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Cardiac troponin T alterations in myocardium and serum of rats after stressful, prolonged intense exercise

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Chen, Yingjie, Robert C. Serfass, Shannon M. Mackey-Bojack, Karen L. Kelly, Jack L. Titus, and Fred S. Apple.
Cardiac troponin T alterations in myocardium and serum of rats after stressful, prolonged intense exercise. *J Appl Physiol* 88: 1749–1755, 2000.—The goal of this study was to determine whether the stress of forced exercise would result in injury to the myocardium. Male rats with 8% of body weight attached to the tail were forced to swim 3.5 h (3.5S), forced to swim 5 h (5S), or pretrained for 8 days and then forced to swim 5 h (T5S). Rats were killed immediately after they swam (0 h PS) and at 3 h (3 h PS), 24 h (24 h PS), and 48 h after they swam (48 h PS). Tissue homogenates of the left ventricle were analyzed by Western blot analysis for cardiac troponin T (cTnT). Serum cTnT was quantified by immunoassay. Results indicated that, in the 3.5S, 5S, and T5S groups, serum cTnT was significantly ($P < 0.01$) increased at 0 and 3 h PS. The 5S group demonstrated a greater increase in serum cTnT than the 3.5S group ($P < 0.01$) and the T5S group ($P < 0.01$) at 0 h PS. Western blot analysis indicated significant decreases ($P < 0.01$) in myocardial cTnT in the 5S group only at 0 h PS ($P < 0.01$) and 3 h PS ($P < 0.05$). Histological evidence of localized myocyte damage demonstrated by interstitial inflammatory infiltrates consisting of neutrophils, lymphocytes, and histiocytes, as well as vesicular nuclei-enlarged chromatin patterns, was observed in left ventricle specimens from the 5S group at 24 and 48 h PS. Our findings demonstrate that stressful, forced exercise induces alterations in myocardial cTnT and that training before exercise attenuates the exercise-induced heart damage.

myocardial damage; exercise stress; histology

EPIDEMIOLOGICAL STUDIES have demonstrated that regular exercise is associated with a reduction in the long-term risk of myocardial events (19, 20, 27, 32). People who exercise regularly not only have a lower baseline risk of myocardial infarction but also they have a lower risk that an infarction will be triggered by heavy physical exertion (26). The overall risk of death from coronary heart disease decreased about twofold in individuals who were physically active compared with less active individuals (20). On the other hand, heavy physical exercise has been recognized as a factor that triggers severe myocardial events such as impaired ventricular performance (29), cardiac ischemia (33, 34), myocardial infarction, and cardiac arrest (37, 40, 42). In symptom-free men, the risk of fatal and nonfatal heart attacks has been shown to be 4–56 times higher during physical exercise than when seated at home reading a book (37, 47). Because 1,500,000 myocardial infarctions occur annually in the United States (2), ≥75,000 infarctions that lead to 25,000 deaths may occur soon after exercise (26, 41). However, no direct experimental data are available to demonstrate that forced exercise can damage the heart. Studies are limited by suitable animal models, as well as by cardiac-specific markers that may be released into the circulation after injury.

Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) have unique amino acid sequences that differentiate them from their respective skeletal muscle isoforms (7, 48). These differences have allowed for the development of specific monoclonal antibodies that have been incorporated into immunoassays for detection of myocardial injury. Serum cTnT and cTnI have been used as specific markers for myocardial infarction (1, 3, 9, 22, 23) and ischemic injury (1, 9, 18, 49). Serum cTnT and cTnI measurements are specific in the assessment of cardiac injury in the presence of skeletal muscle damage (1). Increased concentrations of cTnT and cTnI in serum have been related to the extent of myocardial damage also (1, 9, 22, 23). Decreased myocardial cTnT concentrations also indicate heart damage, as demonstrated in dogs after coronary artery occlusion (34).

The purpose of the present study was threefold. First, we determined whether the stress of prolonged intense exercise would damage the heart. Second, we determined whether increased exercise volume would induce an increase in heart damage. Third, we determined whether training before prolonged intense exercise would have a protective effect on heart damage. Measurement of cTnT concentrations in the serum and myocardium of swimming rats was used as the marker for myocardial injury.

RESEARCH DESIGN AND METHODS

Animals and animal care. Male Sprague-Dawley rats ($n =$ 143), weighing 285–335 g (body weight on the day before

swimming), were used in these experiments. The rats were housed in cages in rooms regulated for temperature (23 ± 1°C), humidity (45–55%), and light cycle (0600–1800) and provided laboratory rat chow and water ad libitum. The research involving rodents in this study conforms with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Animal Care and Use Committee.

Experimental groups and exercise protocol. To attempt to minimize the general stress response of forced swimming, all rats were familiarized with swimming for 20 min (water temperature 35°C), without body weight attached to the rats, 2 days before any swimming protocols. The rats were then divided into three protocols. The first protocol consisted of 32 rats: 6 rats were used as sedentary controls and killed at rest, and 26 rats swam for 3.5 h (3.5S) in a swimming tank with 8% body weight (workload) attached to the tail. One rat was removed from this protocol when it was unable to finish the required forced swimming. After 3.5 h of swimming, the rats were towel dried, weighed, and killed immediately (0 h PS, $n = 6$), 3 h (3 h PS, $n = 7$), 24 h (24 h PS, $n = 6$), and 48 h after swimming (48 h PS, $n = 6$). The second protocol consisted of 63 rats: 12 rats were used as sedentary controls, and 51 rats swam for 5 h (5S) in a swimming tank with 8% body weight attached to the tail (25 or 26 rats swam together at each time). Three rats were removed from this protocol when they were unable to finish the required forced swimming. After the forced swimming, the rats were weighed and killed at 0 h PS ($n = 12$), 3 h PS ($n = 12$), 24 h PS ($n = 12$), and 48 h PS ($n = 12$). The third protocol consisted of 48 rats. The rats were pretrained for 8 days before a 5-h swim (T5S). Pretraining included 25 min of swimming on *day 1* followed by an increase to 60 min on *day 6* with no weight on the tail. The rats swam with a 5% tail weight for 20 min on *day 7* and for 30 min on *day 8*. A 2-day rest period followed the training. Twelve rats were used as the pretrained control group; the other 36 rats swam for 5 h with 8% body weight attached to the tail (each time, 18 rats swam together). After the forced swimming, the rats were weighed and killed at 0 h PS ($n = 12$), 3 h PS ($n = 12$), and 48 h PS ($n = 12$). No rats were killed at 24 h PS in the T5S protocol. In all protocols, the 8% body weight load attached to the tail determined exercise intensity. The water temperature was 35°C, and the water depth was 26 cm.

Most of the rats could keep their noses and mouths constantly above the water surface by paddling with the legs during the first 3 h of swimming. The rats with better swimming ability were able to keep their bodies almost parallel to the water surface. The rats with poor swimming ability swam almost vertically against the water surface. Some of the rats were unable to keep their bodies floating in the water, occasionally would sink to the bottom of the swimming tank, and then would press the tank bottom forcefully with their hindlegs to keep their noses and mouths out the water. Some rats quickly learned this skill by sinking to the bottom for rest and then pushing on the bottom of tank with their hindlegs to keep their noses and mouths out of the water. Immediately after swimming, the rats were very tired but not exhausted, inasmuch as they were able to move around in the cage and soon to actively clean the water on their fur with their front legs and mouths.

In all protocols, the body weights of the 48-h PS groups were monitored up to 48 h after exercise.

Animal death and tissue sampling. Rats were anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip). The abdominal cavity was quickly opened, and 10 ml of blood were drawn out from the abdominal aorta. Serum samples were collected and stored at −80°C for cTnT measurements. The

chest cavity was then quickly opened, and the heart was excised, frozen on dry ice, and stored at −80°C until biochemical analysis for cTnT by Western blot. Within each group, rats were killed within 30 min.

Sample preparation. Frozen myocardial samples were cut into small pieces on dry ice-cooled sample plates and then added to 2 ml of ice-cold buffer [200 mM potassium phosphate (pH 7.4), 5.0 mM EGTA, 5.0 mM β-mercaptoethanol, and 10% (vol/vol) glycerol] (46). The samples were homogenized three times in an ice bath for 10 s each at high speed with a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY) and then centrifuged at 3,000 g for 30 min at 4°C. The procedure yielded >98% recovery of cytosolic and myofibril proteins (46). The supernatants were used for Western blotting. Protein concentrations were determined using a modified Lowry method (21) with BSA as a standard. Serum cTnT measurements were obtained using the Roche second-generation cTnT immunoassay, which is 100% specific for cTnT (5). No cross-reactivity was observed in the cTnT assay at high concentrations of rat skeletal muscle troponin T (TnT; data not shown). Precision (percent coefficient of variation) at the upper reference limit for cTnT (0.1 µg/l) was <10%. The cTnT assay has been shown to be linear from 0.01 ng/ml (lower limit of detection) to 50.0 ng/ml (upper limit of linearity). Furthermore, this assay has been previously validated for use in several laboratory animals, including rats (30).

Histological examination. Left ventricular tissue was removed and immediately placed in 10% buffered Formalin. The apex of the heart was also removed and immediately frozen at −70°C after the animal was killed. The apex heart section remained frozen until preparation for histological examination, at which time it was placed in 10% buffered Formalin and permitted to thaw. One or two hearts from each protocol, including controls, were chosen randomly from the different time periods. From the randomly selected hearts, the entire apical segment and left ventricular tissues were embedded in paraffin, sectioned at 5-µm intervals, and stained with hematoxylin-eosin and/or trichrome stains.

Western blot analysis. Tissue homogenates were size fractionated on 12% SDS-polyacrylamide gels (43) and subsequently transferred to Hybond nitrocellulose membranes (Amersham, Arlington Heights, IL). Nonspecific binding sites were blocked by incubating the membranes in a blocking buffer [5.0% nonfat dry milk in Tris-buffered saline (TBS): 20 mM Tris·HCl (pH 7.6), 137 mM NaCl] for 1 h. A primary antibody specific for human cTnT (a gift from Fortron Bio-Scientific, Morrisville, NC) was diluted (1:1,000) in antibody buffer (1.0% nonfat dry milk in TBS) and incubated with the membranes for 2 h on a rotating cylinder. The membranes were washed three times with Tween-TBS for 30 min. Horseradish peroxidase-labeled secondary antibody (sheep anti-mouse IgG) was then incubated with the membranes for 1 h at a dilution of 1:3,000. The membranes were again washed three times in Tween-TBS buffer before a 1-min incubation with enhanced chemiluminescent substrate (Amersham). Light emission was detected by exposure to Fuji RX autoradiography film in the presence of Cronex intensifying screens. Signal intensities within the linear range were quantitated using laser densitometry (Molecular Dynamics, Sunnyvale, CA). Linearity was established by analysis of a standard curve generated with known amounts of proteins (with use of myocardial muscle homogenate) by Western blot. Myocardial cTnT was linear from 0.5- to 6-µg sample loading amounts of total protein. An excellent correlation ($r = 0.99$) between loading amounts and signal intensities of cTnT was obtained. To ensure that proteins on different sides of the gel were equally transferred, blotted, and detected, a control sample
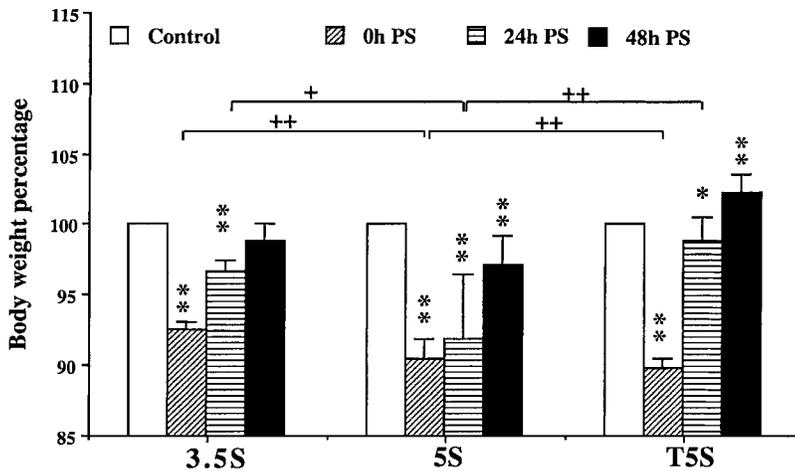
Fig. 1. Body weight alterations of rats after 3.5 h of swimming (3.5S), 5 h of swimming (5S), and 8 days of training before a 5-h swim (T5S). $*P < 0.05$; $**P < 0.01$ compared with before swimming. $^{+}P < 0.05$; $^{++}P < 0.01$ between groups. Data were obtained from rats killed 48 h after swimming (PS) from each protocol and expressed as percentages of body weight of controls before swimming.

(pooled tissue samples) was loaded onto all the gels in at least two lanes as internal control (I) lanes, allowing for blotting quality control. Sample loading amount for cTnT was 2 µg/lane. Although there is a small amount of cross-reactivity against skeletal TnT with the Fortron antibody, rat skeletal TnT isoforms migrate distinctly to lower molecular weights and do not interfere in the determination of cTnT when the Fortron antibody is used (data not shown). However, no skeletal TnT isoforms were observed in the rat heart samples studied.

Statistical analysis. Data from all groups were analyzed using a two-way ANOVA to determine main effects across time after swimming. Tukey's post hoc test for multiple comparisons was used to determine significant differences from preexercise values when significant main effects were found. Significance was set at $P < 0.05$. Values are means ± SD unless stated otherwise. The values of Western blot are presented as percentage of the control group.

RESULTS

Figure 1 shows that body weight was maximally decreased ($P < 0.01$) at 0 h PS for all three protocols: 7.4% after the 3.5S protocol, 9.4% after the 5S protocol, and 9.9% after the T5S protocol. Body weights remained decreased ($P < 0.01$) up to 24 h PS in the 3.5S protocol, up to 48 h PS in the 5S protocol, and up to 24 h PS in the T5S protocol.

Figure 2 shows that serum cTnT concentrations increased significantly ($P < 0.01$) above control concentrations (0.01 µg/l) after all three protocols at 0 and 3 h PS. The largest increases occurred in the 5S protocol, with a peak cTnT concentration of 0.15 ± 0.07 (range 0.06–0.26) µg/l at 0 h PS. This increase was significantly ($P < 0.01$) greater than any other cTnT increase in the 3.5S (mean 0.04 µg/l, range 0.03–0.06 µg/l) and T5S (mean 0.06 µg/l, range 0.03–0.12 µg/l) groups. The increases in the 3.5S and T5S groups at 0 and 3 h PS were not significantly different.

Figure 3 shows a representative Western blot analysis of cTnT in the 0, 3, 24, and 48 h PS groups after the 5S protocol. One cTnT band migrated to a molecular weight position corresponding to ~40 kDa. An internal control sample, I, was used to confirm equivalence of protein transfer across each gel. Skeletal TnT isoforms were not detected.

Figure 4 shows the changes in cTnT determined by Western blot analysis in left ventricles after the 3.5S, 5S, and T5S protocols. cTnT did not significantly decrease after the 3.5S and T5S protocols. However, after the 5S protocol, cTnT decreased 14% (arbitrary scanning units 3,729 ± 153, $P < 0.01$) and 10% (arbitrary scanning units 3,909 ± 156, $P < 0.05$) at 0 and 3 h PS, respectively, compared with controls (arbitrary scanning units 4,349 ± 174). Furthermore, cTnT in the 5S
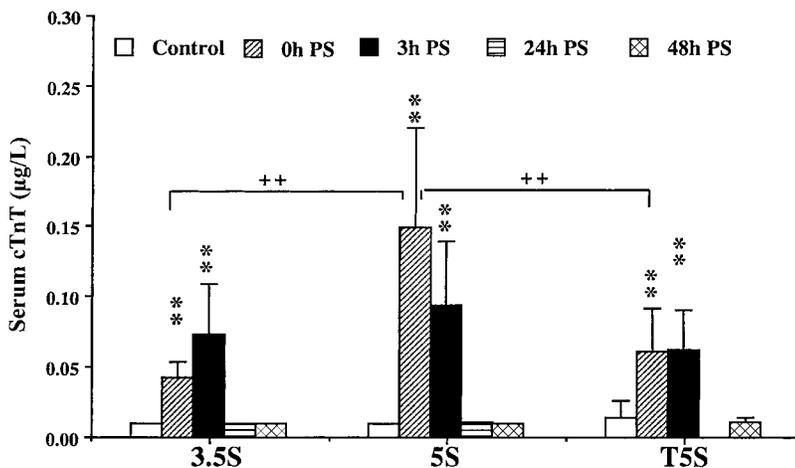

Fig. 2. Serum cardiac troponin T (cTnT) concentrations of rats in 3.5S, 5S, and T5S protocols. $**P < 0.01$ compared with controls. $^{++}P < 0.01$ between groups.

43.9 —   __Control___   __0h PS___   I   __3h PS___   __24h PS___   I   __48h PS___
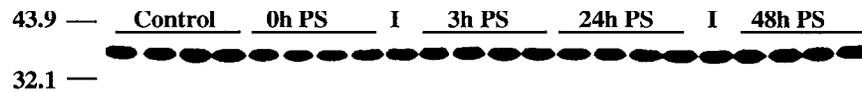
32.1 —

Fig. 3. Representative Western blot analysis of myocardial cTnT from 0, 3, 24, and 48 h PS groups after 5S protocol. One cTnT band migrated to a position corresponding to ~40 kDa. I, internal control (pooled tissue samples) used for determining equivalence of protein transfer across each gel.

protocol was significantly decreased 10% ($P < 0.05$) compared with the same PS groups in the 3.5S and T5S protocols. By 24 h PS, cTnT concentrations returned to preexercise control values.

As shown in Fig. 5, within the wall of the left ventricular myocardium, there are foci of myocyte injury with an interstitial inflammatory infiltrate consisting of neutrophils, lymphocytes, and histiocytes, as well as vesicular nuclei-enlarged chromatin patterns. These foci of myocyte injury in the left ventricles were observed in only four rats in the 5S group at 24 and 48 h PS. Furthermore, no histological evidence of recent or remote ischemic damage was evident in any of the apical sections examined.

DISCUSSION

The present study demonstrates that cTnT is released into the circulation from rat myocardium after 3.5 h of forced swimming, indicative of myocardial injury. Furthermore, an increase in exercise volume from 3.5 to 5 h of forced swimming resulted in a larger increase of circulating cTnT, indicative of a greater amount of myocardial injury compared with the 3.5-h model. However, an 8-day period of conditioning or pretraining before the 5-h forced swim resulted in a significant decrease in the release of cTnT into the circulation (Fig. 2) compared with the 5-h swimming protocol without pretraining, indicative of less myocardial injury than the 5-h model. Concordant with these findings, myocardial (left ventricle) cTnT concentrations were significantly decreased at 0 and 3 h PS in the 5S protocol, and histological evidence of myocyte damage in the 5S group at 24 and 48 h PS was also observed (Fig. 5). However, no measurable myocardial tissue loss of cTnT or histological evidence of damage was noted in the 3.5S or T5S protocol at any time period. These studies appear to be unique in studying the alteration

of cTnT in myocardial tissue and serum after a stressful, forced swimming exercise protocol. Our findings are in agreement with recent findings in humans, in which ultraendurance exercise (Hawaii Ironman Triathlon) was demonstrated to cause myocardial damage, as indicated by increased serum cTnI and cTnT concentrations and alterations in echocardiography in 6 of 23 athletes immediately after the event (36). The cellular nature of damage in this study was not examined.

Our finding raises several questions. First, were the increased serum concentrations of cTnT in the 3.5S and T5S protocols, which do not show loss of myocardial cTnT, due to reversible or irreversible ischemic injury to the heart? Second, do the increased serum concentrations of cTnT in the 5S protocol, which does correspond to myocardial tissue loss of cTnT, represent a necrotic, irreversible injury to the heart? Serum cTnT is a highly sensitive and specific marker for myocardial injury in humans and animals (23, 28, 34, 46, 49). Serum cTnT is measured in the present study by the second-generation cTnT immunoassay from Roche, which is 100% specific for the heart, with no cross-reactivity against human skeletal muscle TnT (5, 35) or rat skeletal muscle TnT (data not shown). It is interesting to observe that serum cTnT concentrations in the 3.5S, 5S, and T5S protocols peaked ~5–6 h after the initiation of exercise. Thus the exercise volume and the release kinetics of cTnT from myocardial tissue into the circulation are likely responsible for the increased serum cTnT. The cTnT release patterns we observed in rats appear similar to those observed after injury to the myocardium after myocardial infarction in humans, in that 4–8 h are required for cTnT to become increased in the serum after the myocardial insult (23, 44, 49). Furthermore, serum cTnT concentrations in the three protocols returned to baseline concentrations by 24 h.
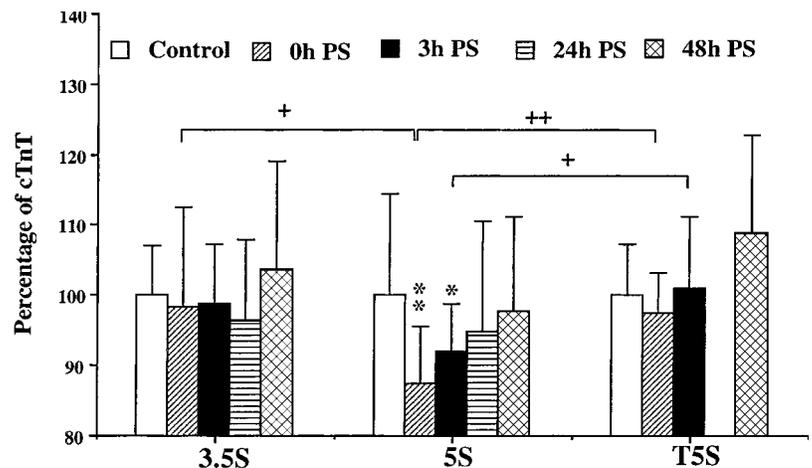
Fig. 4. cTnT alterations determined by Western blot analysis in left ventricles after 3.5S, 5S, and T5S protocols compared with nonswimming controls. *$P < 0.05$; **$P < 0.01$ compared with controls. +$P < 0.05$; ++$P < 0.01$ between groups.
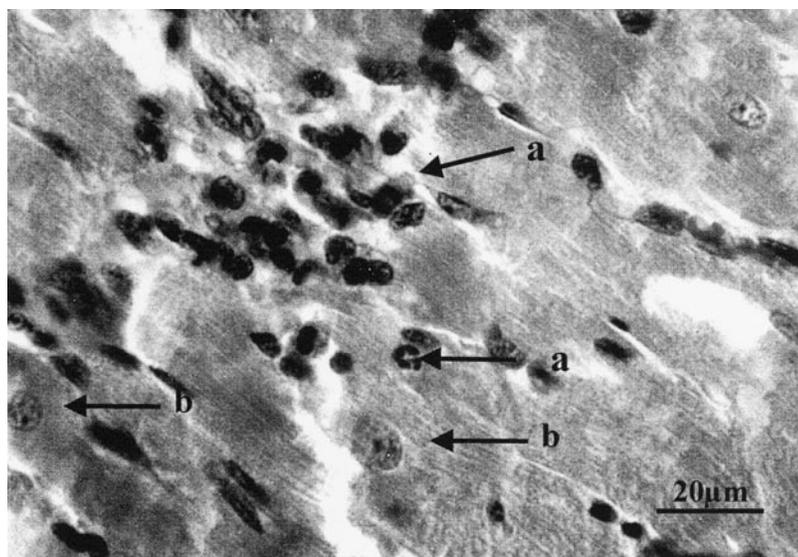
Fig. 5. Representative hematoxylin-eosin-stained cryosection of a left ventricle specimen obtained from a rat subjected to 5S protocol at 48 h PS. Damaged myocardial fibers and damaged intracellular areas are shown by foci of inflammatory infiltrates consisting of neutrophils, lymphocytes, and histiocytes (a) and vesicular nuclei-enlarged chromatin patterns (b).

We hypothesize that the rapid return to baseline suggests that release of cTnT from the exercise-induced myocardial injury is likely from localized minor, irreversible myocyte degeneration, since large irreversible injury to myocardial tissue after myocardial infarction in humans and in animal models remains increased for 5–10 days (22, 34, 49). The rapid increase and normalization of serum cTnT in our exercise model likely represent the loss of the 2–8% cytoplasmic compartment of cTnT, which has a half-life of <6 h (16, 46). The rapid normalization of cTnT to preexercise control values by 24 h of the decreased myocardium at 0 h PS in the 5S protocol likely is due to a reequilibration between the 95% myofibril compartment and the depleted 5% cytoplasmic cTnT compartment.

Histological evidence of myocyte damage in the left ventricles from four rats in the 5S group at 24 and 48 h PS demonstrated by inflammatory infiltrates of neutrophils, lymphocytes, and histiocytes and vesicular nuclei-enlarged chromatin patterns supports the hypothesis that loss of cTnT into the circulation is due to myocardial damage. No ischemic changes were identified histologically in any of the apical heart specimens for any of the time intervals after exercise, regardless of the duration of exercise. It is possible that the apex of the heart is not the best area for histopathological observations. However, the apex specimens were frozen before histological examination, which may have interfered with interpretation. Initial signs of ischemia, myocyte hypereosinophilia, and prominent contraction bands are not evident until 4–6 h after the onset of ischemia. Therefore, ischemic changes were not expected histologically in rats killed 0 or 3 h after exercise. The foci of myocyte injury, as shown in Fig. 5, are likely irreversible, suggestive of myocarditis, and may not always be identifiable by limited light-microscopic examination. No electron-microscopic examination was performed in this study. Mechanisms responsible for the myocyte injury were not studied.

Studies have shown that exercise of short duration induced regional myocardial dysfunction in dogs with severe coronary stenosis or ventricular hypertrophy (13–15). Repetitive episodes of exercise-induced ischemia resulted in cumulative postexercise regional myocardial dysfunction that lasted >2 h after the last episodes of exercise (14). Unfortunately, no serum biochemical markers for myocardial injury were tested in the above studies. Epidemiological studies have confirmed that heavy physical exertion can trigger myocardial infarction (26, 38). Studies also have shown that prolonged intense exercise can induce impaired left ventricular function and silent myocardial ischemia in some athletes (8, 17, 29, 36). However, most studies failed to show elevation of serum cTnT in symptomless athletes after marathon or ultramarathon running (1, 24, 39).

Our findings regarding alterations of the cardiac contractile protein cTnT after the prolonged single bout of forced swimming in rats are likely due to the development of our high-intensity (imposed tail weight) model, as well as the effect of severe stress placed on the rats. Our unpublished data indicated that rats swimming with 8% body weight attached to the tail generate a blood lactate (a marker for exercise intensity) concentration of 7.2 mM (vs. nonswimming control concentration of 3.7 mM), whereas rats run on a treadmill at 25 m/min fail to show any significant increase in blood lactate. A blood lactate level of 7 mM was reported in rats running up a 16° incline at a speed of 50 m/min with O_2 uptake of ~110 $ml \cdot kg^{-1} \cdot min^{-1}$ (4). Furthermore, a maximal O_2 uptake of ~80 $ml \cdot kg^{-1} \cdot min^{-1}$ was obtained from the swimming rats with 2% body weight attached to the tail (25). In comparison, the exercise intensity in our swimming model was four times higher than that with 2% body weight attached to the tail. Our study shows that a 40% increase of total exercise volume from 3.5 to 5 h of swimming resulted in an increase in serum cTnT from 0.04 µg/l to 0.15 ng/l (3.5 times above the cTnT concentration in the 3.5S group) immediately after swimming. The mechanism for this finding is not totally clear. It may relate to the different metabolic processes between

the early and late stages of prolonged exercise, including depletion of glycogen stores (11, 12), alterations in glucose and fatty acid utilization (12), calcium alteration (6), free radical-mediated injury (45), and prolonged elevation of circulating catecholamines. Furthermore, no studies were undertaken to study whether there was evidence of impairment of cardiac function. However, numerous studies in the literature now clearly demonstrate the important prognostic role of increased serum cTnT levels in predicting short and long cardiac events in patients presenting with ischemic chest pain (10, 31). Furthermore, in an acute myocardial infarction study involving a dog model, increases in serum cTnT correlated with loss of myocardial cTnT (34). Thus, in humans, there appears to be an important physiological message for which medical and pharmacological management decisions are now influenced by increased serum cTnT values.

We also demonstrated a significant protective effect on prolonged exercise-induced myocardial damage as a consequence of the pretraining. In this study, the rats were pretrained for 8 days. Immediately after swimming, serum cTnT concentration in pretrained rats was 60% lower than in the untrained rats [0.06 μg/l in trained rats (T5S) vs. 0.15 μg/l in untrained rats (5S)]. Pretraining also blunted any decrease in myocardial cTnT after 5 h of swimming. These data suggest that even a short-term, low exercise volume of physical training could significantly protect the heart from prolonged, intense exercise-induced myocardial damage. Comparatively, in humans, the effect of regular physical exercise (preconditioning) may be very important. Our data may help in understanding the effect of regular physical exercise in reducing the long-term risk of myocardial events and the fact that people who are physically active have a lower risk of myocardial events triggered by heavy physical exertion (26).

In conclusion, our study shows that a single bout of stressful, prolonged, intense exercise induced damage as monitored by cTnT in the heart and serum in the myocardium of rats. Furthermore, an increase in exercise volume induced more myocardial damage, and training before stressful, prolonged, intense exercise provided protection from myocardial damage.

REFERENCES

1. **Adams EJ III, Bodor GS, Davila-Roman VG, Delmez JA, Apple FS, Ladenson JH, and Jaffe AS.** Cardiac troponin I—a marker with high specificity for cardiac injury. *Circulation* 88: 101–106, 1993.
2. **American Heart Association.** *Heart and Stroke Facts.* Dallas, TX: Am. Heart Assoc. Natl. Center, 1992.
3. **Apple FS, Falahati A, Paulsen PR, Miller EA, and Sharkey SW.** Improved detection of minor ischemic myocardial injury with measurement of serum cardiac troponin I. *Clin Chem* 43: 2047–2051, 1997.
4. **Armstrong RB, Laughlin MH, Rome L, and Taylor CR.** Metabolism of rats running up and down an incline. *J Appl Physiol* 55: 518–521, 1983.
5. **Baum H, Braun S, Gerhardt W, Gilson G, Hafner G, Muller-Bardorff M, Stein W, Klein G, Ebert C, Hallermayer K, and Katus HA.** Multicenter evaluation of a second-generation assay for cardiac troponin T. *Clin Chem* 43: 1877–1884, 1997.
6. **Chen YJ, Zhang DT, Guo QF, Wei JP, and Serfass C.** Effects of prolonged high intensity running on myocardium in untrained rats (Abstract). *Med Sci Sports Exerc* 29: 246, 1997.
7. **Dhoot GK, Grearson N, and Perry SV.** Polymorphic forms of troponin T and troponin C and their localization in striated muscle cell types. *Exp Cell Res* 122: 339–350, 1979.
8. **Douglas PS, O'Toole ML, Hiller WD, Hackney K, and Reichek N.** Cardiac fatigue after prolonged exercise. *Circulation* 76: 1206–1213, 1987.
9. **Feng YJ, Chen C, Fallon JT, Lai T, Chen L, Knibbs DR, Waters DD, and Wu AHB.** Comparison of cardiac troponin I, creatine kinase-MB and myoglobin for detection of acute ischemic myocardial injury in a swine model. *Am J Clin Pathol* 110: 70–77, 1998.
10. **Hamm CW, Heeschen C, Goldman B, Vahanin A, Adgey J, and Miguel CM.** Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. *N Engl J Med* 340: 1623–1629, 1999.
11. **Hargreaves M.** Interactions between muscle glycogen and blood glucose during exercise. *Exerc Sport Sci Rev* 25: 21–39, 1997.
12. **Hawley JA, Brouns F, and Jeukendrup A.** Strategies to enhance fat utilization during exercise. *Sports Med* 25: 241–257, 1998.
13. **Hittinger L, Shannon RP, Kohin S, Manders WT, Kelly P, and Vatner SF.** Exercise-induced subendocardial dysfunction in dogs with left ventricular hypertrophy. *Circ Res* 66: 329–343, 1990.
14. **Homans DC, Laxson DD, Sublett E, Lindstrom P, and Bache RJ.** Cumulative deterioration of myocardial function after repeated episodes of exercise-induced ischemia. *Am J Physiol Heart Circ Physiol* 256: H1462–H1471, 1989.
15. **Homans DC, Sublett E, Dai XZ, and Bache RJ.** Persistence of regional left ventricular dysfunction after exercise-induced myocardial ischemia. *J Clin Invest* 77: 66–67, 1986.
16. **Katus HA, Remppis A, Scheffold T, Diederich KW, and Kuebler W.** Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. *Am J Cardiol* 67: 1360–1367, 1991.
17. **Koller A, Mair J, Mair M, Calzolari C, Larue C, and Puschendorf B.** Diagnosis of myocardial injury in marathon runners. *Ann NY Acad Sci* 752: 234–235, 1995.
18. **Kragten JA, Herments WT, and Dieijen-Visser MP.** Cardiac troponin T release into plasma after acute myocardial infarction: only fractional recovery compared with enzymes. *Ann Clin Biochem* 33: 314–323, 1996.
19. **Lee IM, Manson JE, Hennekens CH, and Paffenbarger RS Jr.** Body weight and mortality. A 27-year follow-up of middle-aged men. *JAMA* 270: 2823–2828, 1993.
20. **Leon AS, Connett J, Jacobs DR Jr, and Rauramaa R.** Leisure-time physical activity levels and risk of coronary heart disease and death. The Multiple Risk Factor Intervention Trial. *JAMA* 258: 2388–2395, 1987.
21. **Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ.** Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275, 1951.
22. **Mair J, Genser N, and Morandell D.** Cardiac troponin I in the diagnosis of myocardial injury and infarction. *Clin Chim Acta* 245: 19–38, 1996.
23. **Mair J, Puschendorf B, and Michel G.** Clinical significance of cardiac contractile proteins for the diagnosis of myocardial injury. *Adv Clin Chem* 131: 63–98, 1994.
24. **Mair J, Schobersberger W, Koller A, Bialk P, Villiger B, Frey W, and Puschendorf B.** Risk for exercise-induced myocardial injury for athletes performing prolonged strenuous endurance exercise. *Am J Cardiol* 80: 543–544, 1997.
25. **McArdle WD.** Metabolic stress of endurance swimming in the laboratory rat. *J Appl Physiol* 22: 50–54, 1967.
26. **Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, and Muller JE.** Triggering of acute myocardial

infarction by heavy physical exertion: protection against triggering by regular exertion. *N Engl J Med* 329: 1677–1683, 1993.

27. **Morris JN, Clayton DG, Everitt MG, Semmence AM, and Burgess EH.** Exercise in leisure time: coronary attack and death rates. *Br Heart J* 63: 325–334, 1990.

28. **Newby LK, Christenson RH, Ohman EM, Armstrong PW, Thompson TD, Lee KL, Hamm CW, Katus HA, Cianciolo C, Granger CB, Topol EJ, and Califf RM.** Value of serial troponin T measures for early and late risk stratification in patients with acute coronary syndromes. *Circulation* 98: 1853–1859, 1998.

29. **Niemelä KO, Palatsi I, Ikäheima M, Airaksined J, and Takkunen J.** Impaired left ventricular diastolic function in athletes after utterly strenuous prolonged exercise. *Int J Sports Med* 8: 61–65, 1987.

30. **O'Brien PJ, Dameron GW, and Beck ML.** Differential reactivity of cardiac and skeletal muscle from various species in two generations of cardiac troponin T immunoassays. *Res Vet Sci* 65: 135–137, 1998.

31. **Ohman EM, Armstrong PW, Christenson RH, Granger CB, Katus HA, Hamm CW, O'Hanesian MA, Wagner GS, Kleiman NS, Harrell FE Jr, Califf RM, and Topol EF.** Cardiac troponin T levels for risk stratification in acute myocardial ischemia. *N Engl J Med* 335: 133–141, 1996.

32. **Paffenbarger RS, Wing AL, and Hyde PT.** Physical activity as an index of heart attack risk in college alumni. *Am J Epidemiol* 8: 161–175, 1978.

33. **Rama D, Margaritis I, and Orsetti A.** Troponin I immunoenzymometric assays for the detection of muscle damage: application to the monitoring of a triathlon. *Clin Chem* 42: 2033–2035, 1996.

34. **Ricchiuti V, Sharkey S, Murakami M, Voss E, and Apple FS.** Cardiac troponin I and T alterations in dog hearts with myocardial infarction: correlation with infarct size. *Am J Clin Pathol* 110: 241–247, 1998.

35. **Ricchiuti V, Voss E, Ney A, Odland M, Anderson PAW, and Apple FS.** Cardiac troponin T isoforms expressed in renal diseased skeletal muscle will not cause false positive results by second generation cardiac T assay by Boerhinger Mannheim. *Clin Chem* 44: 1919–1924, 1998.

36. **Rifai N, Douglas PS, O'Toole M, Rimm E, and Ginsburg GS.** Cardiac troponin T and I, electrocardiographic wall motion analyses, and ejection fractions in athletes participating in the Hawaii Ironman Triathlon. *Am J Cardiol* 83: 1085–1089, 1999.

37. **Shephard RJ.** Sudden death—a significant hazard? *Sports Med* 8: 101–110, 1974.

38. **Siegel AJ, Lewandrowski KB, Strauss HW, Fischman AJ, and Yasuda T.** Normal postrace antimyosin myocardial scintigraphy in asymptomatic marathon runners with elevated serum creatine kinase MB isoenzyme and troponin T levels. Evidence against silent myocardial cell necrosis. *Cardiology* 86: 451–456, 1995.

39. **Siegel AJ, Scholar M, Yang J, Dhanak E, and Lewandrowski KB.** Elevated serum cardiac markers in asymptomatic marathon runners after competition: is the myocardium stunned? *Cardiology* 88: 487–491, 1997.

40. **Siscovich DS, Weiss NS, Fletcher RT, and Lasky T.** The incidence of primary cardiac arrest during vigorous exercise. *N Engl J Med* 311: 874–877, 1984.

41. **Tofler GH, Muller JE, and Stone PH.** Modifiers of timing and possible triggers of acute myocardial infarction in the thrombosis in myocardial infarction phase II (TIMI II) Study Group. *J Am Coll Cardiol* 20: 1049–1055, 1992.

42. **Tofler GH, Stone PH, Maclure M, Edelman E, Davis VG, Robertson T, Antman EM, and Muller JE.** Analysis of possible triggers of acute myocardial infarction (the MILIS study). *Am J Cardiol* 66: 22–27, 1990.

43. **Towbin H, Staehelin T, and Gordon J.** Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 76: 4350–4354, 1979.

44. **Tucker JF, Collins RA, Anderson AJ, Haauser J, Kalas J, and Apple FS.** Early diagnostic efficiency of cardiac troponin I and troponin T for acute myocardial infarction. *Acad Emerg Med* 4: 13–21, 1997.

45. **Venditti P and Di Meo S.** Antioxidants, tissue damage, and endurance in trained and untrained young male rats. *Arch Biochem Biophys* 331: 63–68, 1996.

46. **Voss EM, Sharkey SW, Gernert A, Murakami MA, Johnson RB, Hsieh CC, and Apple FS.** Human and canine cardiac troponin T and CK MB distribution in normal and diseased myocardium. *Arch Pathol Lab Med* 119: 799–806, 1995.

47. **Vuori I and Suunakki T.** Risk of sudden death and sport. *Sports Med* 1: 181–186, 1984.

48. **Wilkinson JM and Grand JA.** Comparison of amino acid sequence of troponin I from different striated muscles. *Nature* 271: 31–35, 1978.

49. **Wu AHB, Valdes R, Apple FS, Gornet T, Stone MA, Mayfielf-Stokes S, Ingersoll-Stroubos AM, and Wiler B.** Cardiac troponin-T immunoassay for diagnosis of acute myocardial infarction. *Clin Chem* 40: 900–907, 1994.