Exercise-training-induced alterations in hepatic function in mares

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Dyke, T. M., R. A. Sams, and K. W. Hinchcliff. Exercise-training-induced alterations in hepatic function in mares. J. Appl. Physiol. 85(4): 1442–1447, 1998.—The effects of exercise training on hepatic function in horses were determined by studying the plasma clearance of antipyrine (20 mg/kg iv) in adult mares that either underwent treadmill training for 5 wk (n = 7) or remained in box stalls for the same time period (n = 6). Training consisted of treadmill exercise at 60% (12 min/day) and 90% (3 min/day) of pretraining maximal oxygen consumption (Vo2max) for 6 days/wk for 5 wk. V02max and velocity to obtain a blood lactate concentration of 4 mmol/l were significantly increased (from 129 to 149 ml·min⁻¹·kg⁻¹ and from 5.6 to 6.1 m/s, respectively) as a result of training. The plasma clearance and volume of distribution of antipyrine increased significantly in the trained group (from 5.5 to 6.4 ml·min⁻¹·kg⁻¹ and from 813 to 881 ml/kg, respectively) and decreased significantly in the untrained group. Elimination half-lives did not change as a result of training or box rest. Increases in plasma antipyrine clearance were indicative of an increase in hepatic metabolism of antipyrine. Increases in the volume of distribution of antipyrine suggest that total body water increases as a result of exercise training.

antipyrine; pharmacokinetics; total body water; horse
Animal studies. Thirteen healthy Standardbred mares, ranging from 6 to 9 yr of age and from 411 to 497 kg in weight, were used in these studies. They had spent the previous 3 mo in small paddocks (where they were observed to spend most daylight hours standing, walking, and occasionally cantering) or in box stalls (intermittently for no longer than 2 wk). All horses had been accustomed to treadmill exercise within 6–12 mo of this study. Horses were kept in box stalls for at least 14 days before antipyrine administration and were supplied with mixed grass hay ad libitum, crushed corn, trace mineral salt, and water. All horses were judged to be healthy based on a physical examination, clinical chemistry profile, and hemogram.

**Measurement of \(\dot{V}\text{O}_2\text{max} and the oxygen consumption (\(\dot{V}\text{O}_2\))-speed relationship**. The \(\dot{V}\text{O}_2\text{max} and the relationship between \(\dot{V}\text{O}_2\) and speed were determined for each horse during an incremental exercise test within 7 days of the experiment. The incremental exercise test consisted of each horse running on a treadmill (Sato, BIAB Industrial, Uppsala, Sweden) inclined at 4° for 90 s at 4 m/s, after which the treadmill speed was increased by 1 m/s every 90 s until the horse was unable to maintain its position on the treadmill. \(\dot{V}\text{O}_2\) was measured every 10 s during the exercise test with the use of an open-circuit calorimeter (Oxymax-L; Columbus Instruments, Columbus, OH) as previously described (13). \(\dot{V}\text{O}_2\text{max} was defined as the value at which \(\dot{V}\text{O}_2\) reached a plateau despite further increases in speed. A plateau was defined as a change in \(\dot{V}\text{O}_2\) of <4 ml·min\(^{-1}\)·kg\(^{-1}\) with an increase in speed.

For each horse, the relationship between treadmill speed and \(\dot{V}\text{O}_2\) was plotted (SigmaPlot; Jandel, San Rafael, CA). A regression equation was calculated for the linear part of the curve. The treadmill speeds that were expected to produce 60 and 90% \(\dot{V}\text{O}_2\text{max} were calculated for each horse.

Before exercise, during the last 15 s of exercise at each speed, and at 5 min after exercise, jugular venous blood was collected into lactate 2372 preservative tubes (Yellow Springs Instruments, Yellow Springs, OH) and frozen within 10 min at −20°C. Whole blood lactate concentrations were determined with the use of a lactate analyzer (1500L, Yellow Springs Instruments).

For each horse, the relationship between treadmill speed and whole blood lactate concentration was plotted, and a regression equation was calculated for the curvilinear portion of the curve and interpolated to provide a treadmill speed that produced a whole blood lactate concentration of 4 mmol/l (\(L\text{a}_\text{max}\)) for each horse (SigmaPlot). An estimate of maximal blood lactate concentration (\(L\text{a}_\text{max}\)) was recorded at 5 min after completion of exercise.

**Drug administration.** Antipyrine (Aldrich Chemical, Milwaukee, WI) was dissolved in 0.9% sodium chloride (Baxter Healthcare, Deerfield, IL) at a concentration of ~360 mg/ml and filtered through a 0.22-μm filter (Milllex-GV; Millipore Products Division, Bedford, MA). A dose of 20 mg/kg was injected into the left jugular vein over 30–45 s.

**Blood sampling.** Before drugs were administered, an intravenous catheter (Angiocath; Becton Dickinson Vascular Access, Sandy, UT) was inserted into the right jugular vein. Blood samples were taken before administration of the drug and at 5, 10, 15, 20, 30, and 45 min and 1, 2, 3, 4, 5, 6, 7, and 8 h after antipyrine was administered.

Blood samples were placed in heparinized vacuum collection tubes (Vacutainer collection tubes; Becton Dickinson, Rutherford, NJ), and plasma was harvested within 1 h of collection. Plasma samples were kept at −70°C until analysis.

**Urine sampling.** A Foley catheter was inserted into the urinary bladder of each mare. A urine sample was collected for 10–30 min before the drug was administered. After antipyrine was administered, urine samples were collected for 8 h. Hourly volumes were recorded, and 5- to 50-ml samples from each hourly collection were placed in acetate buffer (0.5 M sodium acetate; Jenneille Chemical, Cincinnati, OH) with 80 g/l sodium metabisulfite (Jenneille Chemical) in a 1:1 (vol/vol) ratio and kept at −70°C until analysis.

**Training protocol.** Horses were randomly allocated to two groups: group 1 underwent a training protocol; group 2 was untrained (control group) and was maintained in box stalls throughout the experiment. Group 1 horses underwent a training protocol consisting of treadmill exercise on 6 days/wk for 5 wk. Group 2 horses were untrained (control group) and were maintained in box stalls throughout the experiment. Exercise consisted of walking for 1 min at 2 m/s and then at 4 m/s for 1 min on a treadmill inclined at a 4° slope. Treadmill speed was increased from 60% \(\dot{V}\text{O}_2\text{max} for 10 min, at which point treadmill speed was increased to a speed calculated to require 90% \(\dot{V}\text{O}_2\text{max} for 3 min. The horses then ran at 60% \(\dot{V}\text{O}_2\text{max} for 2 min before walking at 2 m/s for 1 min. Total exercise time was 18 min, 13 min of which were at relatively high intensity. At all other times, the horses were in box stalls.

All horses were weighed weekly. At the end of the 5-wk period, all horses underwent an incremental exercise test as previously described. At least 24 h after the incremental exercise test, all horses were administered antipyrine as previously described.

**Determination of antipyrine concentrations in plasma.** The normal-phase HPLC method previously described (8) was used to determine the concentration of antipyrine in methylene chloride extracts of alkalized plasma samples, with the use of 4-aminotyramine as the internal standard.

**Determination of concentrations of antipyrine, norantipyrine, and 4-hydroxyantipyrine in urine.** The concentrations of antipyrine, norantipyrine, and 4-hydroxyantipyrine in urine samples were determined by a previously described reverse-phase HPLC analysis of acetonitrile extracts of acidified urine samples, with the use of phenacetin as the internal standard (8).

**Pharmacokinetic analysis.** Methods of analysis of plasma antipyrine concentration vs. time data and analysis of urinary excretion of antipyrine and metabolites data were identical to those previously reported (8).

**Statistical analysis.** Body weights, \(\dot{V}\text{O}_2\text{max}, and blood lactate concentrations were compared within and between groups by using two-way ANOVA and post hoc Student t-tests for paired and independent data, as appropriate. Antipyrine \(C_{\text{pl}}, V_{\text{dss}}, and renal excretion of drug and/or metabolite within and between groups were compared by using Wilcoxon signed-rank tests and Mann-Whitney rank-sum tests, respectively. A P value of <0.05 was considered significant.

**RESULTS**

Although 16 horses began the study, the results of two horses were not included because the horses became lame during training and were removed from the trial. Another horse (150) was initially included in group 2 and then was moved to group 1 for training. We were unable to perform \(\dot{V}\text{O}_2\) tests on horse 46, as it became agitated when the face mask was placed on the face. Therefore, \(\dot{V}\text{O}_2\text{max} and \(\dot{V}\text{O}_2\)-speed regressions were
not completed for this horse, and the speeds estimated
to require 60 and 90% \( \dot{VO}_{2\text{max}} \) for this horse were
extrapolated from values calculated for other horses.
Because blood lactate data were similar to those of the
other horses, deviations in the initial protocol are not
expected to have influenced the results. There were no
significant differences between the trained and un-
trained group at the start of the study with respect to
body weight (456 ± 453 kg, respectively) or \( \dot{VO}_{2\text{max}} \)
(129 ± 9.5 vs. 121 ± 12.2 ml·min\(^{-1} \)·kg\(^{-1} \), respectively).
In addition, there were no differences in \( \text{Cl}_p \) (6.0 vs. 5.0
ml·min\(^{-1} \)·kg\(^{-1} \), respectively) or \( V_{\text{dss}} \) (877 vs. 846 ml/kg,
respectively) of antipyrine between groups at the start
of the study.
In the trained group, body weights decreased an
average of 5% [from 456 ± 25.8 (SD) to 432 ± 24.2 kg].
In the untrained group, body weights decreased an
average of 2%, from 453 ± 30.6 to 445 ± 30.7 kg. Body
weight decreases in the trained group were signifi-
cantly different from those in the untrained group (\( P <
0.005 \)). \( \dot{VO}_{2\text{max}} \) increased after training by 15%, which
was significantly different from an increase of 2% in the
untrained group (\( P = 0.004 \); Fig. 1). Changes in the
treadmill velocity needed to produce a \( V_{\text{La4}} \) in the
trained group were significantly greater after 5 wk
than in the untrained group (9 vs. 4%, respectively; \( P =
0.035 \); Fig. 1). Initially \( V_{\text{La4}} \) was 5.6 ± 0.76 and 4.8 ±
0.52 m/s in the trained and untrained groups, respec-
tively. Changes in blood [La\(_{\text{max}}\)] were not significantly
different between groups (\( P = 0.836 \)).
Mean plasma concentrations of antipyrine vs. time in
both groups (Fig. 2) were representative of data from
each horse. \( \text{Cl}_p \) of antipyrine significantly increased
(from 5.5 to 6.4 ml·min\(^{-1} \)·kg\(^{-1} \), before and after training,
respectively; \( P = 0.031 \)). In the untrained group,
\( \text{Cl}_p \) was significantly less after 5 wk of box rest (5.0 vs.
4.2 ml·min\(^{-1} \)·kg\(^{-1} \); \( P = 0.031 \); Table 1 and Fig. 3). Percent
differences in \( \text{Cl}_p \) before and after the training
period were significantly different between the trained
group (median, +16%) and the untrained group (me-
dian, −12%; \( P = 0.001 \)). When \( \text{Cl}_p \) was not normalized
for body weight (that is, expressed as liters per minute),
similar differences in the two groups were still ob-
served. There was no apparent relationship between
the relative change in \( \dot{VO}_{2\text{max}} \) during training or
rest and the corresponding relative change in \( \text{Cl}_p \) of
antipyrine (\( r < 0.4 \)).
Median \( V_{\text{dss}} \) of antipyrine increased as a result of
training (from 813 to 881 ml/kg; \( P = 0.047 \)) and
decreased significantly in the untrained group (from
846 to 745 ml/kg; \( P = 0.031 \); Table 1 and Fig. 3). Percent
differences in \( V_{\text{dss}} \) after the 5-wk period were signifi-
cantly different between the trained group (+8%) and
the untrained group (−12%; \( P = 0.008 \)). Similar results
were seen when \( V_{\text{dss}} \) was not normalized for body
weight. Elimination half-lives were not significantly
different from each other, either within a group or
between groups (\( P > 0.7 \); Table 1 and Fig. 3).
There was no effect of training or rest on the percent
difference in the excretion of norantipyrine, antipyrine,
4-hydroxyantipyrine, or total excretion of metabolites
(Table 2).
DISCUSSION

The purposes of our study were to determine the effects of an intense 5-wk exercise training period on hepatic function by assessing changes in the hepatic clearance and oxidative metabolism of antipyrine. We demonstrated that 5 wk of training, at submaximal intensities led to a 15% increase in V˙O₂max and a9 % increase in VLa4. This was associated with a 16% increase in Clp of antipyrine and an 8% increase in Vdss of antipyrine; this suggests that both hepatic function and total body water are elevated by training. These changes were significantly greater than those seen in the untrained group. Other studies have demonstrated increases in V˙O₂max during training (12, 14), but an untrained control group was not included in those studies. Clp of antipyrine increased in the trained group by 7% and decreased in the untrained group by 12%.

We suggest that training led to an increase in the oxidative metabolic capacity of the liver to metabolize antipyrine and that a decrease in this capacity occurred in the other group of horses as a result of box rest. The endogenous biochemical stimuli that induce hepatic oxidative capacity during training have not been identified. Expression of cytochrome P-450 proteins can be induced by various factors, including age, gender, disease, and exposure to chemicals, that lead to increased numbers of enzyme molecules and capacity for metabolism by that isozyme. An inducing agent may produce a specific pattern of isozyme synthesis rates, resulting in different isozyme expression and individual changes in rates of metabolism by different isozymes (17).

Table 1. Estimates of pharmacokinetic parameters of antipyrine for trained and untrained horses after intravenous administration of antipyrine before and after 5-wk period of training or box stall rest

<table>
<thead>
<tr>
<th>Variable</th>
<th>Trained, n = 7 mares</th>
<th>Untrained, n = 6 mares</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>C₁, µg/ml</td>
<td>50.3 ± 31.2</td>
<td>57.2 ± 38.6</td>
</tr>
<tr>
<td>C₂, µg/ml</td>
<td>20.8 ± 2.3</td>
<td>19.0 ± 2.3</td>
</tr>
<tr>
<td>λ₁, h</td>
<td>15.6 ± 1.34</td>
<td>18.8 ± 17.7</td>
</tr>
<tr>
<td>λ₂, h</td>
<td>0.39 ± 0.09</td>
<td>0.40 ± 0.08</td>
</tr>
<tr>
<td>AUC, µg·h·ml⁻¹</td>
<td>55.6 (38.7–86.2)</td>
<td>51.9 (35.4–77.0)*</td>
</tr>
<tr>
<td>AUMC, µg·h·ml⁻¹·kg⁻¹</td>
<td>138 (68.9–275)</td>
<td>126 (61.9–252)</td>
</tr>
<tr>
<td>Clp, ml·min⁻¹·kg⁻¹</td>
<td>5.5 (3.9–8.6)</td>
<td>6.4 (4.3–9.4)*</td>
</tr>
<tr>
<td>Vdss, ml/kg</td>
<td>813 (670–1,084)</td>
<td>881 (733–1,118)*</td>
</tr>
<tr>
<td>Elimination half-life, h</td>
<td>1.7 (1.3–2.6)</td>
<td>1.8 (1.3–2.4)</td>
</tr>
</tbody>
</table>

Values are means ± SD or medians (with range in parentheses) after administration of antipyrine (20 mg/kg iv) before and after 5-wk period of training (Trained) or box stall rest (Untrained). C₁ and C₂, y-intercepts; λ₁ and λ₂, rate constants of nonlinear regression of plasma antipyrine concentration vs. time data; AUC, area under plasma antipyrine concentration vs. time curve; AUMC, area under first moment curve; Clp, plasma antipyrine clearance; Vdss, steady-state volume of distribution of antipyrine. *P < 0.05 compared with initial values.

Fig. 3. Plasma clearance (Clp), steady-state volume of distribution (Vdss), and elimination half-life of antipyrine for horses before and after a 5-wk period of training or box stall rest (n = 6 mares). *P < 0.05 compared with initial values.

Table 2. Urinary excretion of norantipyrine, antipyrine, and 4-hydroxyantipyrine in horses 8 h after administration of antipyrine (20 mg/kg iv) before and after a 5-wk period of training or box rest

<table>
<thead>
<tr>
<th>Norantipyrine</th>
<th>Antipyrine</th>
<th>4-Hydroxyantipyrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Trained, n = 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>344 (212–574)</td>
<td>264 (160–336)</td>
<td>51 (21–113)</td>
</tr>
<tr>
<td>Untrained, n = 6</td>
<td></td>
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</table>

Values are medians, with range in parentheses; n, no. of mares.
evidence that hepatic mRNA activity for cytochrome P-450 isoform enzymes changes during training in rats (6). This would result in changes in the maximal capacity of the cytochrome P-450 enzyme pathway, resulting in altered ability of the liver to metabolize certain drugs. In addition, it has been shown that the activity of oxidative enzymes in skeletal muscle (such as citric synthase) increases with training of horses (1). The training-induced increase in the Clp of antipyrine observed in our study demonstrates that training can alter hepatic enzyme function in horses and that the effects of training are not limited to the cardiorespiratory or musculoskeletal systems.

Training also led to an increase of ~8% in the Vdss of antipyrine in our study, presumably because of an increase in the amount of total body water or an increase in total body water relative to body weight (liters/kg body weight). Conversely, box rest led to a 12% decrease in antipyrine Vdss. Training-induced changes in body mass, percent lean body mass, or body fluid compartments have not been studied in horses, except for plasma volume. Plasma volume increased 20–30% after training (14, 15), but our study is the first to document increases in total body water (the putative Vdss of antipyrine) as a result of training.

Elimination half-life is dependent on Vdss and Clp, and this relationship can be expressed as

\[ \text{Elimination half-life} = \frac{(\ln 2 \times \text{Vdss})}{\text{Clp}} \]

The combined increase in the Clp and Vdss of antipyrine had a minimal effect on the elimination half-life of antipyrine in our study; therefore, plasma concentrations of antipyrine were minimally affected by training. Nevertheless, the training-induced increases in Clp and Vdss may have practical consequences. Calculations of loading doses of drugs are dependent on Vdss, and calculations of maintenance doses are dependent on Clp. For therapeutic drugs with kinetic parameters similar to those for antipyrine, both the loading dose and maintenance dose may have to be increased in animals that are exercise trained.

Antipyrine was selected as the model drug for examination of hepatic oxidative metabolism because of its pharmacokinetic properties, including low hepatic extraction, metabolism by numerous cytochrome P-450 pathways, and rapid excretion of metabolites into urine. In the horse, antipyrine is metabolized to at least two metabolites, norantipyrine and 4-hydroxyantipyrine (8). The urinary excretion of both of these metabolites was studied, because production of metabolites was not sufficient to lead to measurable plasma concentrations of major antipyrine metabolites. Demethylation and hydroxylation are involved in the production of these metabolites; therefore, at least two different cytochrome P-450 pathways must be involved in the metabolism of antipyrine. There is evidence in other animal species that certain factors (including other drugs, diet, and environmental factors) can induce individual cytochrome P-450 pathways differently, but this differential induction may be subtle and may not be detected when only plasma concentrations of antipyrine are studied.

Administration of 3-methylcholanthrene to rats increased the formation rate of 4-hydroxyantipyrine and norantipyrine but decreased the formation of 3-hydroxymethylantipyrine (11). However, administration of either phenobarbitone or antipyrine leads to increased formation rate of norantipyrine but not 4-hydroxyantipyrine or 3-hydroxymethylantipyrine (5). In our study, we demonstrated a training-induced increase in antipyrine Clp but did not demonstrate increased formation rates of 4-hydroxyantipyrine or norantipyrine. Therefore, although training increased antipyrine clearance, we did not identify an increase in any one pathway.

Unlike antipyrine recovery in other animal species, only a small percentage of the dose of antipyrine administered is recovered as antipyrine or metabolites in urine of horses (8). This species difference and the considerable interindividual variability in metabolite excretion observed in our studies may make it difficult to detect changes in the urinary excretion of antipyrine metabolites in horses. The biliary excretion of antipyrine and its metabolites in horses has not been investigated, but excretion is likely to be a major route of elimination of antipyrine. Training-induced increases in Clp of antipyrine may be reflected in changes in biliary excretion rather than in urinary excretion of antipyrine metabolites.

This model has some limitations. We used a relatively brief period (5 wk of training) and a constant relative amount of work/wk. However, this period and intensity of training were sufficient to cause training effects, as shown by increases in VO2max and VLa4,1. In addition, the period of acclimation to box stalls was only 2 wk before antipyrine administration. Our horses had minimal paddock activity before this acclimation period, but there is evidence to suggest that horses detrain very slowly and may require many months of rest before they are considered unfit (3). We suggest that, to avoid potential variability caused by previous training, horses should be detrained for extended periods of time before drugs are administered in further studies. To account for these potential confounding variables, we included a control group of horses that were kept in box stalls for the 5-wk period. Over this period of rest, antipyrine Clp and Vdss of antipyrine decreased in these horses, thus demonstrating an effect of inactivity on hepatic function and total body water. Interestingly, significant changes in VO2max and VLa4 were not seen during this 5-wk rest period.

Although training altered the hepatic clearance and Vdss of antipyrine, it would be unrealistic to predict the effects of training on other drugs that are eliminated by hepatic clearance. The hepatic clearance of other drugs that differ greatly from antipyrine with respect to distribution, metabolism, and excretion would not be expected to correlate closely with the hepatic clearance of antipyrine. The purpose of this study was to determine whether exercise training could influence the hepatic metabolism of a model substance, thereby demonstrating that training could affect hepatic function in horses. Caution must be used in extrapolation of these results to predict the training-induced effects on
the hepatic clearance of other drugs in horses, and such extrapolation was not the purpose of this study.

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