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Maximum O_2 uptake, O_2 debt and deficit, and muscle metabolites in Thoroughbred horses

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ROSE, REUBEN J., DAVID R. HODGSON, THOMAS B. KELSO, LAURA JILL MCCUTCHEON, TRACEY-ANN REID, WARWICK M. BAYLY, AND PHILIP D. GOLLNICK. *Maximum O_2 uptake, O_2 debt and deficit, and muscle metabolites in Thoroughbred horses.* J. Appl. Physiol. 64(2): 781–788, 1988.—This study determined maximal O_2 uptake ($\dot{V}O_{2\,max}$), maximal O_2 deficit, and O_2 debt in the Thoroughbred racehorse exercising on an inclined treadmill. In eight horses the O_2 uptake ($\dot{V}O_2$) vs. speed relationship was linear until 10 m/s and $\dot{V}O_{2\,max}$ values ranged from 131 to 153 ml·kg^{-1}·min^{-1}. Six of these horses then exercised at 120% of their $\dot{V}O_{2\,max}$ until exhaustion. $\dot{V}O_2$, CO_2 production ($\dot{V}CO_2$), and plasma lactate (La) were measured before and during exercise and through 60 min of recovery. Muscle biopsies were collected before and at 0.25, 0.5, 1, 1.5, 2, 5, 10, 15, 20, 40, and 60 min after exercise. Muscle concentrations of adenosine 5′-triphosphate (ATP), phosphocreatine (PC), La, glucose 6-phosphate (G-6-*P*), and creatine were determined, and pH was measured. The O_2 deficit was 128 ± 32 (SD) ml/kg (64 ± 13 liters). The O_2 debt was 324 ± 62 ml/kg (159 ± 37 liters), approximately two to three times comparative values for human beings. Muscle [ATP] was unchanged, but [PC] was lower ($P < 0.01$) than preexercise values at ≤10 min of recovery. [PC] and $\dot{V}O_2$ were negatively correlated during both the fast and slow phases of $\dot{V}O_2$ during recovery. Muscle [La] and [G-6-*P*] were elevated for 10 min postexercise. Mean muscle pH decreased from 7.05 (preexercise) to 6.75 at 1.5 min recovery, and the mean peak plasma La value was 34.5 mmol/l. Relating the metabolic mechanisms for the O_2 debt to the changes in muscle metabolites indicated that terminal oxidation of the estimated changes in [La] would require a greater $\dot{V}O_2$ than was measured. Rephosphorylation of PC accounted for <1.5% of the O_2 debt. The role of elevated postexercise temperature as a contributing factor to the O_2 debt could not be assessed.

oxygen debt; oxygen deficit; muscle metabolites; maximal oxygen uptake

CONSIDERABLE INFORMATION exists on maximal O_2 uptake ($\dot{V}O_{2\,max}$), deficit, and debt after exercise in species such as reptiles (7), rats (2, 3), dogs (25), and human beings (9, 11, 20, 30), but none is available for the Thoroughbred horse. Although the racehorse is thought to have a high aerobic capacity, the only report of $\dot{V}O_{2\,max}$ is a mean value of 64.2 l/min in two Russian Standardbred trotters (13), whose body weights were not reported. The $\dot{V}O_{2\,max}$ of the pony reportedly is >120 ml·kg^{-1}·min^{-1} (19).

We examined O_2 transport and O_2 debt and deficit in Thoroughbred horses, since these animals have been selectively bred to exercise at high speeds for periods of 1–3 min. The concentrations of adenosine 5′-triphosphate (ATP), phosphocreatine (PC), and glucose 6-phosphate (G-6-*P*) in muscle and lactate (La) in muscle and blood were determined in the immediate postexercise period to ascertain any relationships with O_2 debt. Because the term O_2 debt implies a payment of debt accumulated during exercise, Gaesser and Brooks (6) proposed the use of the term excess postexercise O_2 uptake. However, in this paper the term O_2 debt is used, since historically this term has been most widely utilized.

MATERIALS AND METHODS

Measurement of O_2 uptake ($\dot{V}O_2$) and $\dot{V}O_{2\,max}$. Eight gelded male Thoroughbred horses, aged 3–6 yr and weighing 440–536 kg [493 ± 29.9 (SD) kg] were used to determine the repeatability of $\dot{V}O_2$ determinations and the measurement of $\dot{V}O_{2\,max}$. The horses had been exposed to a program of light exercise, but not training, for 3 mo. The horses were maintained in box stalls 2 wk before the exercise tests and performed exercise during the experiments other than that associated with the experimental procedures. A rapid incremental treadmill exercise test similar to that used for human beings, was used to determine the $\dot{V}O_2$ vs. speed relationship and $\dot{V}O_{2\,max}$. All horses were acclimated to treadmill exercise and the respiratory gas collection system before the experiments.

After being led onto the treadmill (Säto, Sweden) set at a +10% slope, a cardiotachometer (PEH Horse Tester, Finland) and loose-fitting gas collection system were applied. The gas collection system consisted of a funnel-shaped hood constructed of a rigid frame covered by an impervious plastic material that allowed flexibility during peak expiratory flow (1). Air was drawn through the hood and around the muzzle into a 5.5-m-long, 9-cm-diam flexible hose that led to a 63-liter volume mixing chamber. From the mixing chamber, the gas was channeled into a rigid pipe, at the end of which was a flowmeter. Airflow was generated by fans positioned after the flowmeter, and gas samples for analysis of CO_2 and O_2 were collected into a 600-liter spirometer that was

also used for calibrating the system. The total volume of the system was 225 liters. The fractions of O_2 and CO_2 were determined in the mixed-expired gas samples using an Applied Electrochemistry S-3A O_2 analyzer and a Beckman LB-2 CO_2 analyzer. Flow rates of ~1,500 (resting measurements) and 6,000 l/min (exercise and immediate postexercise measurements) were used. At these flow rates the time constants for gas flow through the collection system were ~9 and 2.25 s at the low and high flow rates, respectively. The flow rates, determined with a hot-wire anemometer (TSI, series 2210), were controlled by regulating the speed of the fans. Precise control of the gas flow rates was possible by regulating the voltage output, using a flow-voltage curve established before the exercise test.

The horse commenced exercise at 4 m/s for 3 min, and thereafter the treadmill velocity was increased every 60 s until the horse could no longer keep pace with the treadmill. The $\dot{V}O_2$ was measured during the last 5 s of each exercise step. Values are corrected to STPD. The additional treadmill speeds were 6, 8, 9, 10, 11, and 12 m/s. Not all horses completed the 12-m/s exercise step. The exercise protocol was repeated four times for each horse with at least 48 h between exercise tests.

Assessment of O_2 deficit and debt. Six of the horses whose $\dot{V}O_{2\,max}$ had been determined were studied. Their mean body weight was 488 ± 36 kg. A treadmill speed that required an energy expenditure equivalent to 120% of $\dot{V}O_{2\,max}$ was calculated for each horse from the previously established individual regression equations.

A 110-cm 8-F pig-tailed catheter was placed into the pulmonary artery via the left jugular vein of each horse before exercise. Catheter placement was confirmed from pressure-wave changes displayed on an oscilloscope as the catheter was advanced through the right ventricle. Samples of blood (5 ml) were collected from each horse at rest into tubes containing NaF and potassium oxalate. A muscle biopsy was also taken from the middle gluteal muscle as described by Lindholm and Piehl (17). The sample was immediately frozen in liquid N_2 for later metabolite and pH measurements.

After collecting the preexercise samples, the horses were led onto the treadmill, which was set at a +10% slope, and resting $\dot{V}O_2$ was measured after a 10-min acclimation to the equipment. The heart rate was measured during this time to ensure that the horse was not excited. The horse was then warmed up for 5 min at a work intensity equivalent to 50% of $\dot{V}O_{2\,max}$ without the gas collection system, and after a 10-min resting-recovery period, the respiratory hood was applied and the exercise test commenced. The treadmill speed was increased at its maximal rate of acceleration to a speed equivalent to 120% of the horse's $\dot{V}O_{2\,max}$ over 55–60 s. During exercise, expired air samples were collected every 15 s and mixed-venous blood every 30 s. The exercise test was terminated when the horse could not maintain its speed on the treadmill, despite encouragement. The horse was then halted and the expired air samples were collected every 15 s during recovery from 0 to 7 min, every 30 s from 7 to 10 min, every 60 s from 10 to 20 min, and every 5 min from 20 to 60 min. Muscle biopsies and blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 5, 10, 15, 20, 40, and 60 min during recovery.

The changes in $\dot{V}O_2$ during exercise and recovery were plotted for each horse, and the areas under the curve were measured with a digitizing planimeter using a Bioquant Digitizing software/hardware package (Bioquant System IV, Biometrics, Nashville, TN) interfaced with an IBM-XT computer. The preexercise resting $\dot{V}O_2$ was used as the base line for these calculations.

Metabolite and pH analyses. Muscle samples were stored at −80°C until they were analyzed. The samples (20–40 mg) were pulverized at the temperature of liquid N_2 according to the technique of Pette and Reichmann (24). The metabolites measured were creatine (Cr), ATP, PC, La, and G-6-*P* as described by Kelso et al. (16). All the muscle metabolites were expressed as millimoles per mole of total Cr (PC+Cr). Plasma La measurements (18) were carried out following an extraction procedure similar to that of muscle, except that alkalization of the sample was not necessary. Muscle pH was measured at 30°C using an Orion model 701A pH meter and a glass electrode (MI-410, Microelectrodes). For this procedure, frozen tissue was crushed (24) and suspended in a medium of 145 mM KCl, 10 mM NaCl, 5 mM iodoacetate, and 0.3 mM 1-fluoro-2,4-dinitrobenzene (FDNB). Iodoacetate and FDNB were included to prevent alterations in pH due to either lactate formation or PC degradation. N_2 was blown over the surface of the suspension to eliminate CO_2 to avoid undeterminable differences in the CO_2 content due to differential losses into the atmosphere. This procedure can lower the PCO_2 of the buffer from 100 to 8 Torr (Instrumental Laboratories 813 pH/blood-gas analyzer) in 3 min, the time the samples were gassed before pH measurements were made. This method may underestimate the pre- to postexercise change in pH, since there probably are differences in the CO_2 content of muscle at rest and after exercise.

Statistical analyses. Linear regression analysis, using the method of least squares, was used to calculate individual regression lines for $\dot{V}O_2$ vs. speed, between 4 and 10 m/s. A plateau in the $\dot{V}O_2$ vs. speed relationship was reached at either 11 or 12 m/s. Correlation coefficients for $\dot{V}O_2$ vs. speed from all four tests were determined for each horse. The overall relationship between $\dot{V}O_2$ and speed was also determined by regression analysis for all eight horses over the four measurement times.

The $\dot{V}O_2$ and $\dot{V}CO_2$ values during exercise and the data from muscle and blood were analyzed using a repeated-measures analysis of variance, followed by a post hoc Tukey's test to determine significant differences. With the muscle and blood samples, significant differences from preexercise values were examined. $\dot{V}O_2$ and $\dot{V}CO_2$ measurements during exercise were compared with those at 90 s, since a plateau existed for these variables at that time.

The postexercise $\dot{V}O_2$ was analyzed with a least-squares regression analysis. The question asked was, at what stage postexercise was there no significant slope in the $\dot{V}O_2$ vs. time regression line? Logarithmic transformation of the postexercise $\dot{V}O_2$ data was carried out to analyze the fast and slow components of $\dot{V}O_2$ (9).

The O_2 requirement ($ml \cdot kg^{-1} \cdot min^{-1}$) for exercise at 120% of $\dot{V}O_{2\,max}$ was estimated from the linear relationship of $\dot{V}O_2$ vs. exercise intensity as calculated for each horse. The O_2 deficit was calculated by subtracting the area under the exercise $\dot{V}O_2$ curve from the estimated total O_2 requirement (15). It was assumed that the power output and $\dot{V}O_2$ relationship was constant from the onset of exercise to 120% of $\dot{V}O_{2\,max}$.

The O_2 debt was calculated from the area under the recovery $\dot{V}O_2$ curve. The duration of the fast and slow components of the O_2 debt was identified after linear regression analysis of the logarithmically transformed data.

Correlations between the $\dot{V}O_2$ values in recovery and metabolites measured at the same times were determined using regression analysis. All values are means ± SD.

RESULTS

The $\dot{V}O_2$ vs. treadmill speed relationship was linear in all horses at ≤10 m/s. The regression equation was as follows: $\dot{V}O_2 = 16.51 \pm 12.04 \cdot V$, where V is treadmill velocity. The correlation between $\dot{V}O_2$ and speed at these exercise intensities was 0.96 for all measurements (Fig. 1). $\dot{V}O_{2\,max}$ ranged from 131.0 to 153.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (141.7 ± 8.2). The coefficients of variation for the repeated $\dot{V}O_{2\,max}$ measurements ranged from 2.8 to 6.3%. The correlation coefficients for the individual horse $\dot{V}O_2$ vs. speed regression lines ranged from 0.95 to 0.99 for the four repeated measurements. The regression equation for heart rate (HR) vs. speed was $HR = 101.4 \pm 11.6 \cdot V$. HR's (beats/min) during the different exercise stages, taken over all four tests were as follows: 4 m/s, 145 ± 11; 6 m/s, 173 ± 10; 8 m/s, 198 ± 10; 9 m/s, 206 ± 8; 11 m/s, 220 ± 6; 12 m/s, 222 ± 5.

The duration of exercise at 120% of $\dot{V}O_{2\,max}$ ranged from 2.25 to 3.15 min. At the onset of supramaximal exercise the $\dot{V}O_2$ increased quickly with a biological half-life ($t_{½}$) of 9.8 ± 2.3 s. $\dot{V}O_2$ had reached a steady state by 75 s. The $\dot{V}O_2$ at 60 s of exercise was 95% of the steady-state value (Fig. 2). The $\dot{V}CO_2$ showed a similar plateau with a $t_{½}$ of 9.5 ± 3.9 s (Fig. 2). Mean respiratory exchange ratios were 1.13 at the conclusion of exercise, 0.878 at 5 min recovery, 0.771 at 30 min recovery, and 0.679 at 60 min recovery. After cessation of exercise, $t_{½}$ for the $\dot{V}O_2$ was 45.5 ± 9.0 s. The fast and slow components of the $\dot{V}O_2$ recovery curve were complete by 1.4 and 18.3 min, respectively (Fig. 3). There was no significant slope of the $\dot{V}O_2$ vs. time regression line after 18.3

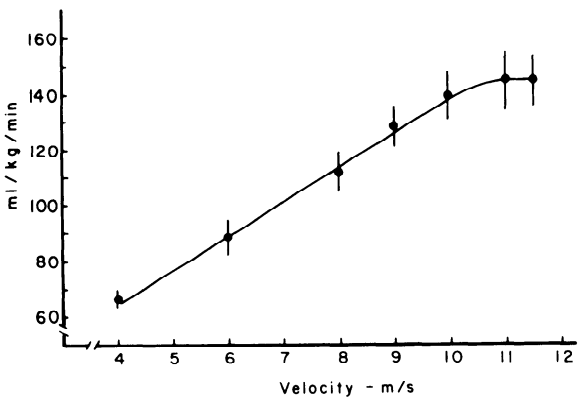

FIG. 1. O_2 uptake ($\dot{V}O_2$) during incremental exercise test in 8 horses. Values are means ± SD. Note plateau in $\dot{V}O_2$ at 11 m/s.

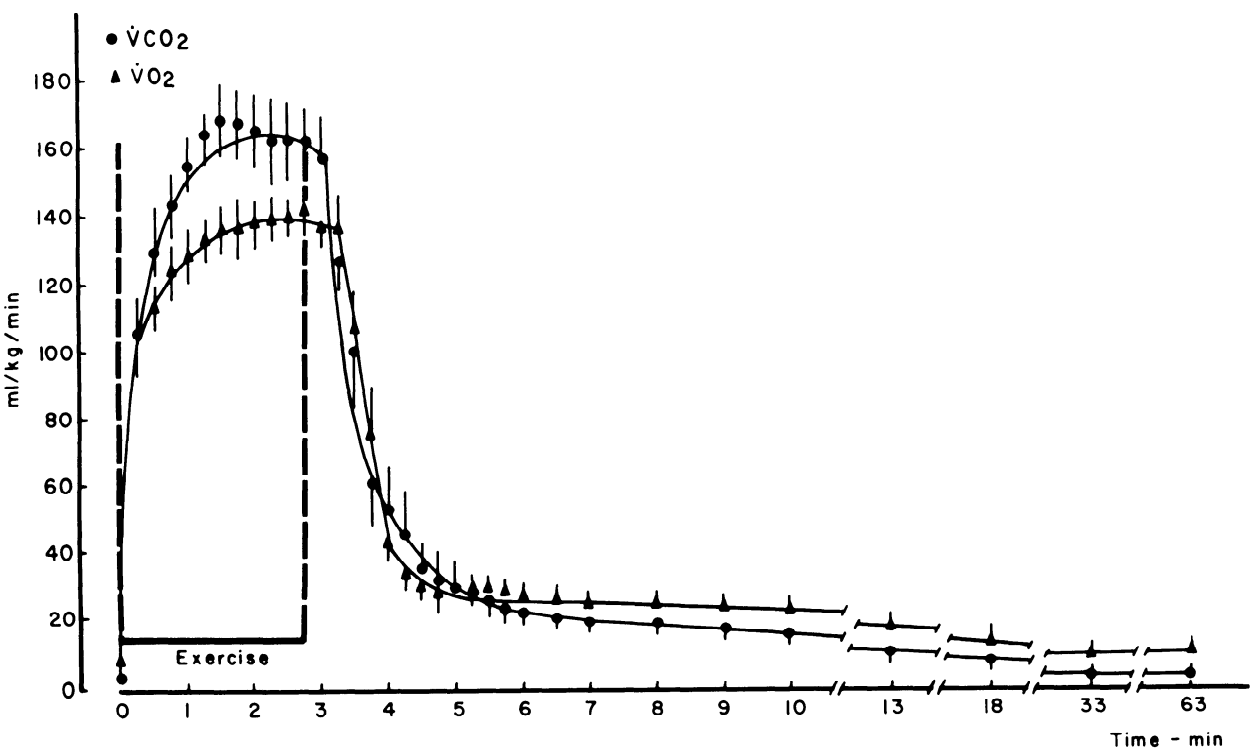

FIG. 2. O_2 uptake ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) in 6 horses during and for 60 min after exercise at 120% maximal $\dot{V}O_2$. Values are means ± SD.

min. The O_2 deficit and debt were 128.2 ± (63.6 ± 13.3 liters) and 324.5 ± 62.1 ml/kg (159.2 ± 36.6 liters), respectively. The $\dot{V}O_2$ and O_2 debt were not correlated at the termination of exercise ($r = 0.003$). O_2 debt-to-O_2 deficit ratio was 2.75 ± 1.18. HR's during the 3rd min of exercise ranged from 206 to 222 beats/min (216 ± 7). The total $\dot{V}O_2$ during exercise was 305.2 ± 57.7 ml/kg (147.2 ± 18.0 liters).

No changes in muscle [ATP] were measured from pre- to postexercise. The [PC] was lower ($P < 0.05$) and the [G-6-P] and [Cr] were higher ($P < 0.05$) than the preexercise values, at ≤10 min postexercise (Fig. 4). Muscle [La] increased ($P < 0.05$) over that of preexercise and remained elevated 10 min postexercise (Fig. 5). Muscle pH was lower ($P < 0.05$) than that of preexercise from 1.5 to 10 min postexercise. Because insufficient muscle was available for pH analysis in all horses from 0.25 to 1.0 min postexercise, statistical analysis was not done. Plasma [La] was higher ($P < 0.01$) than preexercise values during both the exercise and postexercise period (Fig. 6), with peak values of 34.3 mmol/l occurring at 0.25 min postexercise.

Correlation coefficients for $\dot{V}O_2$ and various metabolites between 0 and 1.5 min postexercise (fast component of O_2 debt) were as follows: [PC], −0.68 ($P < 0.01$); muscle [La], 0.40; [G-6-P], 0.58 ($P < 0.05$); pH, −0.31; and blood [La], 0.78 ($P < 0.01$). The correlation coefficients for $\dot{V}O_2$ and metabolites between 1.5 and 18 min

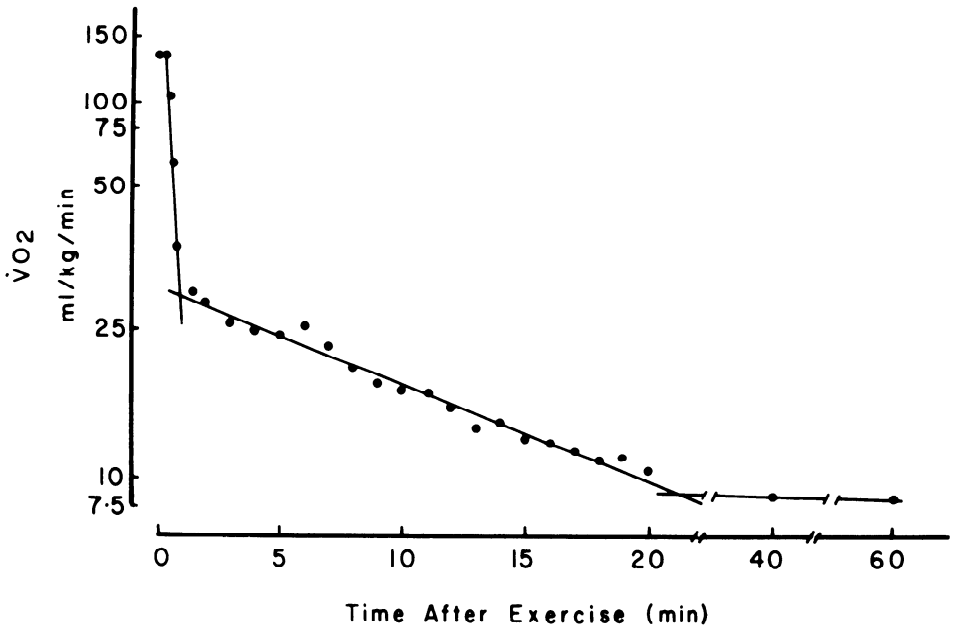

FIG. 3. Mean O_2 uptake ($\dot{V}O_2$) during recovery from exercise at 120% of the maximal $\dot{V}O_2$ in 6 horses. Lines drawn through points represent separate equations derived by linear regression analysis.

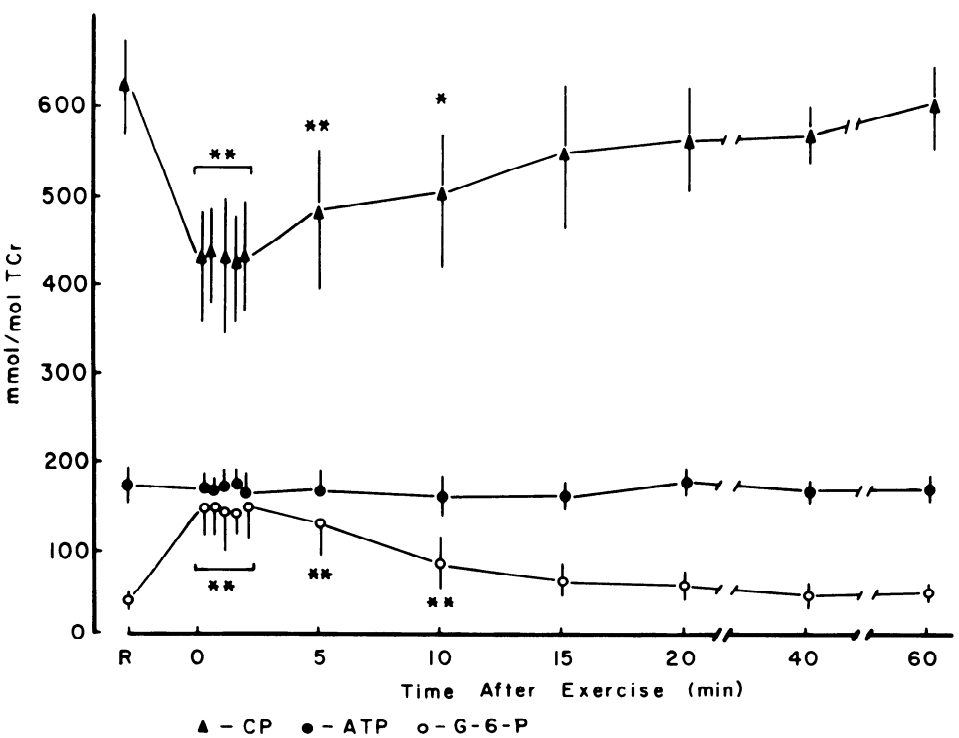

FIG. 4. Concentrations of adenosine 5′-triphosphate (ATP), creatine phosphate (CP), and glucose 6-phosphate (G-6-P) before and after exercise in 6 horses. TCr, total creatine, which is the sum of free creatine plus creatine of CP. Values are means ± SD. * $P < 0.05$. ** $P < 0.01$.

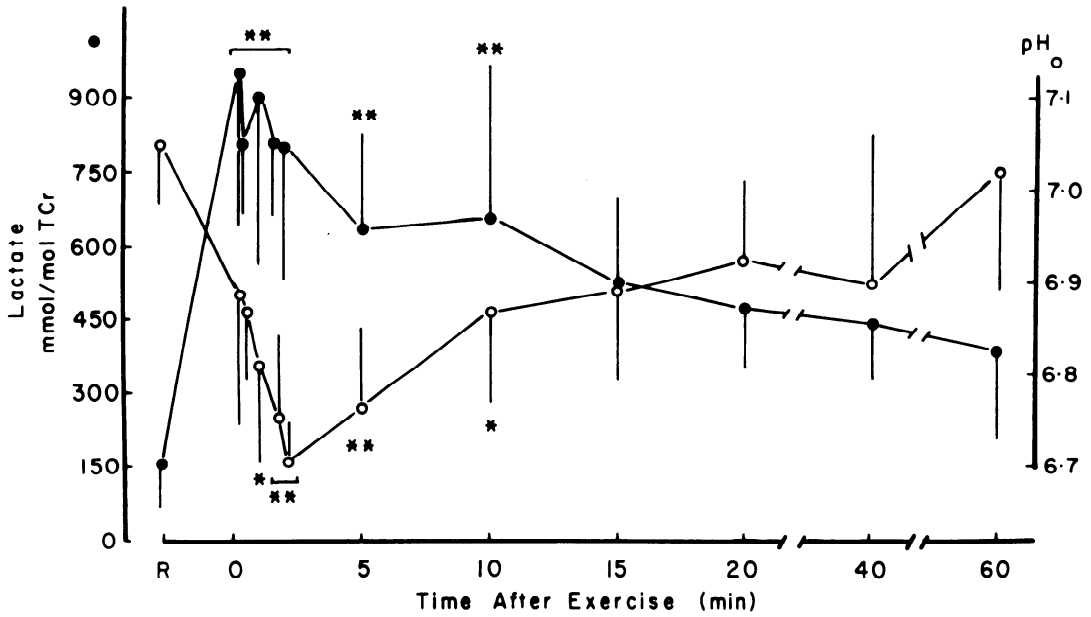

FIG. 5. Results of muscle lactate and muscle pH before and after exercise in 6 horses. TCr, total creatine (see Fig. 4). Values are means ± SD. * $P < 0.05$. ** $P < 0.01$.

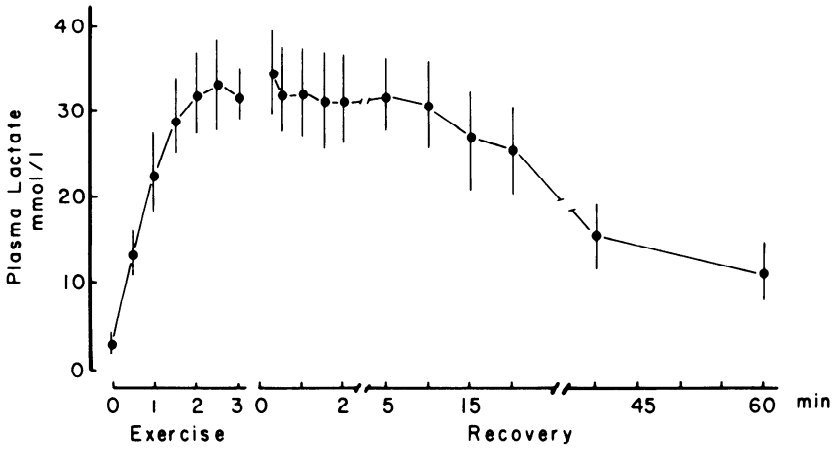

FIG. 6. Results of plasma lactate before, during, and after exercise in 6 horses. Values are means ± SD.

postexercise (slow component of O_2 debt) were as follows: blood [La], 0.18; muscle [La], 0.36 ($P < 0.01$); [PC], −0.54 ($P < 0.01$); [G-6-P], 0.79 ($P < 0.01$); and pH, −0.46 ($P < 0.01$). The correlation between $\dot{V}O_2$ and time during this period was −0.92 ($P < 0.01$).

DISCUSSION

The relationship between $\dot{V}O_2$ and treadmill velocities was linear between 4 and 10 m/s and was highly repeatable. $\dot{V}O_{2\,max}$ was confirmed by the plateau in $\dot{V}O_2$ that occurred at running speeds of 10 or 11 m/s. $\dot{V}O_2$ and HR plateaued concurrently. The $\dot{V}O_{2\,max}$ was ~10 l/min higher than that reported in two Standardbred horses of unknown body weight (13). The $\dot{V}O_{2\,max}$ per kilogram body weight of the untrained Thoroughbred horses was higher than that of several other species. For example, values for untrained human subjects (27) are only one-third to one-half those of the horse. Mean values for the untrained fox hound (22) and pony (19) are 114 and 126 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. These differences in $\dot{V}O_{2\,max}$ are a function of the capacities of O_2 transport systems in these species, each attained in a different manner. In human beings, stroke volume (SV) ranges from 1 to 2 ml/kg, HR from 180 to 200 beats/min, and the arteriovenous O_2 difference ($avDO_2$) from 16 to 18 ml/100 ml (27). In the dog, these values are SV, 2.7 ml/kg; maximal HR (HR_{max}), 300 beats/min; and $avDO_2$, 14 ml/100 ml (22). For the horses of this study exercising for 3 min at 100% of $\dot{V}O_2$, HR_{max} was 220 beats/min; SV 2.7 ml/kg; and $avDO_2$, 22.3 ml/100 ml. The high O_2 delivery capacity of equine blood (23.4 ± 2.4 ml/100 ml) is the result of high erythrocyte concentrations produced by a splenic contraction during exercise (23) and an almost complete O_2 extraction by the working muscles resulting in a mixed-venous O_2 content of 1.1 ± 0.2 ml/100 ml.

The kinetics of $\dot{V}O_2$ were difficult to examine during the high-intensity exercise due to the time taken for the treadmill to reach high speeds. However, $\dot{V}O_2$ after 60 s of exercise at 120% of $\dot{V}O_{2\,max}$ was within 5% of the steady-state value. Ponies reach 95% of the $\dot{V}O_2$ of steady-state value during the first 45–60 s of exercise (5). During the first 30–60 s this increase in $\dot{V}O_2$, $\dot{Q}$, and HR increased to 130% of the steady state (5).

In this study there was no ATP depletion in the middle gluteal muscle after the high-intensity exercise. In human beings there are reports that single bouts of supramaximal exercise decrease the [ATP] of muscle (12, 15). A partial depletion of muscle ATP does not always occur after a single exercise bout. The decline in PC was surprisingly small when compared with that reported in human subjects after very heavy cycle ergometer exercise (12). The reason for this modest decline in PC is unknown, but it may be related to the type and duration of the exercise. Thus the vastus lateralis muscle is used extensively during cycle ergometry, whereas there may have been a differential recruitment of motor units of the middle gluteal muscle with only some fibers becoming depleted of PC. Running on a treadmill at a +10% slope as opposed to a flat natural surface could alter the pattern of muscle involvement. Karlsson and Saltin (15), however, observed only a 55% decline in PC when La was >22 mmol/kg wet wt in the vastus lateralis muscle of human subjects after a 1-min bout of cycle-ergometer exercise at ~170% of the subject's $\dot{V}O_{2\,max}$.

Plasma [La] was higher than that usually found after single bouts of supramaximal exercise in human beings (10, 15) and approached values observed during repeated exercise bouts (10). [La] in muscle (~44 mM when expressed as muscle water) is consistent with a heavy engaged of the muscle during the exercise. This was associated with a reduction in pH. The high rate of glycogenolysis was accompanied by a threefold increase in [G-6-*P*] after exercise. This is consistent with some inhibition of glycolysis.

The O_2 deficits ranging from 78 to 168 ml O_2 equivalents per kilogram were higher than those (60–86 ml of O_2 equivalents per kg) observed in human beings after supramaximal exercise (15, 21). Higher deficits usually exist in sprint-trained rather than endurance-trained subjects (21). Although O_2 deficit may be indicative of anaerobic capacity, its relationship to anaerobic performance has not been established. The mean O_2 debt for the horses of this study was ~2.5 times the deficit, a ratio similar to that of human beings after similar relative efforts (15).

The time course for the return of $\dot{V}O_2$ to the preexercise value was similar to that of human beings, with the bulk of it occurring within 20 min after termination of the exercise (Fig. 3). The early fast phase of recovery has been attributed to the resynthesis of PC in the exercised muscles. However, the restoration of PC in the middle gluteal muscle occurred more slowly and was similar to that of rest only in samples collected after 15 min postexercise (Fig. 4). The time course of recovery for PC and $\dot{V}O_2$ was also similar to that of human beings (8, 25, 28). This, however, appears to be the first study where the concentrations of high-energy phosphates in skeletal muscle, muscle and blood La concentrations, and $\dot{V}O_2$ were followed simultaneously during the 1st min after supramaximal exercise. La was cleared rather slowly from plasma and muscle during recovery (Figs. 5 and 6). Therefore a poor relationship existed between the return of the $\dot{V}O_2$ to rest and the restoration of muscle metabolites to the preexercise state. [La] in muscle exceeded that in blood (44 vs. 36 mM), expressed per liter of water, immediately after exercise and until 3 min postexercise (38 vs. 32 mM), but was similar thereafter. This concentration differential of La between muscle and blood is similar to that reported for human beings (15). [La] remained elevated in muscle and blood after 60 min of recovery, when $\dot{V}O_2$ was only slightly above the preexercise value.

Estimates can be made of the relationships between the amounts of La cleared in muscle and blood and PC restored in muscle and the postexercise $\dot{V}O_2$. Such calculations are based on unproven assumptions concerning the muscle mass engaged in the exercise, the similarity of involvement activity muscle, the representativeness of the biopsy samples, the distribution of La between working muscle and other tissues, the equilibration of La between blood and tissue water, and a lack of La production in the postexercise period.

Estimates can be made of the O_2 required for restoring muscle PC to rest values. PC concentrations 15 s and 60 min after exercise were ~15.5 and 21.7 mmol/kg, respectively (see Ref. 16 for conversion factor for total Cr to kg wet wt). If ~40% of the horse's body weight is skeletal muscle (29), a 500-kg horse, the approximate mean weight of the animals studied, would have ~200 kg of skeletal muscle. If 50% of this muscle mass were used in the exercise, and changes in PC were consistent for all muscles, ~0.62 mol would have been synthesized during recovery. This would have required ~2.3 liters of O_2, or <1.5% of the measured O_2 debt. There was about a 15-s delay between the termination of exercise and collection of the first muscle sample, of which ~7 s was required to stop the horses when they could no longer maintain running speed. The small PC depletion in the muscle after exercise could have been due to some resynthesis of PC occurring in this time. However, the 4% increase in [PC] in four biopsies collected between 15 and 90 s postexercise suggests that any PC resynthesized during the 0- to 15-s postexercise period was small.

Estimates can also be made of the La removed from muscle and blood and how it relates to the postexercise $\dot{V}O_2$. For muscle, a 20.1-mmol/kg wet muscle wt La decline (34.2–14.1 mmol/kg wet muscle wt) occurred in the 60-min postexercise period. If 100 kg of muscle of uniform La dynamics were active in the exercise and the La accumulated only in active muscle, 2.1 mol of La were removed in the 60 min of recovery. In estimating La removal from blood in the postexercise period its equilibration in the extracellular water must be considered. With the use of the formula of Carlson and Harrold (4) and a 100-liter extracellular fluid volume for 500-kg horses (26), a 26 mmol/l H_2O decline in plasma [La] is equal to 2.6 mol of La. Oxidation of 4.6 mol of CO_2 and H_2O consumes ~315 liters of O_2. The actual average $\dot{V}O_2$ during this period was 160 liters for the debt and another 85 liters as the resting value. This could account for 2.4 mol of La oxidized, as within the so-called debt, and 3.64 mol for the total postexercise O_2 uptake if La were the sole fuel oxidized. Therefore it appears that the postexercise $\dot{V}O_2$ could not account for these estimates of La removed during the 60-min recovery period.

In addition to the constraints in interpretation of these calculations described above, it must be recognized that

the estimates of La removed from the body after exercise do not consider its diffusion into inactive muscle (14) or other tissues. The estimation of muscle La also does not account for the ~13% of the muscle weight that is extracellular water. This muscular component was included in the analysis of the muscle samples of La. We also have no support for the assumption that 50% of the muscle was active or that the dynamics of La were similar in all active muscle. The magnitude of the use of La or other fuels also cannot be determined for the resting values, since they are meaningless in the postexercise period where there may be a continued "blowing off" of nonmetabolic CO_2 in the early recovery period followed by its subsequent retention of CO_2 to replenish the bicarbonate buffer pool.

The above calculations suggest that not all the La cleared from muscle and blood in the postexercise period could have been oxidized to CO_2 and H_2O even if it were the sole fuel being metabolized. The question is, Where could it have gone? One possibility is that it could have been converted to glucose via gluconeogenesis in the liver. This process requires a modest $\dot{V}O_{2\,max}$ of ~22.4 l/mol of glucose synthesized. In the present experiments the removal of 1 mol of La from the body via gluconeogenesis would have used only ~11 liters of O_2 presumably in the liver. This is a small part of the O_2 debt, and it would seem that a greater amount of La may have been converted to glucose in the 60-min postexercise period. Conversion of the glucose by gluconeogenesis to glycogen would require only an additional modest $\dot{V}O_2$. For example, 50% of the La cleared from the body could have been converted to glycogen with the use of only 30 liters of O_2.

In summary, the current data suggest that in the 60 min of recovery in the Thoroughbred horse after exercise that results in a large O_2 debt and accumulation of La in muscle and blood, *1*) restoration of the PC concentration in muscle was a minor component of the O_2 debt, *2*) not all the La that was cleared could be accounted for by terminal oxidation, *3*) some La could be involved in gluconeogenesis and glycogen synthesis, and *4*) a large amount of La remains in the body when the total body $\dot{V}O_2$ approached that of rest. The results demonstrate that the horse may be a good experimental animal for studying some of the problems relating to the metabolic bases for the O_2 debt and the postexercise fate of La, since it has both high aerobic and anaereobic capacities and willingly exercises at intensities that produce high O_2 debts and muscle and blood [La].

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