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Furosemide reduces accumulated oxygen deficit in horses during brief intense exertion

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Hinchcliff, K. W., K. H. McKeever, W. W. Muir, and R. A. Sams. Furosemide reduces accumulated oxygen deficit in horses during brief intense exertion. *J. Appl. Physiol.* 81(4): 1550–1554, 1996.—We theorized that furosemide-induced weight reduction would reduce the contribution of anaerobic metabolism to energy expenditure of horses during intense exertion. The effects of furosemide on accumulated O₂ deficit and plasma lactate concentration of horses during high-intensity exercise were examined in a three-way balance randomized crossover study. Nine horses completed each of three trials: 1) a control (C) trial, 2) a furosemide-unloaded (FU) trial in which the horse received furosemide 4 h before running, and 3) a furosemide weight-loaded (FL) trial during which the horse received furosemide and carried weight equal to the weight lost after furosemide administration. Horses ran for 2 min at ~120% maximal O₂ consumption. Furosemide (FU) increased O₂ consumption (ml·2 min⁻¹·kg⁻¹) compared with C (268 ± 9 and 257 ± 9, *P* < 0.05), whereas FL was not different from C (252 ± 8). Accumulated O₂ deficit (ml O₂ equivalents/kg) was significantly (*P* < 0.05) lower during FU (81.2 ± 12.5), but not during FL (96.9 ± 12.4), than during C (91.4 ± 11.5). Rate of increase in blood lactate concentration (mmol·2 min⁻¹·kg⁻¹) after FU (0.058 ± 0.001), but not after FL (0.061 ± 0.001), was significantly (*P* < 0.05) lower than after C (0.061 ± 0.001). Furosemide decreased the accumulated O₂ deficit and rate of increase in blood lactate concentration of horses during brief high-intensity exertion. The reduction in accumulated O₂ deficit in FU-treated horses was attributable to an increase in the mass-specific rate of O₂ consumption during the high-intensity exercise test.

weight carriage; locomotion; energy expenditure

PERFORMANCE OF MUSCULAR work, such as running, requires the expenditure of energy. The amount of energy required to support running is related to the body mass of the animal and the speed of running (27). The effect of weight carriage on energy expenditure can be determined at submaximal work intensities by measurement of the rate of O₂ consumption ($\dot{V}O_2$) (15, 18, 24, 27). Determining the energetic cost of locomotion for work intensities above that inducing maximal $\dot{V}O_2$ ($\dot{V}O_{2\max}$) is problematic, because energy used above that produced by the maximal rate of aerobic metabolism is generated by anaerobic metabolism. Anaerobic capacity may limit exercise at intensities above $\dot{V}O_{2\max}$ (8). The rate of anaerobic energy production during supramaximal exertion can be estimated from the accumulated O₂ deficit, and relative rates of anaerobic energy production can be compared by the rate of appearance of lactate in the blood (13). The effect of increasing or decreasing body weight on the energetic cost of locomotion at exercise intensities above that inducing $\dot{V}O_{2\max}$ has not been investigated to our knowl-

edge. If acute weight reduction is accomplished without a reduction in aerobic capacity, the accumulated O₂ deficit and rate of increase in blood lactate concentration would be less after weight reduction for individuals performing a given supramaximal exercise test.

Furosemide administration to horses denied access to feed and water results in reductions in body weight of ~3–4% over a 4-h period (9, 14, 22). We theorized that furosemide-induced weight reduction would reduce the contribution of anaerobic metabolism to energy expenditure of horses during intense exertion. Specifically, our hypothesis was that furosemide administration would reduce the accumulated O₂ deficit and rate of increase in blood lactate concentration of horses during an intense exercise test of defined duration. Furthermore, we hypothesized that the addition of weight equal to that lost in response to furosemide administration would prevent the effect of furosemide on accumulated O₂ deficit.

MATERIALS AND METHODS

Experimental design. The effects of furosemide-induced weight reduction on $\dot{V}O_2$ and plasma lactate concentration during high-intensity exercise were examined in a three-way balance randomized crossover study. Nine horses participated in each of three trials: 1) a control (C) trial in which the horse was given 10 ml of isotonic saline solution intravenously 4 h before running, 2) a furosemide-unloaded (FU) trial in which the horse received furosemide (50 mg/ml, 1 mg/kg body wt iv; Lasix, Hoeschst-Roussel, Somerville, NJ) 4 h before running, and 3) a furosemide weight-loaded (FL) trial during which the horse received furosemide 4 h before running and while running carried a saddle pad containing weight equal to the urinary and insensible weight losses that occurred over the 3.75 h after furosemide administration. The study could not be blinded because of the need to add weights to FL horses. Trials in individual horses were performed ≥7 days apart.

Subjects. The subjects were nine Standardbred fillies 3–4 yr of age and 383–453 kg body wt. The fillies were maintained at pasture and received no enforced exercise other than running on a treadmill once weekly for the 12 wk preceding the study. During these training periods, horses ran on a treadmill inclined at 4° for 4 min at 4 m/s, at 10–12 m/s until fatigue, and then at 3 m/s for 5 min.

Measurement of $\dot{V}O_{2\max}$. $\dot{V}O_{2\max}$ and the relationship between $\dot{V}O_2$ and speed were determined for each horse during an incremental exercise test within 7 days of the experiment. The incremental exercise test consisted of the horse running on a treadmill inclined at 4° for 90 s at 4 m/s, after which treadmill speed was increased by 1 m/s every 90 s until the horse was unable 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 ml·min⁻¹·kg⁻¹ with an increase in speed.

Experimental protocol. On the day of each trial, between 0800 and 0930, the horse was weighed, saline or furosemide was administered, and the horse was placed in a clean stall. Access to food and water was denied until completion of the trial. A pulmonary artery catheter (PE-240, Becton Dickinson, Parsippany, NJ) for collection of mixed venous blood was aseptically placed through a catheter introducer placed in the right jugular vein. The position of the catheter was confirmed before and after exertion by examination of the pressure waveforms displayed on a physiograph (VR12 Physiologic Monitoring System, PPG Biomedical Systems, Pleasantville, NY). The horse was weighed 3.75 h after furosemide or saline administration, and all feces produced during that time were collected and weighed. Lead weights equal to the body weight lost over the 3.75 h after furosemide administration were added to a saddle pad of mares in the FL trial. The amount of weight added to horses in the FL trials was calculated as the difference between the initial body weight, recorded immediately before furosemide administration, and the body weight recorded 3.75 h later, less the weight of feces produced during the 3.75-h period.

Exercise test. During each experimental trial (C, FL, and FU), horses ran at 3 m/s for 3 min, at a predetermined high speed for 2 min, and at 3 m/s for 5 min (recovery) on a treadmill inclined at 4°. The transition from 3 m/s to the high speed was accomplished in ~10 s. The speed at which each horse performed the high-speed test was determined during pilot trials and was the speed at which the horse could just complete the 2-min high-speed test. The average high speed was 10.8 ± 0.2 (SE) m/s, representing an average intensity of $121 \pm 2.9\%$ $\dot{V}O_{2\max}$. $\dot{V}O_2$ was measured every 10 s during the exercise test (see below).

$\dot{V}O_2$. $\dot{V}O_2$ was measured with an open-circuit calorimeter (Oxymax-XL, Columbus Instruments, Columbus, OH), as previously described (15). Flow through the system was ~1,500 l/min STP with the horse stationary and 10,000 l/min during running. Gas collection systems of this design have been demonstrated not to impair the respiratory function of running horses compared with closed systems, and the overall accuracy of the system was verified repeatedly by nitrogen dilution (7, 10). The O_2 sensor (Electrochemical cell, Columbus Instruments) was calibrated against gases of known composition immediately before the start of each exercise test. Discrepancy between simulated $\dot{V}O_2$ produced by nitrogen dilution and the value measured by the system was $\pm 3\%$ at nitrogen flow rates equivalent to a $\dot{V}O_2$ of 54 l/min (~140 ml \cdot min⁻¹ \cdot kg⁻¹ for a 385-kg horse).

Calculation of O_2 deficit. Accumulated O_2 deficit was calculated as the difference between the expected $\dot{V}O_2$ and the actual $\dot{V}O_2$ during the 2-min high-speed run with use of previously described assumptions (19). Actual $\dot{V}O_2$ was calculated using the trapezoidal rule (11). Expected $\dot{V}O_2$ was calculated from the speed- $\dot{V}O_2$ relationship determined during the incremental exercise test and the speed of the horse during the experimental trials. The speed- $\dot{V}O_2$ relationship was determined using $\dot{V}O_2$ rates below $\dot{V}O_{2\max}$.

Blood collection. Mixed venous blood samples for measurement of hematocrit, plasma total protein, and lactate concentration were collected with the horse standing on the treadmill, after the warm-up period, at the end of the high-speed run, and at the end of the 5-min recovery period. Plasma for measurement of lactate concentration was collected after centrifugation (1,500 g for 20 min) of mixed venous blood collected into evacuated glass tubes containing sodium fluoride and potassium oxalate (Vacutainer, Becton Dickinson). Lactate concentration was measured electrochemically (model 23L, Yellow Springs Instruments, Yellow Springs, OH). He-

matocrit of blood collected into evacuated glass tubes containing EDTA (Vacutainer) was measured in triplicate by the microhematocrit technique. Plasma total protein was measured by refractometry.

Interpretation of plasma lactate concentration is confounded by changes in blood volume (13). Therefore, plasma lactate concentrations after furosemide treatment were adjusted for the effect of the furosemide-induced reduction in plasma volume (14) by the formula

$$[\text{Lac}]_{\text{adj}} = (\text{TP}_c/\text{TP}_f) \cdot [\text{Lac}]$$

where $[\text{Lac}]_{\text{adj}}$ is the plasma lactate concentration adjusted for the effect of furosemide on plasma volume, TP_c is the concentration of plasma protein immediately before exercise in the control treatment (C), TP_f is the plasma protein concentration immediately before exercise after furosemide treatment (FU and FL), and $[\text{Lac}]$ is the unadjusted lactate concentration at each sampling point. The use of plasma total protein to correct for the effect of a reduction in plasma volume on blood lactate concentration is appropriate, because changes in plasma total protein concentration of resting horses accurately reflect changes in plasma volume induced by furosemide (14). The effect of the exercise test on plasma protein concentration was similar after all treatments (see Fig. 4) and would not affect the between-group comparison of blood lactate concentration. Rate of increase in blood lactate concentration was calculated from the recovery lactate concentration ($[\text{Lac}]_{\text{adj}}$) and was normalized to body weight

$$R_{\text{Lac}} = [\text{Lac}]_{\text{adj}}/2 \text{ min}/\text{BW}$$

where R_{Lac} is the rate of increase in blood lactate concentration, BW is the body weight of the horse immediately before running, and 2 min is the duration of the high-speed exercise test.

Statistical analysis. Data from this study were analyzed as a three-way crossover design by use of a two-way repeated-measures analysis of variance [repeated measures on treatment (i.e., C, FL, or FU) and time factors] or as a one-way repeated-measures analysis (repeated measures on the treatment factor) depending on the data being analyzed (12, 20). Significance was defined as $P < 0.05$ for each of the main effects (treatment or time) and as $P < 0.1$ for the interaction. Results are expressed as the means of each group at specified times and the standard error of the mean or of the mean differences (20). All mass proportional variables ($\dot{V}O_2$, O_2 deficit) for the C and FU treatments are expressed relative to the body weight of the horse 3.75 h after furosemide or saline administration. Mass proportional variables for the FL treatment are expressed relative to the sum of the 3.75-h weight and the weight added to the horse.

RESULTS

$\dot{V}O_{2\max}$ and speed- $\dot{V}O_2$ relationship. $\dot{V}O_{2\max}$ of the nine horses was 137.7 ± 4.3 ml \cdot min⁻¹ \cdot kg⁻¹ at a treadmill speed of 9.1 ± 0.2 m/s. The correlation coefficient for the speed- $\dot{V}O_2$ regression averaged 0.998 ± 0.0001 ($P < 0.01$), the slope of the regression line was 0.258 ± 0.01 ml $O_2 \cdot$ m⁻¹ \cdot kg⁻¹, and the ordinate intercept was 5.1 ± 0.7 ml $O_2 \cdot$ min⁻¹ \cdot kg⁻¹.

Accumulated O_2 deficit. Work intensity during the high-speed run was $121 \pm 2.9\%$ $\dot{V}O_{2\max}$. Calculated O_2 demand during the 2-min high-speed run was 348.7 ± 18.3 ml/kg. FL-treated horses carried 16.1 ± 1.6 kg of weight during the exercise test. Body weight of horses 3.75 h after furosemide or saline administration was

401 ± 7, 391 ± 8, and 393 ± 8 kg for C, FU, and FL, respectively. $\dot{V}O_2$ ($\text{ml} \cdot 2 \text{ min}^{-1} \cdot \text{kg}^{-1}$) during the high-speed run was significantly affected by furosemide administration and weight carriage ($P < 0.02$; Fig. 1). Furosemide administration (FU) increased ($P < 0.05$) the mass-specific $\dot{V}O_2$ rate, whereas the combination of furosemide treatment and weight carriage (FL) did not affect the mass-specific rate of $\dot{V}O_2$ compared with saline treatment. Accumulated O_2 deficit was significantly ($P < 0.05$) lower after furosemide administration (FU) than after saline treatment (Fig. 2). There was no effect ($P > 0.05$) of the combination of furosemide treatment and weight carriage (FL) on accumulated O_2 deficit compared with saline treatment.

Absolute $\dot{V}O_2$ (liters of O_2 per horse) during the high-speed test for FU and FL did not differ significantly ($P > 0.05$) from C: 103 ± 4, 104 ± 4, and 99 ± 4 l $O_2/2 \text{ min}$ for C, FU, and FL treatments, respectively.

The proportion of energy derived from anaerobic sources, calculated as the difference between estimated and actual $\dot{V}O_2$ during the high-speed run, differed significantly ($P < 0.03$) among treatments. Anaerobic energy sources supplied 25.6 ± 2.0, 22.6 ± 2.3, and 27.0 ± 2.1% of energy during the high-speed run after C, FU, and FL treatments, respectively. Anaerobic energy sources supplied significantly less energy during the FU trial than during the C or FL trials.

Plasma lactate, hematocrit, and total protein. Hematocrit and plasma total protein concentration were significantly ($P < 0.01$) greater after furosemide treatment (FU and FL) than after the control treatment (Figs. 3 and 4). Plasma lactate concentration, after correction for furosemide-induced hemoconcentration, was significantly ($P < 0.05$) lower during recovery after FU treatment than after C or FL treatments (Fig. 5). The rates of increase in blood lactate concentration of C and FU, but not of FL, were significantly different ($P < 0.05$) during the high-speed test: 0.061 ± 0.001, 0.058 ± 0.001, and 0.061 ± 0.001 mmol/kg for C, FU, and FL, respectively.

DISCUSSION

This study demonstrated that administration of furosemide decreased the accumulated O_2 deficit and rate

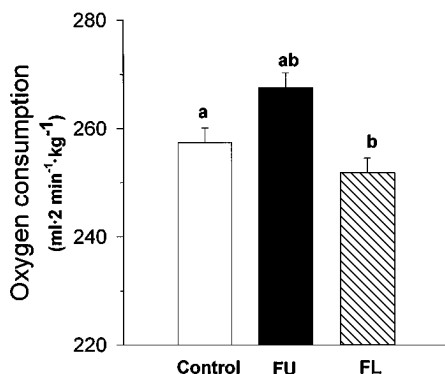


Fig. 1. O_2 consumption (means ± SE) of 9 horses during a high-speed exercise test after control (C) treatment, treatment with furosemide and weight carriage (FL), and treatment with furosemide alone (FU). Values with similar superscripts (a, b) differ significantly ($P < 0.05$).

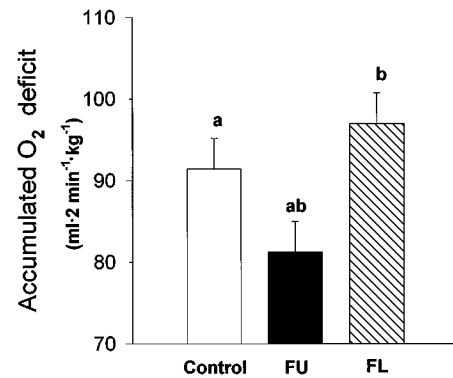


Fig. 2. Accumulated O_2 deficit in 9 horses during an intense exercise test. See legend of Fig. 1 for further explanation.

of increase of blood lactate concentration of horses during brief high-intensity exertion. The reduction in accumulated O_2 deficit in furosemide-treated horses that did not carry added weight was attributable to an increase in the mass-specific rate of $\dot{V}O_2$ during the high-intensity exercise test. Therefore, acute weight reduction, with preservation of the maximal rate of aerobic metabolism (expressed as liters of O_2 per individual) resulted in a reduction in energy supplied by anaerobic metabolism during brief intense exertion of defined duration. Addition of weight increased the accumulated O_2 deficit and rate of increase in blood lactate concentration, suggesting that the effect of furosemide was mediated by weight reduction. If the duration of intense exertion is limited by anaerobic capacity, then acute weight reduction may exert an ergogenic effect by decreasing the anaerobic cost of exertion at a given speed, provided that the absolute aerobic capacity of the individual is maintained.

Addition of weight to various quadrupedal species, including horses, increases the energy cost of locomotion at speeds below that eliciting $\dot{V}O_{2\text{max}}$ (26, 28). Although the energy cost of locomotion above $\dot{V}O_{2\text{max}}$ has not been precisely defined, it is generally assumed that the energy (expressed as O_2 equivalent)-speed relationship developed for submaximal exercise applies during supramaximal exercise (13, 19). We are not aware of studies that have examined the effect of acute weight reduction on the energy cost of locomotion at

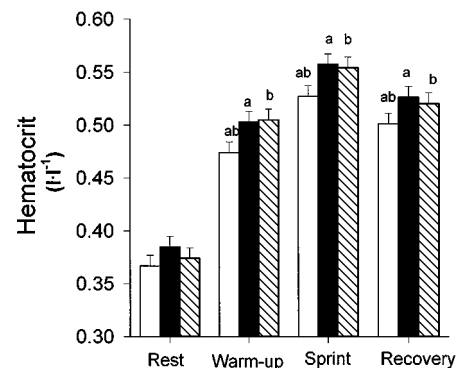


Fig. 3. Hematocrit of 9 horses during an intense exercise test. Open bars, control; filled bars, furosemide unloaded; hatched bars, furosemide loaded. See legend of Fig. 1 for further explanation.

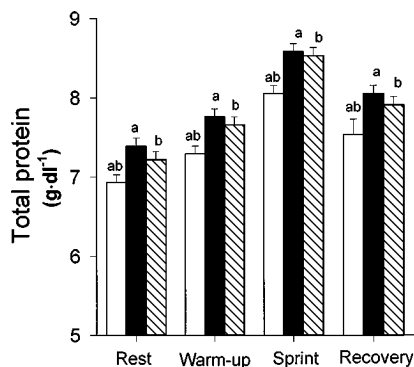


Fig. 4. Plasma protein concentration of 9 horses during an intense exercise test. See legends of Figs. 1 and 3 for further explanation.

intensities above $\dot{V}O_{2\max}$. Our results suggest that the acute weight reduction induced by furosemide is responsible for the lower O_2 deficit of horses during brief intense exercise. Although furosemide has potent dose-dependent hemodynamic effects in stationary and running horses (16, 21, 22), furosemide administration did not affect the maximal aerobic capacity of the horse, as indicated by similar absolute (liters of O_2 per horse) $\dot{V}O_2$ rates of horses after each treatment during the high-speed test. Therefore, the reduction in O_2 deficit appears to be due to a decrease in body weight, and therefore absolute O_2 demand, with no reduction in the absolute maximal aerobic capacity of the horse. The effect of the decrease in body weight is to reduce the difference between the absolute O_2 demand and the absolute $\dot{V}O_2$, hence reducing the absolute O_2 deficit. O_2 deficit, in relative and absolute terms, is reduced by the reduction in body weight.

The effect of furosemide to reduce accumulated O_2 deficit is consistent with the reduced rate of increase in blood lactate concentration measured in the furosemide-treated unloaded horses. A lower accumulated O_2 deficit implies a lesser requirement for energy production from anaerobic sources, which is consistent with the lower rate of increase in blood lactate concentration.

Furosemide, a potent diuretic, is administered to performance horses as prophylaxis for exercise-induced pulmonary hemorrhage (17). Because of the widespread use of furosemide in racehorses, there is concern that furosemide may alter athletic performance of

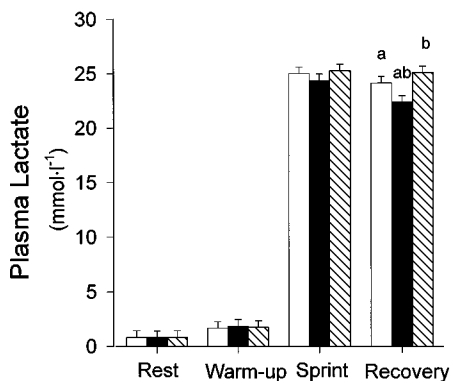


Fig. 5. Plasma lactate concentration of 9 horses during an intense exercise test. See legends of Figs. 1 and 3 for further explanation.

these animals separate from any effect on the incidence or severity of exercise-induced pulmonary hemorrhage, a putative performance-limiting condition (25). Addition of weight equal to that of the furosemide-induced acute weight loss prevented the effect of furosemide administration on O_2 deficit and rate of increase in blood lactate concentration. This suggests that any ergogenic effect of furosemide may be attributable to its reduction of body weight, although other mechanisms, such as altered acid-base status, cannot be ruled out.

Our values for O_2 deficit are similar to those previously reported for exhaustive exercise in horses (128 ml O_2 equivalents/kg) (23) but are substantially greater than the maximal accumulated O_2 deficit of 32 ml O_2 equivalents/kg reported by other investigators for Thoroughbred horses (6). The reason for the differences between the values for accumulated O_2 deficit reported by us and Rose et al. (23) and those reported by Eaton et al. (6) is not apparent.

A methodological concern with estimation of the O_2 deficit is that the $\dot{V}O_2$ -speed relationship developed for work intensities below $\dot{V}O_{2\max}$ may not be valid for work intensities above $\dot{V}O_{2\max}$. This issue is addressed elsewhere (13) but does not seem to have been problematic in the estimation of maximal accumulated O_2 deficit of horses or humans (6, 19, 23). A related concern in this study is that furosemide may have altered the $\dot{V}O_2$ -speed relationship. We previously demonstrated that furosemide does not alter the aerobic efficiency (ml $O_2 \cdot kg^{-1} \cdot m^{-1}$) of running, as demonstrated by the virtually identical $\dot{V}O_2$ of horses at various intensities during incremental exertion, including that producing peak $\dot{V}O_2$ (15). Therefore, our estimates of the $\dot{V}O_2$ -speed relationship developed in the untreated horses should be valid for use in furosemide-treated horses.

Another concern is that furosemide may have altered $\dot{V}O_{2\max}$ and therefore the intensity at which the high-speed test was performed. Bayly et al. (3) reported that furosemide increased peak or maximal $\dot{V}O_2$, whereas others have not detected an effect of furosemide on mass-specific peak $\dot{V}O_2$ of horses (15, 18). Furosemide does not affect or decreases $\dot{V}O_{2\max}$ in humans (1, 2, 4). However, an effect of furosemide on $\dot{V}O_{2\max}$ does not affect interpretation of the results of this study. The variable of primary interest was the O_2 deficit incurred during a high-speed test of defined duration. The choice of speed was determined to be the speed at which the horses could just complete the high-speed test, not the speed associated with a specific relative work intensity. The primary dependent variable was therefore not dependent on the relative intensity of the exertion, but rather on the absolute work intensity.

In conclusion, the choice of exercise test was based on our hypothesis that furosemide would reduce the O_2 deficit during an exercise test of defined duration. This hypothesis was chosen because athletic capacity of horses to which furosemide is administered often is judged by their performance in races of ~ 2 -min duration. The intensity at which the horses in this study completed their high-speed test ($120\% \dot{V}O_{2\max}$) is similar to that at which racehorses compete (5). It should be emphasized

that we did not measure athletic or anaerobic capacity of the horses in this study and therefore did not demonstrate an ergogenic effect of furosemide.

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