit, %							
C	32.3 ± 1.0	43.8 ± 1.2	43.9 ± 1.1	42.8 ± 1.2	47.5 ± 1.1	48.3 ± 1.1	48.5 ± 1.0
P	32.5 ± 0.8	45.5 ± 1.4	45.7 ± 1.3	45.7 ± 1.0	50.0 ± 1.4	51.0 ± 1.4	51.5 ± 1.6
TPP, g/l							
C	65.2 ± 2.3	68.2 ± 1.9	67.8 ± 2.2	66.5 ± 2.4	68.1 ± 2.4	69.2 ± 2.5	70.2 ± 2.7
P	64.8 ± 1.9	67.8 ± 2.4	67.0 ± 2.2	66.1 ± 1.9	67.8 ± 2.2	68.5 ± 2.1	69.3 ± 2.2
Lactate, mM							
C	0.82 ± 0.09	0.67 ± 0.07	0.54 ± 0.07	0.59 ± 0.08	1.75 ± 0.14	3.01 ± 0.35	5.04 ± 0.49
P	0.76 ± 0.04	0.65 ± 0.06	0.54 ± 0.04	0.75 ± 0.17	2.01 ± 0.18	3.52 ± 0.57	4.41 ± 0.32
$T_{\text{re}}, ^\circ\text{C}$							
C	37.3 ± 0.1	37.5 ± 0.1	38.4 ± 0.2	39.0 ± 0.2	39.9 ± 0.2	40.5 ± 0.2	41.2 ± 0.2
P	37.3 ± 0.2	37.8 ± 0.2	38.9 ± 0.3	$39.8 \pm 0.2^*$	$40.5 \pm 0.2^*$	$41.4 \pm 0.2^*$	$42.0 \pm 0.2^*$

Values are means \pm SE for 6 horses. Propranolol was administered at 0.22 mg/kg iv. C, control; P, propranolol; TPP, total plasma protein; T_{re} , rectal temperature. Values for P trial are at the end of exercise ($49.9 \pm 1.2 \text{ min}$) rather than at 60 min. *Significantly different from C, $P < 0.05$.

Table 2. Heart rate responses to intravenous isoprenaline challenge 10 min after exercise under control conditions and after administration of propranolol

	0 min	1 min	5 min	10 min
Heart rate, beats/min				
C	70 \pm 5	195 \pm 8*	108 \pm 8*	67 \pm 4
P	70 \pm 4	92 \pm 5	65 \pm 8	59 \pm 5

Values are means \pm SE for 6 horses. Horses were challenged with isoprenaline at 1.0 μ g/kg; propranolol was administered at 0.22 mg/kg iv. *Significantly greater than P, $P < 0.05$.

peaking at the end of exercise (Fig. 1A). In contrast, plasma glucose rose rapidly in the P trial and was significantly greater than in the C trial after 20 min of exercise. Despite the doubling of tracer infusion rate at the start of exercise, in both trials there was a progressive decrease in plasma isotopic enrichment (Fig. 1B). HGP and glucose R_d were similar at rest between trials (Fig. 2). In the C trial, HGP increased progressively

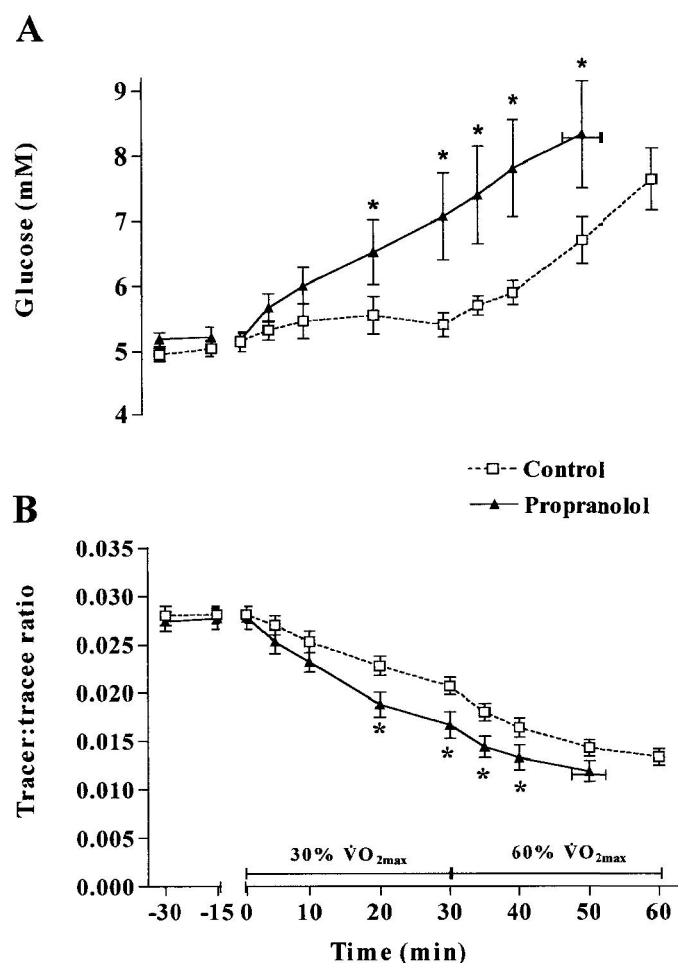


Fig. 1. Plasma glucose concentration (A) and isotopic enrichment (B) at rest and during exercise at 30 and 60% of maximum O_2 uptake ($\dot{V}O_{2max}$) in the control trial and after administration of propranolol (0.22 mg/kg, 15 min before exercise). Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from control, $P < 0.05$.

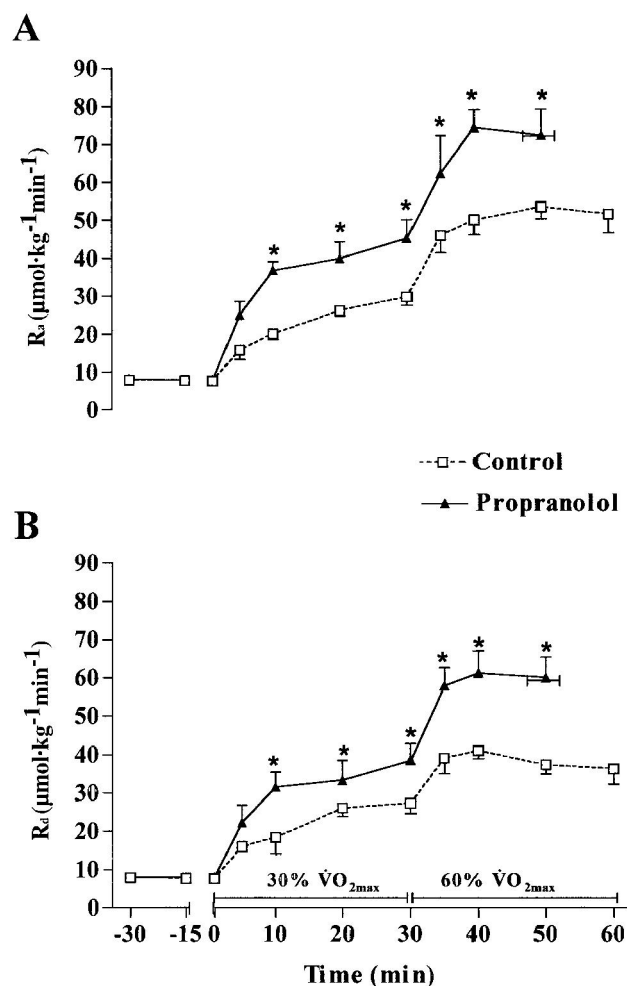


Fig. 2. Rate of plasma glucose appearance (R_a , A) and disappearance (R_d , B) at rest and during exercise at 30 and 60% $\dot{V}O_{2max}$ in the control trial and after administration of propranolol (0.22 mg/kg, 15 min before exercise). Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from control, $P < 0.05$.

during exercise at $\sim 30\%$ $\dot{V}O_{2max}$; at 30 min HGP was almost fourfold higher than preexercise values ($30.5 \pm 3.6 \mu$ mol·kg⁻¹·min⁻¹). The doubling of work intensity was accompanied by a proportional increase in HGP with peak values of $54.4 \pm 4.0 \mu$ mol·kg⁻¹·min⁻¹ at 50 min (Fig. 2A). Although the pattern of change in HGP was similar between trials, mean HGP was 40–50% higher in the P trial than in the C trial between 10 min and the end of exercise (Fig. 2A).

During exercise at 30% $\dot{V}O_{2max}$ in the C trial, the increase in HGP was matched by a quantitatively similar increase in glucose R_d (Fig. 2B). However, glucose R_d did not match HGP during the higher workload, thus accounting for the progressive increase in plasma glucose between 30 and 60 min. Notably, glucose R_d rose significantly more in the P trial than in the C trial with peak values (40 min of exercise) that were 50% higher than those observed in the C trial. However, HGP was consistently higher than glucose R_d during exercise in the P trial (Fig. 2), as evidenced by the almost linear increase in plasma glucose concen-

tration in this trial (Fig. 1A). MCR was similar at rest in the two trials (Fig. 3). During exercise, MCR was significantly greater in the P trial than in the C trial between 5 and 20 min of the lower workload and between 35 and 50 min of the higher workload. In the C trial, the transition to the higher workload was accompanied by a small initial increase in MCR, but thereafter MCR declined, such that mean values were not different from those during exercise at the lower workload (Fig. 3).

Plasma hormone concentrations. Basal serum IRI and plasma IRG were very similar in the C and P trials (Fig. 4). During exercise in the C trial, serum IRI did not change significantly, whereas there was a small but significant decrease in IRI during exercise in the P trial (Fig. 4A). Serum IRI was significantly higher in the C trial than in the P trial throughout exercise. Plasma IRG did not change significantly during exercise in the P trial. In contrast, in the C trial there was a progressive increase in IRG during exercise at 30% $\dot{V}O_{2\max}$, with a more substantial increase during exercise at the higher workload (Fig. 4B). Consequently, plasma IRG was significantly higher in the C trial than in the P trial at several time points during exercise. The glucagon-to-insulin molar ratio (G/I) increased during exercise in both trials (Fig. 5). However, as a consequence of the decline in serum IRI, G/I was higher ($P < 0.05$) in the P trial than in the C trial during exercise at 30% $\dot{V}O_{2\max}$.

Preexercise Epi and NE concentrations did not differ in the two trials (Fig. 6). Plasma catecholamine concentrations increased during exercise in both trials. However, the exercise-associated increases in plasma Epi ($P < 0.001$) and NE ($P < 0.005$) were significantly greater in the P trial than in the C trial. In the C trial,

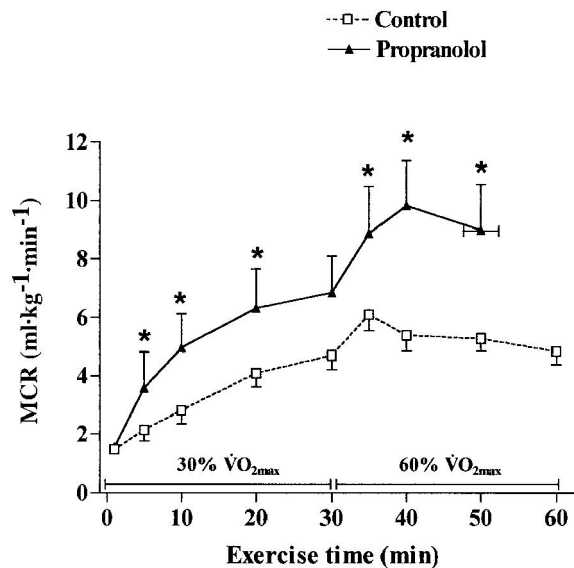


Fig. 3. Metabolic clearance rate of glucose (MCR) during exercise at 30 and 60% $\dot{V}O_{2\max}$ in the control trial and after administration of propranolol (0.22 mg/kg, 15 min before exercise). Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from control, $P < 0.05$.

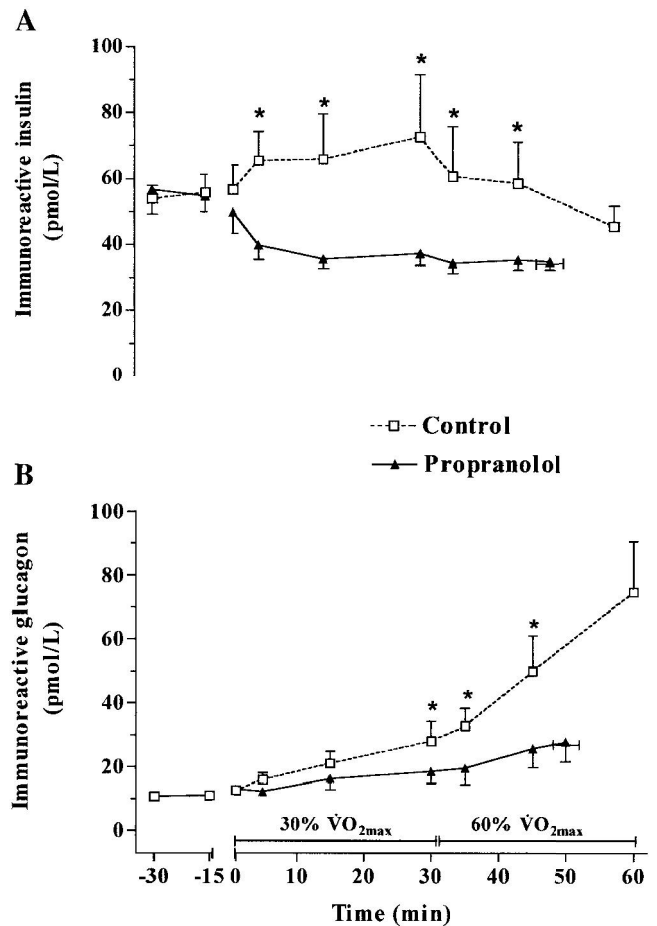


Fig. 4. Serum immunoreactive insulin (A) and plasma immunoreactive glucagon (B) at rest and during exercise at 30 and 60% $\dot{V}O_{2\max}$ in the control trial and after administration of propranolol (0.22 mg/kg, 15 min before exercise). Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from propranolol, $P < 0.05$.

plasma Epi rose from 0.98 ± 0.20 to 4.60 ± 1.1 nmol/l at 30 min of exercise, with a further increase to 13.80 ± 4.30 nmol/l at the end of exercise. Corresponding values for plasma Epi in the P trial were 1.10 ± 0.30 ,

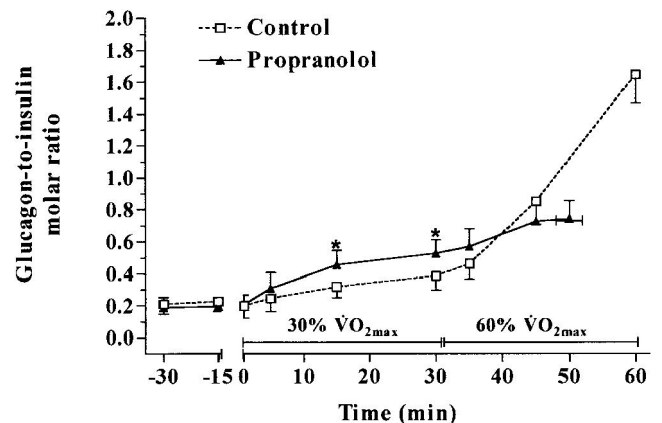


Fig. 5. Glucagon-to-insulin ratio during graded exercise in the control trial and after administration of propranolol. Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from control, $P < 0.05$.

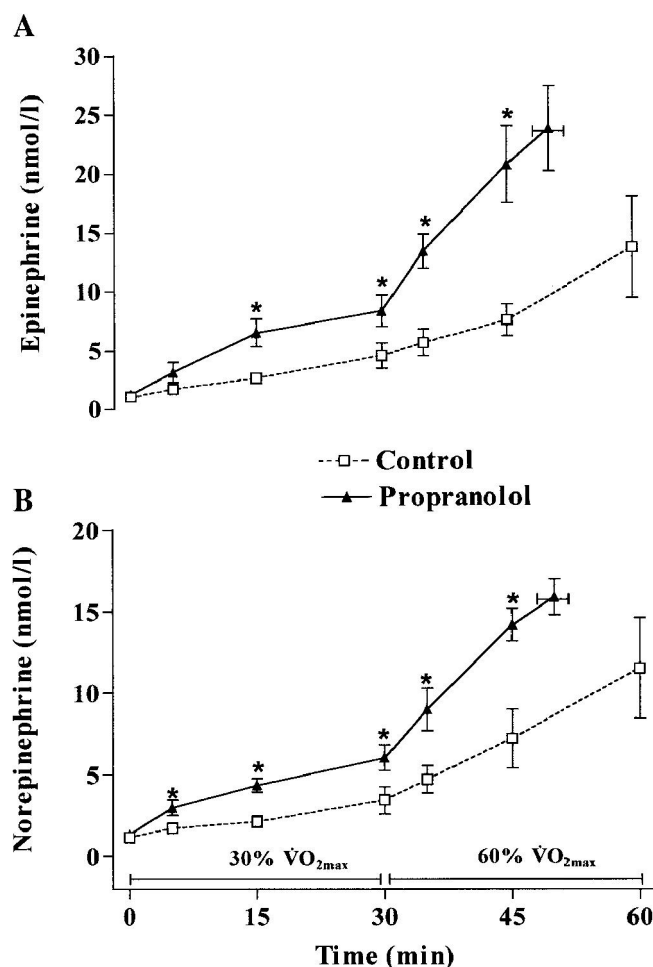


Fig. 6. Plasma epinephrine (A) and norepinephrine (B) at rest and during exercise at 30 and 60% $\dot{V}O_{2max}$ in the control trial and after administration of propranolol (0.22 mg/kg, 15 min before exercise). Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from control, $P < 0.05$.

8.40 \pm 1.4, and 24.1 \pm 3.60 nmol/l, respectively (Fig. 6A). In the C trial, plasma NE rose from 1.20 \pm 0.22 to 11.50 \pm 3.1 nmol/l at the end of exercise, representing an \sim 10-fold increase. In contrast, there was a 15-fold increase in plasma NE during exercise under β -blockade, with a peak concentration of 15.9 \pm 1.5 nmol/l. Plasma NE was significantly higher in the P trial than in the C trial at all time points during exercise (Fig. 6B).

Hematocrit, plasma total protein, lactate, glycerol, and NEFA. Hematocrit was significantly increased by 5 min of exercise and increased further coincident with the increment in workload. Plasma total protein followed a pattern similar to that for hematocrit (Table 1). Hematocrit and plasma total protein were similar between the two trials. Plasma lactate concentrations were also similar in the two trials. Plasma lactate decreased slightly during exercise at 30% $\dot{V}O_{2max}$ and then increased to reach 5.04 \pm 0.7 and 4.45 \pm 0.65 mmol/l in the C and P trials, respectively (Table 1). Plasma glycerol and NEFA concentrations during exercise

were significantly lower in the P trial than in the C trial. Although there were progressive increases in plasma glycerol and NEFA throughout exercise in the C trial, glycerol concentration was unchanged in the P trial, whereas plasma NEFA was decreased relative to preexercise values (Fig. 7).

Respiratory gas exchange and whole body substrate oxidation. Tables 3 and 4 show the steady-state gas exchange data and the total calculated CHO_{ox} and fat oxidation rates during the two trials. Whereas $\dot{V}O_2$ was similar in both trials, RER was significantly greater in the P trial than in the C trial between 20 and 50 min of exercise (Table 3). The total rate of energy expenditure was similar between trials during exercise at \sim 30% $\dot{V}O_{2max}$ (0.21 \pm 0.03 kcal \cdot kg $^{-1}\cdot$ min $^{-1}$) and \sim 60% $\dot{V}O_{2max}$ (0.40 \pm 0.04 kcal \cdot kg $^{-1}\cdot$ min $^{-1}$; Fig. 8). During the lower workload in the C trial, there was a progressive decrease in CHO_{ox} that was matched by a similar increase in the rate of fat oxidation (Table 4). The subsequent increase in workload was associated with an almost threefold increase in CHO_{ox} compared with

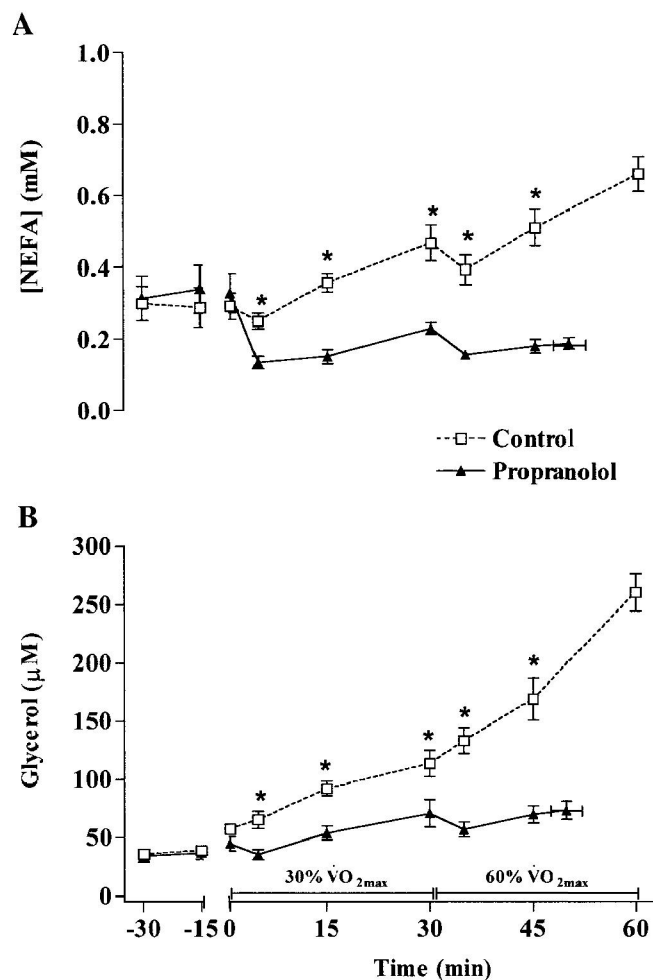


Fig. 7. Plasma nonesterified fatty acid concentration ([NEFA], A) and glycerol (B) at rest and during exercise at 30 and 60% $\dot{V}O_{2max}$ in the control trial and after administration of propranolol (0.22 mg/kg, 15 min before exercise). Values are means \pm SE for 6 horses. Horizontal error bar indicates SE for exercise duration in propranolol condition. *Significantly different from propranolol, $P < 0.05$.

Table 3. Steady-state gas exchange data during exercise at 30% and 60% $\dot{V}O_{2max}$ under control conditions or after administration of propranolol

	30% $\dot{V}O_{2max}$			60% $\dot{V}O_{2max}$		
	10 min	20 min	30 min	40 min	50 min	60 min
$\dot{V}O_2$, l/min						
C	18.9 \pm 0.4	19.0 \pm 0.4	19.4 \pm 0.4	34.8 \pm 1.0	34.6 \pm 0.8	34.9 \pm 0.9
P	19.1 \pm 0.8	19.5 \pm 0.8	19.9 \pm 0.7	35.2 \pm 1.0	35.2 \pm 0.8	ND
RER						
C	0.862 \pm 0.01	0.843 \pm 0.01	0.836 \pm 0.01	0.932 \pm 0.01	0.930 \pm 0.01	0.924 \pm 0.01
P	0.886 \pm 0.01	0.872 \pm 0.01*	0.874 \pm 0.02*	0.958 \pm 0.01*	0.952 \pm 0.01*	ND

Values are means \pm SE for 6 horses. Propranolol was administered at 0.22 mg/kg iv. $\dot{V}O_{2max}$, maximal O_2 uptake; RER, respiratory exchange ratio; $\dot{V}O_2$, O_2 uptake; ND, not determined. Values for P trial are at the end of exercise (49.9 \pm 1.2 min), rather than 50 min. *Significantly different from C, $P < 0.05$.

exercise at 30% $\dot{V}O_{2max}$. However, absolute rates of fat oxidation remained unchanged during exercise at the higher workload. Compared with the control condition, propranolol treatment resulted in significant suppression of fat oxidation between 20 and 50 min of exercise. In contrast, rates of CHO_{ox} were significantly higher in the P trial than in the C trial between 30 and 50 min (Table 4). Muscle glycogen (and lactate) oxidation, calculated as the difference between total CHO_{ox} and glucose R_d , was not different between trials (Table 5). Therefore, the higher CHO_{ox} in the P trial than in the C trial can be attributed to greater use of plasma glucose for energy production.

Estimates of the absolute and relative caloric contributions from plasma glucose, other CHO sources (muscle glycogen, lactate), and lipid during the 20- to 30-min and 35- to 45-min periods of exercise are shown in Figs. 8 and 9. During the 20- to 30-min period in the C trial, the relative energy expenditure from fat, plasma glucose, and other CHO sources (muscle glycogen, lactate) was 56 \pm 3, 12 \pm 3, and 32 \pm 4%, respectively (Fig. 9). Compared with the C trial, β -adrenergic blockade reduced fat oxidation, representing 40 \pm 3% of the total energy, whereas the contribution of plasma glucose increased to 20 \pm 3% ($P < 0.05$ vs. C trial) with no change in the contribution of other CHO sources (36 \pm 3%). In both trials, the higher workload resulted in a

marked shift in substrate utilization, with muscle glycogen (and lactate) accounting for 68 \pm 3 and 70 \pm 4% of the energy expenditure in the C and P trials, respectively, during the 35- to 45-min period of exercise (Figs. 8 and 9). Conversely, the relative contribution of fat oxidation to energy expenditure decreased, representing 25 \pm 2 and 17 \pm 2% in the C and P trials, respectively. In the C trial, the absolute contribution of plasma glucose to total energy expenditure was unchanged (Fig. 8), whereas the relative contribution of glucose was decreased compared with the 20- to 30-min period (12 \pm 3 vs. 7 \pm 1.5%). The absolute and percent contribution of plasma glucose to energy expenditure was significantly higher in the P trial than in the C trial during the 40- to 50-min period (Figs. 8 and 9).

DISCUSSION

This report represents one of the first descriptions of glucose turnover in the horse during sustained exertion. Furthermore, this study is the first to evaluate the role of β -adrenergic mechanisms in the regulation of glucose kinetics and whole body substrate utilization in this species during exercise. The most significant findings were 1) a $>40\%$ increase in glucose R_a and R_d during exercise after induction of β -adrenergic blockade, 2) increased reliance on plasma glucose for energy

Table 4. Rates of CHO and fat oxidation during the control and propranolol trials

	10 min	20 min	30 min	40 min	50 min	60 min
CHO oxidation, g/min						
C	13.0 \pm 1.5	10.4 \pm 1.7	7.9 \pm 1.3	36.1 \pm 1.2	35.9 \pm 1.3	34.9 \pm 1.1
P	13.7 \pm 1.9	12.4 \pm 1.9	11.8 \pm 2.1*	40.2 \pm 1.6*	39.9 \pm 1.1*	ND
CHO oxidation, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$						
C	161.5 \pm 15.4	130.7 \pm 20.2	115.2 \pm 20.7	452.3 \pm 17.9	450.6 \pm 19.4	437.5 \pm 15.3
P	167.6 \pm 19.2	152.1 \pm 20.3	159.4 \pm 22.5*	503.4 \pm 23.8*	493.9 \pm 19.7*	ND
Fat oxidation, g/min						
C	5.15 \pm 0.30	5.94 \pm 0.40	6.75 \pm 0.50	5.0 \pm 0.50	4.60 \pm 0.40	5.36 \pm 0.50
P	4.37 \pm 0.24	3.99 \pm 0.25*	4.10 \pm 0.36*	3.45 \pm 0.44*	3.15 \pm 0.45*	ND
Fat oxidation, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$						
C	40.9 \pm 4.4	47.4 \pm 5.1	56.1 \pm 4.8	37.9 \pm 4.6	36.1 \pm 2.4	42.3 \pm 4.3
P	35.9 \pm 3.7	33.3 \pm 2.1	38.7 \pm 4.3*	23.6 \pm 3.1*	23.7 \pm 2.4*	ND

Values are means \pm SE for 6 horses. CHO, carbohydrate. Rates of carbohydrate and fat oxidation were calculated from respiratory gas exchange data, averaged over the preceding 10-min interval. Values for P trial are at the end of exercise (49.9 \pm 1.2 min), rather than 50 min. *Significantly different from C, $P < 0.05$.

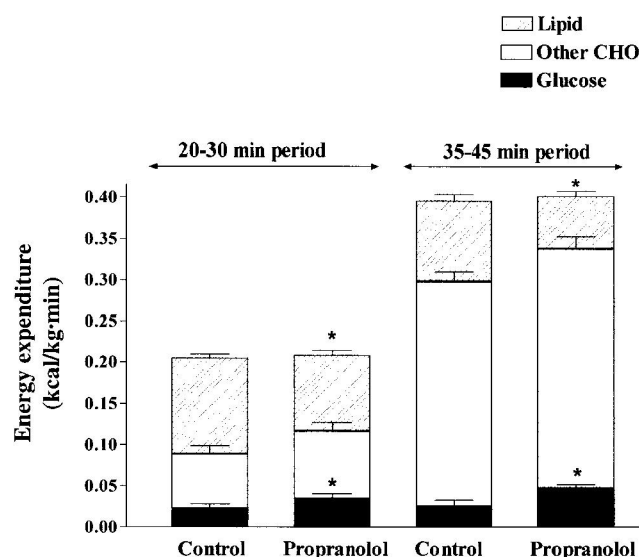


Fig. 8. Contribution of energy from different substrate sources during the 20- to 30-min and 35- to 45-min periods of exercise under control conditions and after administration of propranolol. Values are means \pm SE for 6 horses. CHO, carbohydrate. *Significantly different from control, $P < 0.05$.

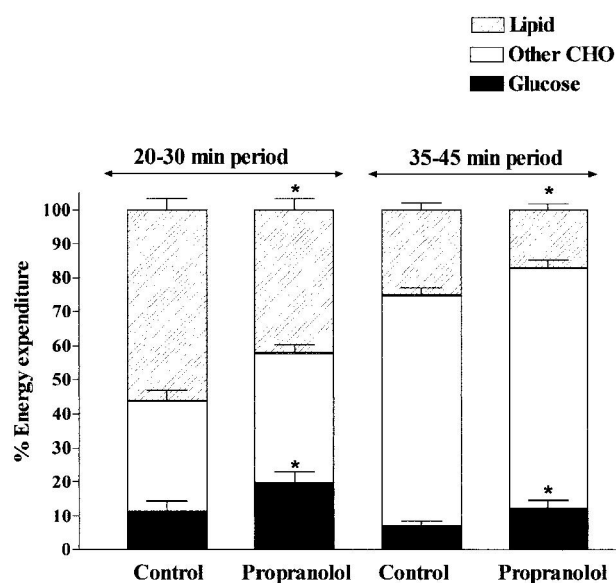


Fig. 9. Percent energy expenditure derived from plasma glucose, other CHO (muscle glycogen, lactate), and lipid during the 20- to 30-min and 35- to 45-min periods of exercise under control conditions and after administration of propranolol. Values are means \pm SE for 6 horses. *Significantly different from control, $P < 0.05$.

transduction during exercise under β -blockade, 3) a β -blockade-associated reduction in endurance capacity, as reflected by the decrease in exercise duration in the P trial, and 4) no change in the rate of fat oxidation after the transition from exercise at $\sim 30\%$ $\dot{V}O_{2\max}$ to exercise at $\sim 60\%$ $\dot{V}O_{2\max}$, such that the additional energy expenditure at the higher workload was met solely by an increase in CHO_{ox} .

Critique of methods. A graded exercise protocol was chosen to examine the effects of two work intensities on our measures of glucose kinetics and substrate utilization. Inasmuch as the two workloads were completed in a single exercise protocol, it is possible that some of the observed alterations in dependent measures were due to time-related, rather than exercise intensity-related, effects. Nonetheless, the large increments in glucose R_a and R_d observed after the step change in workload suggest that exercise intensity was an important determinant of the response.

Table 5. Total CHO oxidation, glucose R_d , and muscle glycogen (and lactate) oxidation during the control and propranolol trials

	20–30 min		35–45 min	
	C	P	C	P
Total CHO oxidation	122 \pm 19	149 \pm 17*	451 \pm 16	495 \pm 20
Glucose R_d	27.3 \pm 2.4	39.5 \pm 4.2*	37.4 \pm 2.6	61.5 \pm 5.3*
Muscle glycogen oxidation	96 \pm 21	109 \pm 18	414 \pm 16	427 \pm 18

Values (means \pm SE for 6 horses) are expressed as $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. R_d , rate of disappearance. Muscle glycogen (plus lactate) oxidation was estimated from the difference between total CHO oxidation and glucose R_d . *Significantly different from C at corresponding time period, $P < 0.05$.

Inasmuch as glucose R_d increases as a function of relative exercise intensity (32), a potential confounding factor in the present study was a decrease in $\dot{V}O_{2\max}$ under β -blockade with a concomitant increase in the relative workload for any given $\dot{V}O_2$ during exercise. In humans, nonselective β -blockade can result in a reduction in $\dot{V}O_{2\max}$ due to decreases in cardiac output and O_2 delivery (3). However, the effects of β -blocker drugs on hemodynamics and $\dot{V}O_{2\max}$ are dependent on the dose administered (34) and the training status of the subject (5). Several studies have reported no change in the peak $\dot{V}O_2$ of healthy male subjects receiving ~ 0.4 – 0.5 mg/kg propranolol once daily (34) or as a single preexercise treatment (5, 39). Similarly, in one previous study of horses in which the propranolol dosing regimen was the same as that employed in the present study (0.22 mg/kg iv, 15 min before exercise), the $\dot{V}O_2$ response to supramaximal exercise (105% of untreated $\dot{V}O_{2\max}$) was not different in the control and β -blockade conditions (36). Thus, although we did not determine the effect of propranolol administration on $\dot{V}O_{2\max}$, it is probable that there was minimal or no change in aerobic capacity, such that the relative workloads in the C and P trials were similar.

Another concern with the experimental model was the duration of β -blockade after a single intravenous dose of propranolol. Previous studies in dogs (28) and in humans (21, 39) have also used single, bolus dose methods to study the effect of β -blockade on plasma glucose metabolism. In the present study, maintenance of β -blockade was evidenced by the markedly attenuated cardiac response to isoprenaline challenge, administered 10 min after completion of exercise. This finding is in agreement with the results of a previous study of horses, wherein β -blockade was maintained

for 60–75 min after a 0.1 mg/kg iv dose of propranolol (51). The complete suppression of the increases in plasma NEFA and glycerol during exercise provided further evidence for the maintenance of β -adrenergic blockade. Inasmuch as IRI concentrations were lower in the P trial than in the C trial throughout exercise, it is likely that these differences in substrate concentrations reflect the inhibitory effects of β -blockade on lipolysis (58).

Plasma hormones. Catecholamine (Epi and NE) concentrations were higher in the P trial than in the C trial, whereas β -blockade attenuated the exercise-associated rise in IRG concentrations and lowered IRI levels. Previous studies in humans at rest and during exercise (1, 17, 46) have demonstrated increased catecholamine concentrations during β -blockade. Our data do not allow for definition of the mechanism for this increase in Epi and NE. However, in humans during moderate-intensity exercise, β -blockade decreases hepatic (6) and splanchnic (1) blood flow. Inasmuch as the gut and liver are the primary sites of catecholamine clearance at rest and during exercise (10), it is possible that the increased plasma Epi and NE result from decreased clearance. It is likely that similar mechanisms accounted for the increased Epi and NE observed in the P trial.

The changes in the plasma concentrations of IRI and IRG likely reflect the effects of β -blockade on pancreatic secretion. In humans, β -blockade decreases insulin secretion at rest (43) and exacerbates the exercise-induced fall in plasma insulin concentrations (17). This decrease has been attributed to intensified α -receptor-mediated inhibition of insulin secretion (16, 43). β -Adrenergic blockade also inhibits glucagon secretion in humans (43), and it is probable that a similar mechanism explains the attenuation of the rise in plasma IRG during exercise in the P trial.

Glucose kinetics. In accord with findings in humans and other animals (53), during low-intensity exercise in the C trial, the increase in HGP was closely matched to the increment in glucose R_d , and plasma glucose concentration was largely unchanged. Conversely, heavier exercise ($\sim 60\% \dot{V}O_{2\max}$) resulted in a mismatch between glucose R_a and R_d (Fig. 2), and plasma glucose increased progressively (Fig. 1A). We hypothesized that β -blockade would alleviate this mismatch by enhancement of glucose uptake; indeed, glucose R_d and MCR were ~ 40 and $\sim 50\%$ higher, respectively, in the P trial than in the C trial, and the difference in glucose R_d was greatest during exercise at $\sim 60\% \dot{V}O_{2\max}$ (Fig. 2B). However, throughout exercise in the P trial, HGP exceeded glucose R_d by $\sim 20\%$, and plasma glucose concentrations were ~ 1.2 – 2.0 mM higher in the P trial than in the C trial between 20 and 50 min of exercise. Thus the increment in HGP under β -blockade cannot be due solely to increased demand for hepatic glucose supply, and it is likely that β -blockade enhanced some component of a feedforward mechanism for regulation of HGP.

Some (39, 46), but not all (47), studies in humans have also demonstrated an increase in HGP during

exercise under β -blockade. However, as in the present study, the aforementioned effects of systemic β -blockade on splanchnic hemodynamics, pancreatic hormone secretion, catecholamine clearance, and fat metabolism complicate elucidation of the mechanism for the increase in HGP during β -blockade. In our study, possible mechanisms include intensified stimulation of hepatic α -adrenergic receptors and lower IRI concentrations and/or a greater increment in G/I. During moderate exercise (<50 – $60\% \dot{V}O_{2\max}$) in humans and dogs, the fall in insulin (53, 54) and increase in glucagon (55) are the primary stimuli for the increase in HGP. Although G/I increased during exercise in both trials, during exercise at $30\% \dot{V}O_{2\max}$ the increment was greater during β -blockade because of the decrease in IRI (Fig. 5C). If it is assumed that alterations in these pancreatic hormones also affect HGP in horses during exercise, the higher G/I in the P trial, at least during exercise at $30\% \dot{V}O_{2\max}$, probably contributed to the increase in the glucose R_a response under β -blockade. However, inasmuch as G/I was similar in the two trials during exercise at $60\% \dot{V}O_{2\max}$, other mechanisms must have contributed to the higher HGP in the P trial during heavier exercise.

As indicated previously, it has been hypothesized that sympathoadrenergic mechanisms, both direct (hepatic sympathetic nerves) and indirect (circulating catecholamines), play an important role in the regulation of HGP during heavy (>60 – $70\% \dot{V}O_{2\max}$) exercise (8, 46). However, although there is a strong correlation between the increase in plasma catecholamines and the rise in glucose R_a during intense exercise (8, 46), mechanistic studies have demonstrated that the exercise-induced increment in glucose R_a is not dependent on adrenergic receptor stimulation, at least in dogs and humans. The absence of hepatic innervation does not affect the exercise-induced increment in glucose R_a in dogs (56), rats (49), or humans (31). In addition, the glucose R_a response to heavy exercise was unchanged in dogs during selective blockade of hepatic α - and β -adrenergic receptors (11). Furthermore, in bilaterally adrenalectomized humans, the overall exercise-induced increase in HGP was unchanged during experiments in which Epi was infused to achieve concentrations within the physiological range (26).

Although adrenergic receptor stimulation does not play an essential role in mediating the increase in HGP during exercise, it is important to recognize that the catecholamines can stimulate an increase in hepatic glucose output. Indeed, infusions of Epi that resulted in moderate (25) and high (30) physiological concentrations augmented HGP in healthy humans during moderate exercise. Furthermore, in dogs at rest, α -adrenergic stimulation via a selective rise in liver sinusoidal NE induced a twofold increase in glucose R_a that was due to enhanced hepatic glycogenolysis (7). Similarly, infusion of NE at rest resulted in a 60–80% increase in plasma glucose concentration in horses (2). In the present study, plasma NE was highly correlated with the increase in HGP in the C trial ($r = 0.90$, $P < 0.01$) and in the P trial ($r = 0.95$, $P < 0.01$). Therefore, it is

possible that hepatic sympathetic activation of α -adrenoceptors is an important mechanism for stimulation of HGP in horses during exercise. Moreover, given that plasma NE concentrations were ~ 90 – 130% higher in the P trial than in the C trial and the potential for an unmasking of α -adrenoceptor stimulation during β -blockade (46), it is possible that direct sympathetic stimulation of hepatic glycogenolysis contributed to the increased HGP in the P trial.

An important finding was the marked increase in glucose uptake (Fig. 2B) during exercise in the P trial. Because MCR was also higher in the P trial than in the C trial (Fig. 3), it is likely that factors other than higher prevailing glucose concentrations contributed to the increase in glucose uptake during β -blockade. Possible mechanisms underlying this increase in glucose clearance include abrogation of β -adrenergic-mediated inhibition of glucose transport into muscle and of β -adrenergic-mediated increases in plasma NEFA concentrations.

Studies in humans have demonstrated that physiological increases in Epi concentrations inhibit glucose clearance at rest (38) and during moderate exercise (26, 35). It also has been suggested that high catecholamine concentrations restrain glucose R_d during heavy exercise (45, 46). That this Epi-induced reduction in glucose R_d can be mitigated by propranolol infusion implicates β -adrenergic mechanisms in the inhibition of glucose uptake (38). Our data from the P trial also lend weight to the hypothesis that β -adrenoceptors restrain glucose uptake during exercise. Furthermore, consistent with our working hypothesis, we suggest that the relatively small increase in glucose R_d in the C trial after the step change in workload also reflects, in part, a β -adrenergic-mediated restraint of glucose uptake into muscle.

β -Adrenergic stimulation may directly inhibit glucose uptake into skeletal muscle by alterations in glucose transporter recruitment and/or activity. In vitro studies have demonstrated that Epi inhibits glucose uptake in rat skeletal muscle (19), although it is not known whether this effect of Epi is mediated by β -adrenoceptors. Alternatively, this β -adrenergic effect is mediated by stimulation of muscle glycogenolysis, which, because of the resultant increase in intracellular glucose 6-phosphate and inhibition of hexokinase, impedes uptake of glucose from the circulation (29). However, the similarity in estimates of rates of muscle glycogen oxidation between the C and P trials argues against this mechanism underlying the increment in glucose R_d during exercise in the P trial.

Finally, suppression of circulating NEFA availability augments whole body and leg glucose uptake in dogs during exercise (4). Conversely, increases in plasma NEFA to >1 mM have been shown to decrease leg glucose uptake in exercising men (20). Therefore, because β -blockade abolished the exercise-induced increase in plasma NEFA, it is possible that the lower NEFA concentrations also contributed to the difference in glucose R_d and MCR between the P and C trials.

Substrate utilization. β -Blockade augmented CHO_{ox} during exercise at both workloads (Table 4, Figs. 8 and 9). Furthermore, given the increase in blood glucose uptake in the P trial and the similar calculated rates of muscle glycogen oxidation in the P and C trials, our data indicate that the increase in total CHO_{ox} was due to increased utilization of plasma glucose. Previous studies in humans (39, 46) and in dogs (27) also have demonstrated an increased reliance on plasma glucose for energy production during exercise after β -blockade. In the present study, the lower rates of fat oxidation during exercise in the P trial may be explained by a decrease in fatty acid availability. The lower plasma NEFA and glycerol concentrations in the P trial (Fig. 7) are consistent with a β -blockade-mediated decrease in lipolysis (58). Furthermore, in human subjects, β -blockade decreases intramuscular triglyceride breakdown in skeletal muscle during submaximal exercise (52). Taken together, these mechanisms would limit fatty acid availability in active skeletal muscle during β -blockade. Alternatively, the increase in glucose uptake during exercise in the P trial may have inhibited fatty acid oxidation by more direct mechanisms. Recent studies in humans have demonstrated reduced long-chain fatty acid transport and oxidation within muscle mitochondria under conditions of increased plasma glucose uptake and accelerated glycolytic flux (12).

Although glucose uptake (and presumably oxidation) increased after the step change in workload, in both trials the relative contribution by plasma glucose to energy expenditure was lower at $\sim 60\%$ than at $\sim 30\% \dot{V}\text{O}_{2\text{max}}$. Furthermore, the absolute rates of fat oxidation were little changed with the increase in workload, such that the relative contribution of lipid fuels to total energy expenditure was decreased by $>50\%$ during exercise at $\sim 60\% \dot{V}\text{O}_{2\text{max}}$. The decrease in the combined contribution of these substrates was compensated by a large increase in muscle glycogen (and lactate) oxidation. Similar patterns of fuel selection as a function of exercise intensity have been demonstrated in humans (41), dogs, and goats (40). In dogs and in goats, maximal rates of fat oxidation are reached at an exercise intensity of $\sim 40\% \dot{V}\text{O}_{2\text{max}}$. Therefore, only CHO metabolism is upregulated to cover the increase in fuel demand at higher workloads. However, in dogs, rates of plasma glucose oxidation are similar during exercise at 40, 60, and $85\% \dot{V}\text{O}_{2\text{max}}$, such that increased utilization of intracellular substrate deposits (i.e., muscle glycogen) is required to meet the increased energy demands (57).

Exercise duration. In the present study, β -blockade resulted in an $\sim 17\%$ reduction in exercise duration. Previous studies in humans and horses (48) and in ponies (44) have firmly established that β -adrenoceptor blocking agents impair exercise performance, although the mechanism(s) responsible for this decrement in performance is not well understood. In the present study, a likely explanation for the reduction in exercise duration in the P trial is an impairment in thermoregulation. Despite the reduction in exercise time, end-exercise T_{re} was $\sim 0.8^\circ\text{C}$ higher in the P trial

than in the C trial. Similarly, in a study by Sexton and Erickson (44), end-exercise pulmonary artery blood temperature was $\sim 1.2^{\circ}\text{C}$ higher in ponies that had received propranolol. Hyperthermia has been implicated in the development of fatigue during submaximal exercise (24). In horses, fatigue during exercise at $\sim 65\% \dot{V}\text{O}_{2\text{ max}}$ coincides with a pulmonary artery blood temperature of $\sim 42.5^{\circ}\text{C}$ (24). Inasmuch as T_{re} is ~ 0.5 to 1.0°C lower than central blood temperature in horses during submaximal exercise (23), it is likely that central blood temperature was approaching 42.5 – 43°C at the end of exercise in the P trial. Given that sweating in the horse is mediated via β_2 -adrenoceptors (23), this exacerbation of exercise hyperthermia can, in part, be explained by a decrease in evaporative heat loss during exercise under β -blockade.

In summary, this study demonstrated that nonselective β -blockade resulted in a $>40\%$ increase in glucose R_a and whole body glucose uptake in horses during exercise at ~ 30 and $\sim 60\% \dot{V}\text{O}_{2\text{ max}}$. β -Blockade augmented total CHO_{ox} during exercise at both workloads, and, inasmuch as estimated muscle glycogen use was similar in control and β -blockade trials, increased utilization of plasma glucose accounted for the increment in CHO_{ox} . An intensified α -adrenergic stimulation of hepatic glycogenolysis and/or an altered G/I probably contributed to the increased HGP with propranolol treatment. The increase in glucose use (R_d and MCR) during exercise under β -blockade supports the contention that β -adrenergic mechanisms restrain glucose uptake into contracting muscle.

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REFERENCES

- Ahlborg G and Juhlin-Dannfelt A. Effect of β -receptor blockade on splanchnic and muscle metabolism during prolonged exercise in men. *J Appl Physiol* 76: 1037–1042, 1994.
- Anderson MG and Aitken MM. Biochemical and physiological effects of catecholamine administration in the horse. *Res Vet Sci* 22: 357–360, 1977.
- Bassett DR Jr and Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32: 70–84, 2000.
- Bracy DP, Zinker BA, Jacobs JC, Lacy DB, and Wasserman DH. Carbohydrate metabolism during exercise: influence of circulating fat availability. *J Appl Physiol* 79: 506–513, 1995.
- Brusasco V, Violante B, and Bucchini G. Effect of β -adrenergic blockade on response to exercise in sedentary and active subjects. *J Appl Physiol* 67: 103–109, 1989.
- Christensen NJ, Trap-Jensen J, Clausen JP, Noer I, and Krogsgaard AR. Effect of β -adrenoceptor blockade on heart rate, hepatic blood flow and circulating noradrenaline during exercise in man. *Acta Physiol Scand* 95: 62A–63A, 1975.
- Chu C, Sindelar DK, Neal DW, Allen EJ, Donahue EP, and Cherrington AD. Effect of a selective rise in sinusoidal norepinephrine on HGP is due to an increase in glycogenolysis. *Am J Physiol Endocrinol Metab* 274: E162–E171, 1998.
- Coggan AR, Comasio AR, Gastaldelli A, Williams BD, and Wolfe RR. Regulation of glucose production during exercise at 80% of $\dot{V}\text{O}_{2\text{ peak}}$ in untrained humans. *Am J Physiol Endocrinol Metab* 273: E348–E354, 1997.
- Coggan AR, Kohrt WM, Spina RJ, Bier DM, and Holloszy JO. Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men. *J Appl Physiol* 68: 990–996, 1990.
- Coker RH, Krishna MG, Lacy DB, Allen EJ, and Wasserman DH. Sympathetic drive to liver and nonhepatic splanchnic tissues during heavy exercise. *J Appl Physiol* 82: 1244–1249, 1997.
- Coker RH, Mahesh GK, Brooks Lacey D, Bracy DP, and Wasserman DH. Role of hepatic α - and β -adrenergic receptor stimulation on hepatic glucose production during heavy exercise. *Am J Physiol Endocrinol Metab* 273: E831–E838, 1997.
- Coyle EF, Jeukendrup AE, Wagenmakers AJM, and Saris WHM. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *Am J Physiol Endocrinol Metab* 273: E268–E275, 1997.
- Eaton MD. Energetics and performance. In: *The Athletic Horse: Principles and Practice of Equine Sports Medicine*, edited by Hodgson DH and Rose RJ. Philadelphia, PA: Saunders, 1994, p. 49–61.
- Fedak MA, Rome L, and Seeherman HJ. One-step N_2 -dilution technique for calibrating open-circuit VO_2 measuring systems. *J Appl Physiol* 51: 772–776, 1981.
- Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55: 628–634, 1983.
- Galbo H. *Hormonal and Metabolic Adaptation to Exercise*. New York: Thieme-Stratton, 1983.
- Galbo H, Holst JJ, Christensen NJ, and Hilsted J. Glucagon and plasma catecholamines during β -receptor blockade in exercising man. *J Appl Physiol* 40: 855–863, 1976.
- Geor RJ, Hinchcliff KW, and Sams RA. Glucose infusion attenuates endogenous glucose production and enhances glucose use of horses during exercise. *J Appl Physiol* 88: 1765–1776, 2000.
- Han XX and Bonen A. Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle. *Am J Physiol Endocrinol Metab* 274: E700–E707, 1998.
- Hargreaves M, Kiens B, and Richter EA. Effect of increased plasma free fatty acid concentrations on muscle metabolism in exercising men. *J Appl Physiol* 70: 194–201, 1991.
- Hargreaves M and Proietto J. Glucose kinetics during prolonged exercise in trained men. *Acta Physiol Scand* 150: 221–225, 1994.
- Hinchcliff KW, McKeever KH, Muir WW, and Sams RA. Effect of furosemide and weight carriage on energetic responses of horses to incremental exertion. *Am J Vet Res* 54: 1500–1504, 1993.
- Hodgson DR, McCutcheon LJ, Byrd SK, Brown WS, Bayly WM, Brengelman GL, and Gollnick PD. Dissipation of metabolic heat in the horse during exercise. *J Appl Physiol* 74: 1161–1170, 1993.
- Hodgson DR, Rose RJ, Kelso TB, McCutcheon LJ, Bayly WM, and Gollnick PD. Respiratory and metabolic responses in the horse during moderate and heavy exercise. *Pflügers Arch* 417: 73–78, 1990.
- Howlett K, Febbraio M, and Hargreaves M. Glucose production during strenuous exercise in humans: role of epinephrine. *Am J Physiol Endocrinol Metab* 276: E1130–E1135, 1999.
- Howlett K, Galbo H, Lorentsen J, Bergeron R, Zimmerman-Belsing T, Bulow J, Feldt-Rasmussen U, and Kjaer M. Effect of adrenaline on glucose kinetics during exercise in adrenalectomized humans. *J Physiol (Lond)* 519: 911–921, 1999.
- Issekutz B. Role of β -adrenergic receptors in mobilization of energy sources in exercising dogs. *J Appl Physiol* 44: 869–876, 1978.
- Issekutz B. Effect of epinephrine on carbohydrate metabolism in exercising dogs. *Metabolism* 34: 457–464, 1985.
- Katz A, Sahlin K, and Broberg S. Regulation of glucose utilization in human skeletal muscle during moderate dynamic exercise. *Am J Physiol Endocrinol Metab* 260: E411–E415, 1991.

30. **Kjaer M, Engfred K, Fernandez A, Secher N, and Galbo H.** Regulation of hepatic glucose production during exercise in humans: role of sympathoadrenergic activity. *Am J Physiol Endocrinol Metab* 265: E275–E283, 1993.
31. **Kjaer M, Keiding S, Engfred A, Rasmussen K, Sonne B, Kirkegaard P, and Galbo H.** Glucose homeostasis during exercise in humans with a liver or kidney transplant. *Am J Physiol Endocrinol Metab* 268: E636–E644, 1995.
32. **Kjaer M, Kiens B, Hargreaves M, and Richter EA.** Influence of active muscle mass on glucose homeostasis. *J Appl Physiol* 71: 552–557, 1991.
33. **MacDonald IA and Lake DM.** An improved technique for extracting catecholamines from body fluids. *J Neurosci Methods* 13: 239–248, 1985.
34. **McKelvie RS, Jones NL, and Heigenhauser GJ.** Factors contributing to increased muscle fatigue with β -blockers. *Can J Physiol Pharmacol* 69: 254–261, 1991.
35. **Mora-Rodriguez R and Coyle EF.** Plasma epinephrine reduces blood glucose clearance during low-intensity exercise (Abstract). *Med Sci Sports Exerc* 31: S126, 1999.
36. **Plummer C, Knight PK, Ray SP, and Rose RJ.** Cardiorespiratory and metabolic effects of propranolol during maximal exercise. In: *Equine Exercise Physiology 3*, edited by Persson SGB, Lindholm A, and Jeffcott LB. Davis, CA: ICEEP, 1991, p. 465–474.
37. **Reimers TJ, Cowan RG, McCann JP, and Ross MW.** Validation of rapid solid-phase radioimmunoassay for canine, bovine, and equine insulin. *Am J Vet Res* 43: 1274–1278, 1982.
38. **Rizza RA, Cryer PE, Haymond MW, and Gerich JE.** Adrenergic mechanisms for the effects of epinephrine on glucose production and clearance in man. *J Clin Invest* 65: 682–689, 1980.
39. **Roberts AC, Reeves JT, Butterfield GE, Mazzeo RS, Sutton JR, Wolfel EE, and Brooks GA.** Altitude and β -blockade augment glucose utilization during submaximal exercise. *J Appl Physiol* 80: 605–615, 1996.
40. **Roberts TJ, Weber J-M, Hoppeler H, Weibel ER, and Taylor CR.** Design of the oxygen and substrate pathways. II. Defining the upper limits of carbohydrate and fat oxidation. *J Exp Biol* 199: 1651–1658, 1996.
41. **Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, and Wolfe RR.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Metab* 265: E380–E391, 1993.
42. **Rose RJ and Hodgson DH.** Hematology and biochemistry. In: *The Athletic Horse: Principles and Practice of Equine Sports Medicine*, edited by Hodgson DH and Rose RJ. Philadelphia, PA: Saunders, 1994, p. 63–78.
43. **Samols E and Weir GC.** Adrenergic modulation of pancreatic A, B, and D cells. *J Clin Invest* 63: 230–238, 1979.
44. **Sexton WL and Erickson HH.** Effects of propranolol on cardiopulmonary function in the pony during submaximal exercise. *Equine Vet J* 18: 485–489, 1986.
45. **Sigal RJ, Fisher S, Halter JB, Vranic M, and Marliss EB.** The roles of catecholamines in glucoregulation in intense exercise as defined by islet cell clamp technique. *Diabetes* 45: 148–156, 1996.
46. **Sigal RJ, Purdon C, Bilinski D, Vranic M, and Marliss EB.** Glucoregulation during and after intense exercise: effects of β -blockade. *J Clin Endocrinol Metab* 78: 359–366, 1994.
47. **Simonson DC, Koivisto V, and Sherwin RS.** Adrenergic blockade alters glucose kinetics during exercise in insulin-dependent diabetics. *J Clin Invest* 73: 1648–1658, 1984.
48. **Snow DH, Summers RJ, and Guy PS.** The actions of the β -adrenoreceptor blocking agents propranolol and metoprolol in the maximally exercised horse. *Res Vet Sci* 27: 22–29, 1979.
49. **Sonne B, Mikines KJ, Richter EA, Christensen NJ, and Galbo H.** Role of liver nerves and adrenal medulla in glucose turnover of running rats. *J Appl Physiol* 59: 1640–1646, 1985.
50. **Steele R.** Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci* 82: 420–430, 1959.
51. **Tavernor WD and Lees P.** The influence of propranolol on cardiovascular function in conscious and anaesthetized horses. *Arch Int Pharmacodyn* 180: 89–99, 1969.
52. **Van Baak MA.** β -Adrenoreceptor blockade and exercise. An update. *Sports Med* 4: 209–225, 1988.
53. **Wasserman DH.** Regulation of glucose fluxes during exercise in the postabsorptive state. *Annu Rev Physiol* 57: 191–218, 1995.
54. **Wasserman DH, Spalding JA, Bracy D, Lacy DB, and Cherrington AD.** Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. *Am J Physiol Endocrinol Metab* 256: E500–E509, 1989.
55. **Wasserman DH, Spalding JA, Lacy DB, Colburn CA, Goldstein RE, and Cherrington AD.** Glucagon is a primary controller of hepatic glycogenolysis and gluconeogenesis during muscular work. *Am J Physiol Endocrinol Metab* 257: E108–E117, 1989.
56. **Wasserman DH, Williams PE, Lacy DB, Bracy D, and Cherrington AD.** Hepatic nerves are not essential to the increase in hepatic glucose production during muscular work. *Am J Physiol Endocrinol Metab* 259: E195–E203, 1990.
57. **Weber J-M, Roberts TJ, Vock R, Weibel ER, and Taylor CR.** Design of the oxygen and substrate pathways. III. Partitioning of energy provision from carbohydrates. *J Exp Biol* 199: 1659–1666, 1996.
58. **Wijnen JAG, van Baak MA, de Haan C, Struijker-Boudier HAJ, Tan FS, and van Bortel MAB.** β -Blockade and lipolysis during endurance exercise. *Eur J Clin Pharmacol* 45: 101–105, 1993.
59. **Wolfe RR.** *Radioactive and Stable Isotope Tracers in Biomedicine*. New York: Wiley-Liss, 1992.