

Effect of a tart cherry juice blend on exercise-induced muscle damage in horses

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Objective—To evaluate whether administering a tart cherry juice blend (TCJB) prior to exercise would reduce skeletal and cardiac muscle damage by decreasing the inflammatory and oxidative stress response to exercise in horses.

Animals—6 horses.

Procedures—Horses were randomly allocated into 2 groups in a crossover study with a 2-week washout period and orally administered either TCJB or a placebo solution (1.42 L, twice daily) in a double-masked protocol for 2 weeks prior to a stepwise incremental exercise protocol. Horses were tested for serum activities of creatine kinase and aspartate aminotransferase (AST) and concentrations of cardiac troponin I (cTnI), thiobarbituric acid reactive substances (TBARS; an indicator of oxidative stress), and serum amyloid A (SAA; an indicator of inflammation). To ensure that treatment would not result in positive results of an equine drug-screening protocol, serum samples obtained from each horse prior to and after 2 weeks of administration of TCJB or the placebo solution were tested.

Results—All horses had negative results of drug screening at both sample times. The exercise protocol resulted in a significant increase in TBARS concentration, SAA concentration, and serum AST activity in all horses. Administration of TCJB or placebo solution was not associated with an effect on malondialdehyde or SAA concentrations. However, administration of TCJB was associated with less serum activity of AST, compared with administration of placebo solution.

Conclusions and Clinical Relevance—Administration of TCJB may diminish muscle damage induced by exercise. (*Am J Vet Res* 2009;70:758–763)

It has been established that exercise causes oxidative stress in humans^{1,2} and rats.³ Therefore, reducing oxidative stress (ie, free radical production) and inflammation during exercise may improve recovery. This has led to several investigations evaluating the effect of antioxidant supplementation in humans during exercise.^{4–18} Several studies^{4–11} have revealed reductions in markers of oxidative stress with antioxidant supplementation, compared with placebo treatments. However, some studies^{15–18} have detected no effect of antioxidant supplementation on markers of running-induced oxidative stress. Most studies^{5,6,16,17,19,20} have detected no effect of antioxidant supplementation on markers of muscle damage after running, although some have.^{18,21} Several studies^{6,7,17,19,22} have detected no effect of antioxidant supplementation on markers of inflammation after pro-

ABBREVIATIONS

AST	Aspartate aminotransferase
CK	Creatine kinase
cTnI	Cardiac troponin I
SAA	Serum amyloid A
TBARS	Thiobarbituric acid reactive substances
TCJB	Tart cherry juice blend

longed running. Ingestion of a TCJB reduced markers of exercise-induced muscle damage in humans.²³ The actions of cyclooxygenase-inhibiting flavonoids^{24,25} and anthocyanins associated with high antioxidant and anti-inflammatory activities of tart cherries^{24,26,27} were thought to be responsible for this effect, although this was not investigated.

Horses in various athletic competitions, such as endurance horses^{28–30} and racehorses,^{31,32} are exposed to oxidative stress and muscle damage. Shortening the recovery time after an exercise event would yield a substantial advantage to owners, trainers, and horses. Certain markers have been used in horses to estimate oxidative stress and inflammation. The TBARS test measures lipid peroxidation that results from oxidative stress, which results in formation of free radicals that react intracellularly to form other substances, such as malondialdehyde.^{29,32,33} Such substances can cause cellular damage that varies from mild to severe. Many al-

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dehydes formed during lipid peroxidation, particularly malondialdehyde, are used for estimation of lipid peroxidation in biological membranes.³⁴

Although plasma fibrinogen concentration has been used as a marker of exercise-induced inflammation in horses, the time between onset of inflammation and increases in plasma fibrinogen limits this marker's usefulness.³⁵⁻³⁷ Serum amyloid A has been identified^{38,39} as an inflammation marker that correlates well with clinical conditions in humans, and use of SAA concentration was recently validated for clinical application in horses with bacterial pneumonia.⁴⁰ Acute-phase proteins such as SAA have been proposed as the most sensitive indicators of inflammation in horses.^{38,39}

The purpose of the study reported here was to determine whether administering a TCJB to horses prior to exercise would reduce skeletal and cardiac muscle damage by influencing the inflammatory and oxidative stress response to exercise. A secondary objective was to determine whether administration of a TCJB would cause a positive test result in a routine equine drug-screening protocol.

Materials and Methods

Experimental design—A heparinized blood sample for drug screening^{a,b} was obtained from the left jugular vein of each horse upon entry into the study. Horses were then randomly allocated to receive either TCJB or placebo solution orally twice daily for 14 days. On day 14, a second blood sample for drug screening was obtained before submitting horses to a stepwise incremental exercise protocol. In addition, blood samples were obtained and separated into 4 aliquots to measure markers of skeletal muscle damage (via serum activities of CK and AST), oxidative stress (via the measurement of TBARS), and inflammation (via concentration of SAA). These blood samples were obtained prior to exercise, at each exercise intensity level, hourly after exercise for 4 hours, and then daily for 5 days. Finally, heparinized blood samples were obtained for assessment of cardiac muscle damage (via plasma concentration of cTnI) prior to exercise and 1, 3, 24, 48, 72, and 96 hours after exercise. After a 2-week washout period, each horse received the alternative treatment (TCJB or placebo) and the experiment was repeated.

Horses—Six unfit (ie, not in a training program) adult (mean age, 10 years; range, 5 to 17 years) sexually intact female horses (5 Thoroughbreds and 1 Warmblood [mean \pm SD weight, 493 \pm 33 kg]) were used in the study. The horses were determined to be in good condition via physical examination, video endoscopic examination of the upper portion of the respiratory tract, and evaluation of a CBC. Horses were kept in box stalls and were walked daily at 1.8 m/s for 1,600 m to become acclimatized to the treadmill and its surroundings. All procedures complied with federal and state regulations and approved local institutional animal care and use procedures.

Exercise protocol—A standardized exercise test reported to increase CK and AST in horses was used.⁴¹ Briefly, at time 0, the treadmill was started and accelerated to 4 m/s. At 2 minutes, the treadmill was inclined to a 6.3% slope. At 4 minutes, the treadmill was accel-

erated to 6 m/s and kept at that speed for 1 minute. At each subsequent minute, the treadmill was accelerated by 1 m/s until the horse was no longer capable of maintaining its position near the front of the treadmill.

Solutions—Solutions (1.42 L of a proprietary TCJB^c or a placebo [1.42 L of a cherry-flavored solution]) were administered orally twice daily until the morning of the exercise test. Each solution had a 13% sugar content and was mixed with 2.7 kg of a concentrate (14% protein and 6% fat) for voluntary consumption by each horse.^d In addition, horses were fed a timothy-clover grass hay mix.

cTnI, CK, and AST analyses—One sample per sampling time was analyzed for cTnI concentration in a validated point-of-care machine^e by use of a 2-site ELISA that uses 2 monoclonal antibodies (caprine and murine) directed against human cTnI. Analytic sensitivity of the assay is 0.02 ng/mL. The reference range in horses is 0.00 to 0.06 ng/mL.^f Sera for determination of CK and AST activities were submitted directly to the Clinical Pathology Laboratory at Cornell University for standard analyses.^g

TBARS test—Concentration of malondialdehyde was estimated by the measurement of TBARS. Standards and plasma samples were prepared according to directions of the assay kit,^h and absorbance was measured in triplicate at 532 nm.ⁱ

SAA—The SAA concentration was measured in duplicate by use of the latex agglutination method. Equine standard sera with SAA concentrations ranging from 0.8 to 400.0 μ g/mL that were produced according to standard methods were used.⁴²⁻⁴⁴ The assay was performed on an automated analyzer^j with polyclonal rabbit and monoclonal murine antibodies covalently bound to polystyrene latex particles.^k The assay coefficients of variation at 35.5 μ g/mL ($n = 20$ samples) and 257.3 μ g/mL (20) were 0.9% and 0.8%, respectively.⁴⁰ The assay detection limit was 0.1 μ g/mL.

Statistical analysis—To control for variation among horses in the study and meet the assumptions of normality, response to administration of placebo solution or TCJB was measured as the difference from baseline values. To ensure comparability between the treatment and control groups prior to exercise, the outcomes (activities or concentrations of CK, AST, cTnI, TBARS, and SAA) were compared in samples obtained immediately prior to exercise by use of a 2-sample *t* test. An overall effect of treatment on each of these variables, while controlling for sampling time, was assessed by use of regression analysis with appropriate transformation (if deemed necessary). Thereafter, the experimental period was stratified into 2 phases (exercise phase and recovery phase) and stratified analysis was performed to determine whether the concentrations or enzyme activities measured during the 2 phases differed. Results are expressed as mean \pm SEM values. For all comparisons, a value of $P < 0.05$ was considered significant.

Results

All horses consumed all of the feed mixture containing the TCJB or placebo solution. Some stall wall

staining as well as red discoloration of the nose was evident in 5 of the 6 horses during administration of both solutions. Volume loss was estimated to be < 30 mL (approx 2% daily) for the placebo and TCJB solutions. No adverse effects were detected. There were no differences immediately prior to exercise between the 2 groups in any of the variables measured. During exercise, the maximum speeds reached after administration of the TCJB (mean, 11.3 ± 0.5 m/s) and the placebo solution (mean, 11.7 ± 0.7 m/s) were not significantly different. Maximum heart rates during each trial after TCJB (mean, 206 ± 4.7 beats/min) and placebo administration (mean, 212 ± 5.6 beats/min) were also not significantly different. By use of the equine drug-screen panel, no positive results were detected at entry into the study or after 2 weeks of administration of TCJB or placebo solution.

cTnI, CK, and AST—There was no significant effect of treatment or exercise on cTnI concentration in either group (Figure 1; Table 1). There was great variation in exercise-induced muscle damage among horses (Figures 2 and 3). The time to peak increase of AST

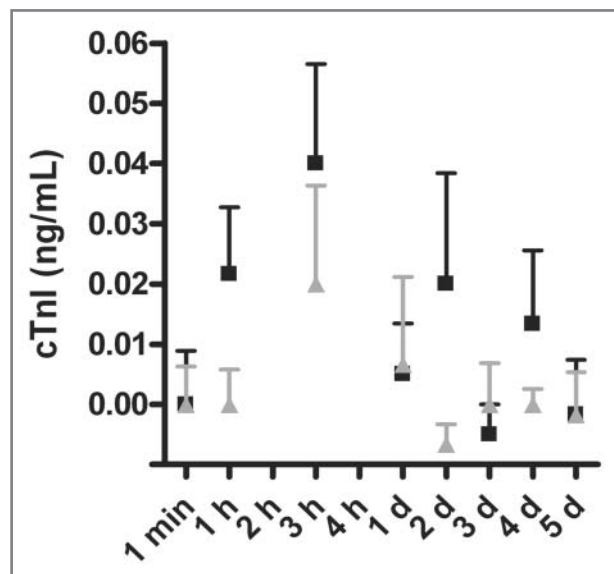


Figure 1—Mean ± SEM concentrations of cTnI in 6 horses treated orally with TCJB (gray triangles) or a placebo solution (black squares) for 2 weeks before strenuous treadmill exercise. Values on the x-axis correspond to sampling times after horses attained certain speeds on the treadmill and after completion of the exercise protocol at various times.

Table 1—Results of linear regression analysis of variables measured during strenuous treadmill exercise and a 5-day recovery period for 6 horses treated orally with TCJB or a placebo solution for 2 weeks prior to exercise.

Variable	Constant (intercept)	Effect of treatment (P value) ^a	Regression coefficient for sampling time (P value)
CK	-294.122	606.499 (0.063) ^a	42.8607 (0.090) ^a
AST	-102.228	122.558 (0.017) ^a	12.9030 (0.001)
cTnI	0.00450	0.00167 (0.737)	0.00001 (0.894)
TBARS	0.11909	0.42071 (0.104)	0.14867 (< 0.001)
SAA	-0.13236	0.06956 (0.565)	0.02344 (0.014)

^aP value for test of variable significance.

activity was unaffected by treatment (placebo, 1,211 ± 686 minutes; TCJB, 490 ± 304 minutes). Activity of AST increased significantly (*P* = 0.011) with time, principally because of increase during the recovery phase. Compared with the control group, there was significantly less exercise-induced increase in AST activity, as measured from baseline, in TCJB-treated horses (*P* = 0.017). When the analysis was stratified, this difference was significant during the exercise (*P* = 0.009) and recovery (*P* = 0.014) phases.

Similarly, the time to peak increase of CK activity was unaffected by treatment (placebo, 180 ± 40.0 minutes; TCJB, 159 ± 49.6 minutes [*P* = 0.2]). During the exercise phase, there was a mild increase in CK activity over time (*P* < 0.001) and no treatment effect was detected. As expected, most of the increase in CK activity occurred during the recovery phase in both groups, and horses treated with TCJB had an increase of lesser magnitude than control horses, although the difference did not reach significance (*P* = 0.054).

TBARS test—The exercise protocol resulted in oxidative stress, as measured via TBARS concentration,

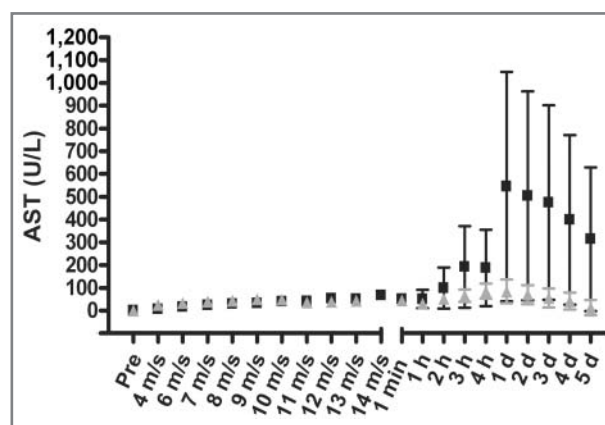


Figure 2—Mean ± SEM activities of AST (referenced to preexercise [Pre] value) in the same horses as in Figure 1. The x-axis is divided, representing samples obtained during the exercise phase (left segment) and the recovery phase (right segment). See Figure 1 for remainder of key.

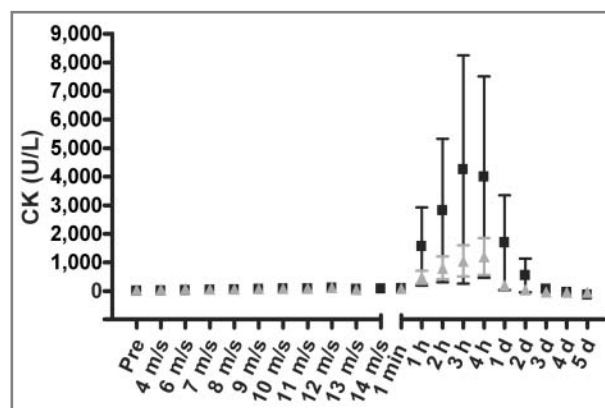


Figure 3—Mean ± SEM activities of CK (referenced to preexercise value) in the same horses as in Figure 1. The x-axis is divided, representing samples obtained during the exercise phase (left segment) and the recovery phase (right segment). See Figure 1 for remainder of key.

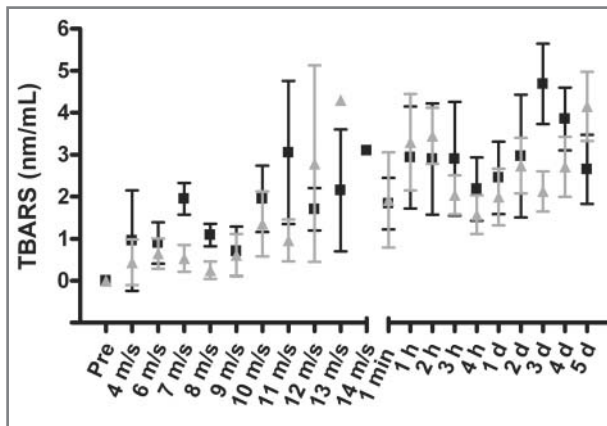


Figure 4—Mean \pm SEM concentrations of TBARS (referenced to preexercise value) in the same horses as in Figure 1. The x-axis is divided, representing samples obtained during the exercise phase (left segment) and the recovery phase (right segment). See Figure 1 for remainder of key.

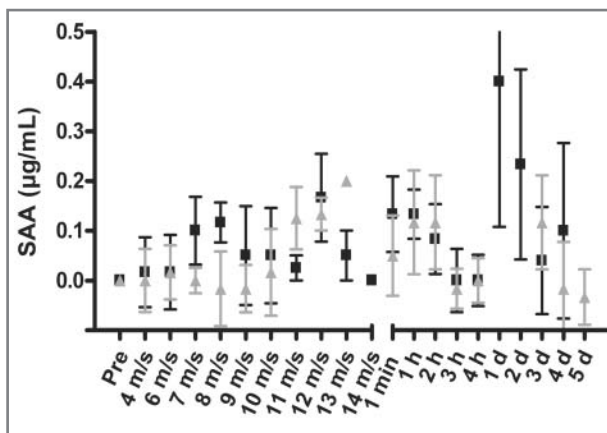


Figure 5—Mean \pm SEM concentrations of SAA (referenced to preexercise value) in the same horses as in Figure 1. The x-axis is divided, representing samples obtained during the exercise phase (left segment) and the recovery phase (right segment). See Figure 1 for remainder of key.

which increased overall with time ($P < 0.001$) because of increase during the exercise phase ($P < 0.001$) in both treatment groups. Although the TBARS value remained increased during the recovery phase, it did not change significantly during that phase in either group (Figure 4). There were no significant differences between TCJB and placebo groups at any time.

SAA—Exercise induced a significant ($P = 0.014$) increase in SAA concentration in both groups (Figure 5), which was not affected by treatment (Table 1). There were no effects of treatment (TCJB or placebo) on SAA values at any time.

Discussion

The strenuous exercise protocol used in the present study resulted in increased muscle enzyme activities in the unfit horses, as reported elsewhere.⁴¹ In addition, the exercise protocol was also associated with increased concentrations of SAA and TBARS, which are indicators of inflammation and oxidative stress, respectively. However, SAA concentrations increased only to

a small degree, compared with other inflammatory processes such as pneumonia.⁴⁰ Therefore, substantial inflammation might not have occurred during the study protocol.

A randomized crossover design was used because equine muscle enzyme activities,^{32,45} the key indicators of muscle damage used in this study, are known to have large individual variations. It is noteworthy that no horses had clinical signs of muscle damage, despite increased muscle enzyme activities, a finding that was also reported in another study.²⁹

It has been hypothesized that oxidative stress, through free radical production, changes the permeability of muscle membranes.^{32,45,46} The TBARS test has been used as a measure of oxidative stress, and a wide range of values has been reported from measurements in humans^{18,47} and horses.^{28,32} In the present study, TBARS test values did not change significantly during the recovery period, which is similar to results reported for endurance horses.³²

Increases in cTnI concentrations after exercise were mild, were not significant, and did not exceed the upper reference limit (0.12 ng/mL)^{48,f} in any horse; increases were also not associated with sampling time. Therefore, it seems unlikely that substantial cardiac stress or damage was induced by the experimental protocol. Equine skeletal muscle has a weak cross-reactivity with the cTnI assay, and that cross-reactivity has been estimated to contribute 0.05% to 0.1% of the total cTnI concentration.⁴⁹ Therefore, the mild increase in cTnI concentrations could have been caused by skeletal muscle damage.

Use of antioxidants such as vitamins E and C to modify oxidative stress, which is a proposed source of exercise-induced muscle damage, has not been associated with modifications of CK and AST activities in horses undergoing an 80-km endurance race.²⁹ In the present study, oral administration of TCJB for 2 weeks prior to short-term strenuous exercise, closer in duration to Thoroughbred and Standardbred racing conditions than endurance competition, resulted in a lower magnitude of increase in the activity of AST, compared with administration of a placebo solution. Whether endurance horses would also have this effect if administered TCJB remains to be evaluated. Although the numerous antioxidants and anti-inflammatory agents in tart cherries have been proposed to be responsible for such effects,²³ no direct treatment effect of TCJB on the markers of oxidative stress or inflammation was detected in the present study.

Because administration of TCJB was associated with increased AST activity of lower magnitude than in horses administered a placebo solution, it may have potential as a treatment for horses with exercise-induced myopathy. In addition, studies in fit horses are warranted.

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- i-STAT 1, Heska Corp, Loveland, Colo.

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- h. OXltek, ZeptoMetrix Corp, Buffalo, NY.
- i. Safire reader and Magellan software, Tecan, Zurich, Switzerland.
- j. Hitachi 7020, Hitachi, Tokyo, Japan.
- k. LZ test Eiken SAA, Eiken Chemical, Tokyo, Japan.

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