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PROGRESSIVE EXERCISE PRECONDITIONING PROTECTS AGAINST CIRCULATORY SHOCK DURING EXPERIMENTAL HEATSTROKE

Ching-Hsia Hung,[*,†] Nen-Chung Chang,[‡] Bor-Chih Cheng,[§] and Mao-Tsun Lin[†]

[*]Department of Physical Therapy, National Cheng Kung University, Tainan, Taiwan 701; [†]Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan 710; [‡]Division of Cardiology, Taipei Medical University/Hospital, Taipei, Taiwan 118; and [§]Department of Surgery, Chi-Mei Medical Center, Tainan, Taiwan 710

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ABSTRACT—Heat shock protein (HSP) 72 expression protects against arterial hypotension in rat heatstroke. HSP72 can also be induced in multiple organs, including hearts from rats with endurance exercise. We validated the hypothesis that progressive exercise preconditioning may confer cardiovascular protection during heatstroke by inducing the overexpression of HSP72 in multiple organs. To deal with the matter, we assessed the effects of heatstroke on mean arterial pressure, heart rate, cardiac output, stroke volume, total peripheral vascular resistance, colonic temperature, blood gases, and serum or tissue levels of tumor necrosis factor-α (TNF-α) in urethane-anesthetized rats pretreated without or with progressive exercise training for 1, 2, or 3 weeks. In addition, HSP72 expression in multiple organs was determined in different groups of animals. Heatstroke was induced by exposing the rats to a high blanket temperature (43°C); the moment at which mean arterial pressure decreased from the peak value was taken as the time of heatstroke onset. Previous exercise training for 3 weeks, but not 1 or 2 weeks, conferred significant protection against hyperthermia, arterial hypotension, decreased cardiac output, decreased stroke volume, decreased peripheral vascular resistance, and increased levels of serum or tissue TNF-α during heatstroke and correlated with overexpression of HSP72 in multiple organs, including heart, liver, and adrenal gland. However, 10 days after 3 weeks of progressive exercise training, when HSP72 expression in multiple organs returned to basal values, the beneficial effects exerted by 3 weeks of exercise training were no longer observed. These results strongly suggest that HSP72 preconditioning with progressive exercise training protects against hyperthermia, circulatory shock, and TNF-α overproduction during heatstroke.

KEYWORDS—Arterial hypotension, stroke volume, hyperthermia, exercise, heat shock protein, heat stress

INTRODUCTION

When the heat loss mechanisms in the body are defective, the excessive accumulation of extra heat production results in heatstroke (1). For example, heatstroke afflicts many humans engaged in firefighting, severe exercising, some military or mining activities, and animals whose behavior is restricted by livestock in a feedlot or during transport (1–5). A variety of factors may increase susceptibility to heatstroke. Important among these are salt and water depletion and fever after immunization in normal persons, and many predisposing diseases (such as cardiovascular disease, diabetes mellitus, malnutrition, acute or chronic alcoholism, hyperthyroidism, and impaired sweat production) (1, 6). Unless promptly recognized and treated, severe hyperthermia, arterial hypotension, respiratory distress syndrome, disseminated intravascular coagulation, aspiration pneumonia, pulmonary edema, circulatory and renal failure, severe electrolyte disturbances, central nervous system dysfunctions (such as restlessness, delirium, and coma), and multiple organ damage may occur and result in high rate of mortality (1, 5–10).

During heatstroke, rodents display hyperthermia, arterial hypotension, intracranial hypertension, cerebral ischemia, neuronal damage, and overproduction of inflammatory cytokines (including tumor necrosis factor-α and interleukin 1β) (11). However, the above-mentioned heatstroke syndromes are attenuated by induction of heat shock protein (HSP) 72 in rat brain (12, 13). Other evidence has also demonstrated that HSP72 can be detected in skeletal muscle, heart, liver, and adrenal gland from rats with endurance exercise training for 7 to 12 weeks (14–20). This raises the possibility that pretreatment of rats with progressive exercise induces HSP72 and improves cardiovascular dysfunction and survival during heatstroke.

To deal with the matter, we investigated the effects of heat stress on survival time, colonic temperature (T_{CO}), mean arterial pressure (MAP), stroke volume (SV), total peripheral resistance (TPR), cardiac output (CO), heart rate (HR), blood gases, and extent of tissue or serum tumor necrosis factor-α (TNF-α) in rats with or without previous chronic exercise training. In addition, the levels of the HSP72 expression in multiple organs, including hearts, were determined in rats with or without previous exercise training.

MATERIALS AND METHODS

Experimental animals

Adult male Wistar rats weighing between 250 and 350 g were purchased from the Animal Resource Center of the National Science Council of the Republic of China (Taipei, Taiwan). The animals were housed in groups of four at an ambient

Address reprint requests to Mao-Tsun Lin, DDS, PhD, Department of Medical Research, Chi-Mei Medical Center, Yung-Kang, Tainan Hsien, Taiwan 710.

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temperature of 24°C ± 1°C and were maintained under a normal light-dark cycle (14:10 h; lights on at 6:00 a.m.). Pelleted rat chow and tap water were allowed *ad libitum*.

Exercise training protocol

Before heatstroke induction, the rats in the exercise groups were trained to run on a motor-driven treadmill (Treadmill Simplex II; Columbus Instruments, Columbus, OH) 5 days a week for 1 to 3 weeks. The exercise protocol was performed according to the previously described method (14) with some modifications. The animals were placed on the already-moving belt facing away from the electrified grid, and they ran in the direction opposite of the movement of the belt. Although some stimulations of 1.0 mA by electric shock (Stimulus Controller, model D48E; DRI Co., Taipei, Taiwan) were necessary to keep them running, they continued to run without stimulation thereafter. Initially, the rats were acclimated to run 15 min at 20 m/min per day for 3 days. The duration and intensity of exercise were then increased progressively. In the first week, the animals were running 30 min at 20 m/min per day for 5 consecutive days. In the second week, the animals were running 30 min at 30 m/min per day for 5 consecutive days. In the third week, the animals were running 60 min at 30 m/min per day for 5 consecutive days. During exercise, the slope was always maintained at 0%. To avoid any possible confounding effects of external factors, sedentary (SED) animals were stimulated by electric shock in the same manner as the exercise groups and were placed daily on a nonrunning treadmill.

Animal surgery and physiological parameter monitoring

The bilateral femoral arteries of adult Wistar rats, under urethane anesthesia (1.4 g/kg intraperitoneally), were cannulated with polyethylene tubing (PE50). One tube was used for continuous monitoring of MAP and HR via a pressure transducer, whereas the other was used for collecting blood samples to determine the animal's arterial pH, arterial partial pressure of CO_2 ($PaCO_2$), O_2 (PaO_2), O_2 saturation (SO_2), hematocrit (Hct), hemoglobin (Hb), lactate, and glucose levels. The MAP and HR were recorded using a polygraph (Gould Polygraph, model 2107; Cleveland, OH), and blood gas and biochemical assays were conducted via a blood gas analyzer (Nova Biochemical, Waltham, MA). T_{CO} was monitored continuously by a thermocoupler. All experiments and animal care were approved by the Institutional Animal Care and Use Committee of the Chi-Mei Medical Center (Tainan, Taiwan), and were conducted according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinch throughout the course of all experiments (approximately 3 h) after a single intraperitoneal dose of urethane. The rectal temperatures of the anesthetized rats were maintained at about 36°C with a thermal mat except during heat stress. Heatstroke was induced by exposing the animals to the heating pad temperature (T_{pad}) of 43°C. The moment at which MAP began to decrease from the peak level was taken as the onset of heatstroke (13, 21). Once the onset of heatstroke occurred during the heat stress, the heating pad was removed and the animals were allowed to recover at room temperature (24°C). At the end of the experiments, all experimental animals were killed with an overdose of urethane.

Measurement of cardiovascular parameters

The animal was put in a supine position. Aseptic rat surgical procedures were used. The animal's trachea was intubated for artificial ventilation (Small Animal Ventilator, model 683; Harvard Apparatus, Holliston, MA) at 50 breaths/min with tidal volume of 20 mL and inspiration-to-expiration ratio at 1:2. A Transonic Flowprobe (1RB2596; Transonic Systems, Ithaca, NY) was implanted around the ascending aorta as described by Smith (22). Briefly, the chest was opened at the third intercostal space to expose the heart. A small section (1 cm long) of the ascending aorta was freed from connective tissue. The flow probe was then implanted around the root of the ascending aorta. The chest incision was closed, and a negative intrathoracic pressure was restored. Aortic blood flow was recorded on the polygraph using a Transonic Flowmeter (T206; Transonic Systems). The CO was calculated from the aortic blood flow, and the TPR was calculated by dividing MAP by CO. SV was expressed as CO divided by HR.

Experimental groups

Animals were randomly assigned to one of the following five groups: SED control group; rats 1 day after exercise training for 1 week (1wk1D); rats 1 day after exercise training for 2 weeks (2wk1D); rats 1 day after exercise training for 3 weeks (3wk1D); and rats 10 days after exercise training for 3 weeks (3wk10D). Each one of the above-mentioned groups were divided into two subgroups. One subgroup of rats was exposed to a T_{pad} of 43°C to obtain the latency for the onset of heatstroke. In the SED group, the latency of the onset of heatstroke was found to be 55 ± 3 min. The other subgroup of rats was exposed to a T_{pad} of 43°C for only 55 min, and was then kept at 24°C until death. The survival time (interval between the onset of heatstroke and animal death) was obtained for each group.

Different groups of animals were used for the different sets of experiments: determination of latency and survival time for SED, 1wk1D, 2wk1D, 3wk1D, and 3wk10D rats; determination of blood pH, $PaCO_2$, PaO_2, SO_2, Hct, Hb, lactate, and glucose in SED rats at 24°C, SED rats at 43°C, and 3wk1D rats at 43°C; determination of HSP72 in SED, 1wk1D, 2wk1D, 3wk1D, and 3wk10D rats; determination of serum or tissue levels of TNF-α in SED rats at 24°C, SED rats at 43°C, 3wk1D rats at 43°C, and 3wk10D rats at 43°C; and determination of T_{pad}, T_{CO}, MAP, HR, CO, TPR, and SV in SED rats at 24°C, SED rats at 43°C, and 3wk1D rats at 43°C.

Determination of TNF-α in serum and organs

For determination of rat TNF-α levels, blood samples and organs were taken 60 min after initiation of heat stress (or 5 min after the onset of heatstroke) once for each animal. The blood samples were allowed to clot for 2 h at room temperature and were then centrifuged (at 2000*g* for 20 min at 4°C). The supernatants were then harvested and the organ samples were prepared according to previous reports (23, 24). The organs were disintegrated in 5 volumes of ice-cold Ripa buffer. The homogenates were incubated on ice for 30 min and were then centrifuged (at 15,000*g* for 30 min at 4°C) twice. The supernatants were stored at −70°C until measurement. The concentrations of TNF-α in serum and organ lysates were determined using double-antibody sandwich enzyme-linked immunoabsorbant assay (ELISA; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Western blot analysis

The animals were killed by decapitation for detection of HSP72 with Western blotting technique. The heart, aorta, lung, liver, kidney, and adrenal gland were quickly removed, placed into Eppendorf tubes, and stored at −70°C until analysis. For detection of HSP72, the methods detailed previously were adopted in the present study (19). Briefly, the tissues were homogenized individually in homogenization buffer (100% sucrose, 0.5 M Tris-HCl, pH 6.8, and Pefabloc SC). These protein samples were denatured in SDS sample buffer (0.5 M Tris-HCl, pH 6.8, 10% SDS, 0.1% bromphenol blue, 2-mercaptoethanol, and glycerol). Protein contents were assayed with a Bio-Rad kit (Bio-Rad, Hercules, CA) and an ELISA reader at 630 nm. Linearity was analyzed by standard curve. Equal amounts (100 μg) of protein extract were loaded and separated by 9% SDS-PAGE. After electrophoresis at 120 V for 6 h in SDS-PAGE running buffer, proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA) under semidry transfer conditions. The membrane was blocked in TTBS (20 mM Tris, 500 mM NaCl, and 0.1% Tween 20, pH7.5) containing 5% skim milk (Difco, Detroit, MI) for 1 h and was incubated at 4°C overnight with mouse monoclonal anti-HSP72 primary antibody (SPA 810; StressGen Biotechnologies, Victoria, British Columbia, Canada), diluted 1:2000 in 5% skim milk. The membrane was then washed four times with TTBS (5 min per wash) and was incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse secondary antibody diluted 1:5000 in 5% skim milk at 4°C. Immunodetection for HSP72 was performed using the enhanced chemiluminescence protocol with Renaissance reagent (NEN Life Science Products, Boston, MA) and then the membrane was exposed to the x-ray film (Fuji, Tokyo, Japan) for 2 to 3 min. Mouse antiactin monoclonal antibody was used as internal control. Quantification of bands from blots was performed by scanning with a scanner (Image Scanner, Amersham Pharmacia Biotech AB, Uppsala, Sweden) and using the computer program ImageMaster TotalLab 1D Elite (version 2.01; Amersham Pharmacia, Piscataway, NJ).

Statistical analysis

Data from experiments were expressed as means ± SEM for each point. Statistical analysis was conducted by using analysis of variance (ANOVA) for factorial experiments, and Duncan's multiple-range test was used for *post hoc* multiple comparisons among means. ANOVA for repeated measures was used for the analysis of data for Figure 5. Pearson correlation coefficient (r) was used as a measure of linear association between two variables. A P value <0.05 was considered statistically significant.

RESULTS

Figure 1 shows time course for the expression of HSP72 in heart, lung, liver, and adrenal gland during exercise training. It can be seen from the figure that the HSP72 expression begins to rise at 1 week of exercise training, to reach its peak level at 3 weeks of exercise training, and to decline to its basal level at 10 days after the termination of 3 weeks of exercise training. However, the HSP72 expression in the aorta and kidney starts to rise at 3 weeks of exercise training and returns to its basal level at 10 days after a 3-week exercise training. In addition, Figure 2 shows that 5 to 10 days after a 3-week exercise

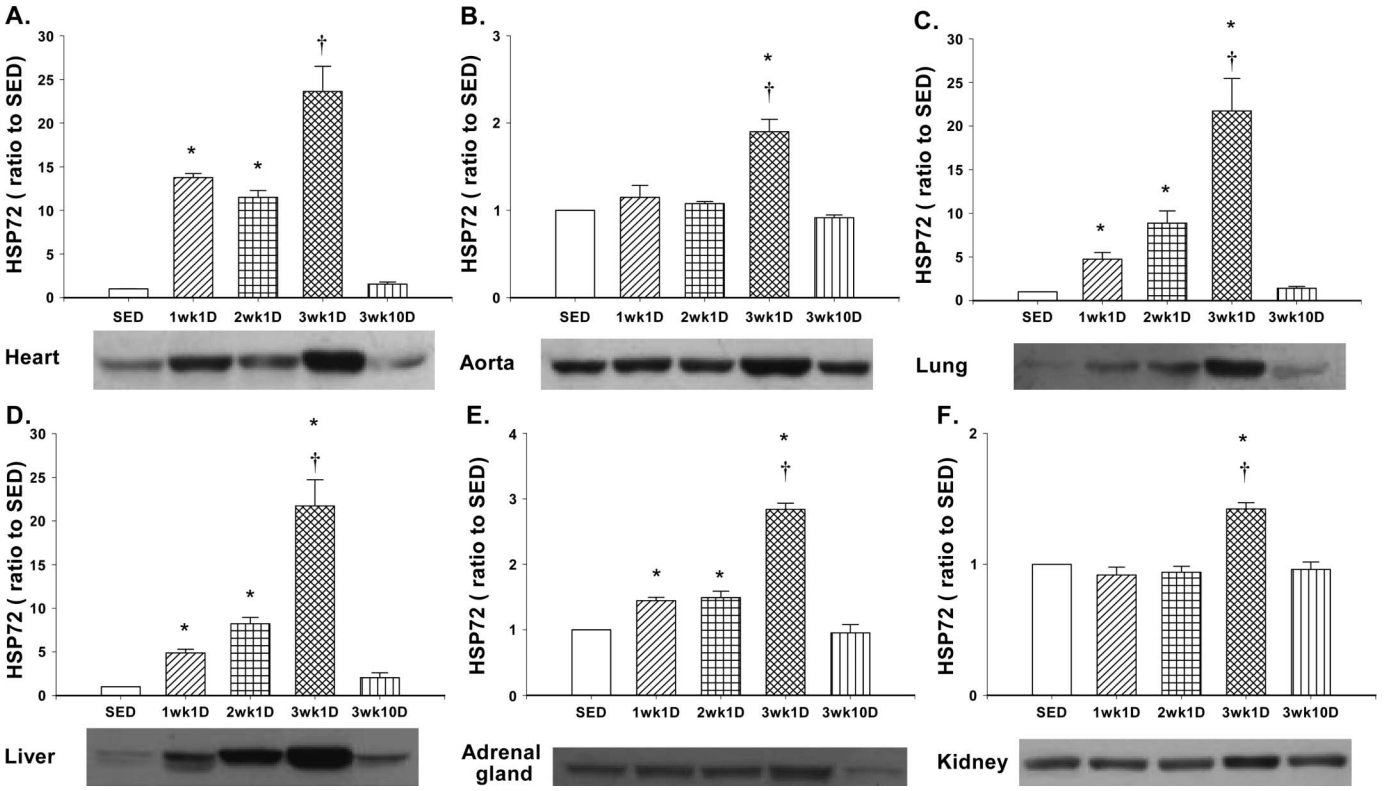

FIG. 1. **The expression of HSP72 (by Western blot analysis) in multiple organs of different groups of rats.** Protein levels are expressed as the ratios to the SED group. Bars are the means ± SEM of three determinations from eight rats per group. Below each column is a representative Western blot of HSP72. *$P < 0.05$ compared with the corresponding values of SED group (ANOVA, followed by Duncan's test). †$P < 0.05$ compared with the corresponding values of 1wk1D, 2wk1D, or 3wk10D group (ANOVA, followed by Duncan's test).

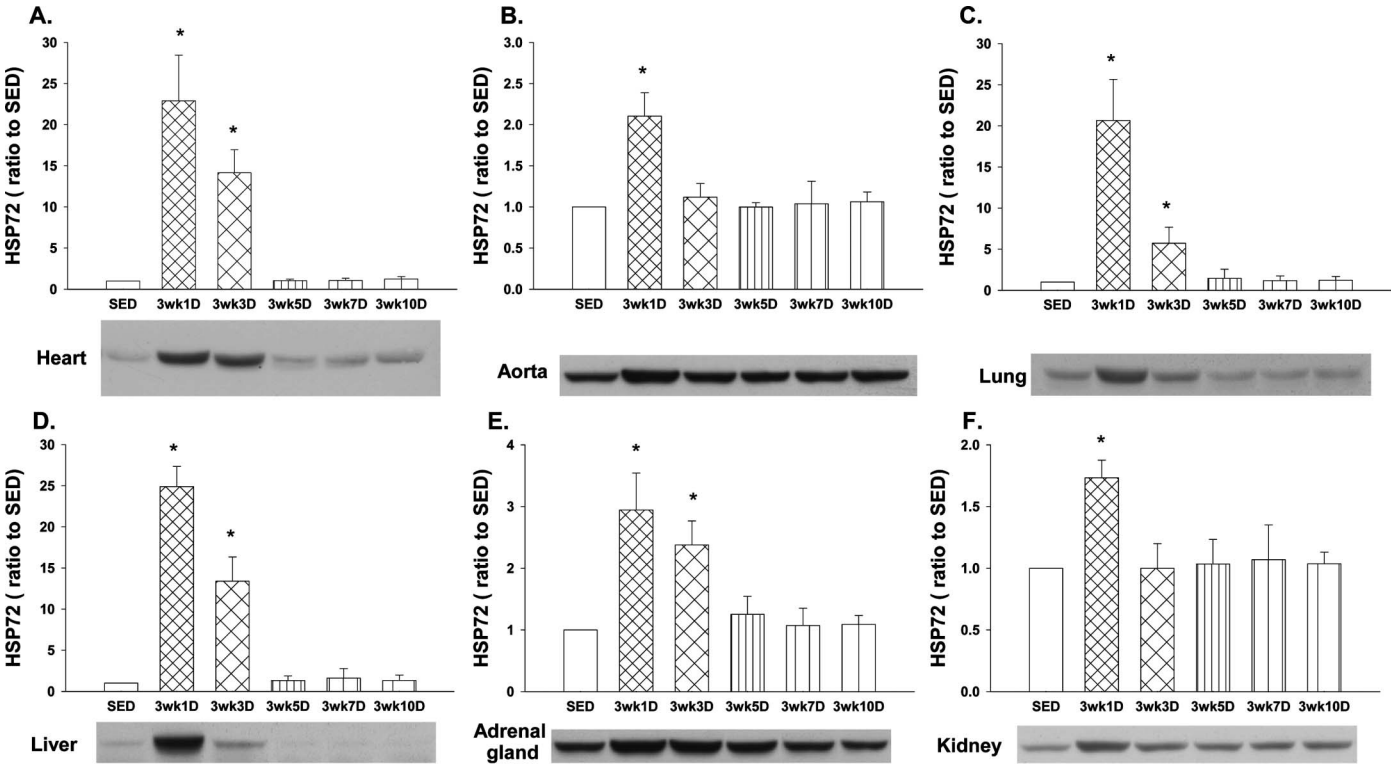

FIG. 2. **The expression of HSP 72 in multiple organs in different groups of rats.** Bars represent means ± SEM of eight rats per group obtained 60 min after initiation of heat exposure (HE, rats kept at 43°C for 55 min). *$P < 0.05$ compared with SED group (ANOVA, followed by Duncan's test). †$P < 0.05$ compared with the corresponding control values of SED + HE or 3wk10D + HE (ANOVA, followed by Duncan's test).

TABLE 1. **Effects of HE (T_b = 43°C) on the latency for onset of heatstroke and survival time in different groups or rats**

Treatment	Latency (min)	Survival Time (min)*
SED rats at 43°C	55 ± 1	18 ± 3
1wk1D rats at 43°C	54 ± 3	19 ± 2
2wk1D rats at 43°C	56 ± 2	32 ± 10
3wk1D rats at 43°C	64 ± 2*	181 ± 25†
3wk10D rats at 43°C	55 ± 2	23 ± 4

Data are means ± SEM for eight rats per group.
*All group exposed to 43°C had HE withdrawn at 55 min.
†$P < 0.05$ compared with SED rats at 43°C (one–way ANOVA, followed by Duncan's test).

training program, HSP72 expression in heart, lungs, liver, and adrenal glands returned to its basal level. Three weeks of exercise training is able to maintain a high level of HSP72 in several vital organs for only 3 to 4 days.

Table 1 summarizes the latency and survival time for SED and exercise-pretreated rats during heatstroke. The values of latency and survival time for 1wk1D or 2wk1D rats were not distinguishable from those of SED rats. However, as compared with those of SED, 1wk1D, 2 wk1D, or 3wk10D rats, the values for the latency and survival time were significantly greater in those of 3wk1D rats.

Figures 3 and 4 depict that the levels of TNF-α in heart, liver, adrenal glands, or serum are significantly increased at 60 min after initiation of HE in SED + HE, 1wk1D + HE, 2wk1D + HE, or 3wk10D + HE rats. As compared with those of SED, 1wk1D, 2wk1D, or 3wk10D rats, the levels of TNF-α in these organs were significantly lower in 3wk1D rats obtained 60 min after initiation of HE. In addition, a negative correlation of TNF-α with HSP72 levels (ratio to SED) in hearts, livers, and adrenal glands of rats sacrificed without exposure to the acute experiment are depicted in Figure 5. However, HE in SED, 1wk1D, 2wk1D, 3wk1D, and 3wk10D caused indistinguishable TNF-α responses in aorta, lung, and kidney (Fig. 4).

Figure 6 depicts the time course for the various cardiovascular parameters in SED + NT, SED + HE, or 3wk1D + HE rats. Five minutes after the onset of heatstroke or 60 min after initiation of HE, the values of MAP, HR, CO, TPR, and SV in SED + HE rats were significantly lower than those of SED + NT. In contrast, the values of T_{CO} in SED + HE rats obtained 60 min after initiation of HE were significantly higher than those of SED + NT. When exposed to the same extent of heat stress, 1wk1D + HE or 2wk1D + HE rats shared with the SED + HE rats the same MAP, HR, CO, TPR, and SV responses (the data are not shown here). As compared with those of SED + HE rats, the values of MAP, HR, CO, TPR, and SV were significantly greater, but the values of T_{CO} were significantly lower in 3wk1D + HE rats (Fig. 6).

Table 2 contains means and SE values for pH, $PaCO_2$, PaO_2, SO_2, Hct, Hb, lactate, and glucose levels obtained from SED rats kept at 24°C, SED rats kept at 43°C, 3wk1D rats kept at 43°C, or 3wk10D rats kept at 43°C. The values of blood pH, $PaCO_2$, PaO_2, SO_2, and glucose in heatstroke SED rats that received no chronic exercise pretreatment obtained at 60 min after initiation of HE were significantly lower than those of the NT controls. In contrast, the values of blood lactate were significantly greater at 60 min after initiation of HE in SED rats. As compared with those at the same time point of SED + HE rats, the values of pH, $PaCO_2$, and glucose levels in 3wk1D rats, but not in 3wk10D rats, were significantly greater, whereas the values of blood lactate were significantly lower. However, the values of Hct and Hb were indistinguishable among the SED rats kept at 24°C, SED rats kept at 43°C, 3wk1D rats kept at 43°C, and 3wk10D rats kept at 43°C.

DISCUSSION

In the present study, we have first demonstrated that progressive exercise preconditioning protects against heatstroke in rats. This protection in the form of survival time is

TABLE 2. **Effects of HE to 43°C for 55 min on pH, $PaCO_2$,PaO_2, SO_2, Hct, Hb, lactate, and glucose measured at different time points in different groups of rats**

Groups/Time Course	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	SO_2 (%)	Hct (%)	Hb (g/dL)	Lactate (mmol/L)	Glucose (mg/dL)
SED rats kept at 24°C								
0 min	7.44 ± 0.01	35.6 ± 1.2	97 ± 2	98 ± 0	41.1 ± 0.8	13.5 ± 0.4	1.89 ± 0.16	172 ± 19
55 min	7.45 ± 0.01	36.4 ± 1.0	99 ± 2	98 ± 0	41.4 ± 0.7	13.8 ± 0.3	1.75 ± 0.09	169 ± 16
60 min	7.44 ± 0.01	36.2 ± 1.4	98 ± 1	98 ± 0	41.3 ± 0.5	13.6 ± 0.1	1.79 ± 0.11	170 ± 13
SED rats kept at 43°C								
0 min before HE	7.44 ± 0.01	34.9 ± 1.8	98 ± 1	97 ± 0	39.8 ± 0.7	13.2 ± 0.2	1.79 ± 0.13	160 ± 17
55 min after HE	7.41 ± 0.02	27.0 ± 2.6*	86 ± 5*	96 ± 0	41.0 ± 0.8	13.7 ± 0.3	2.89 ± 0.32*	146 ± 16
60 min after HE	7.33 ± 0.03*	22.8 ± 1.6*	79 ± 3*	94 ± 1*	40.7 ± 1.1	13.7 ± 0.2	4.06 ± 0.67*	101 ± 12*
3wk1D rats kept at 43°C								
0 min before HE	7.44 ± 0.01	33.6 ± 1.1	98 ± 2	98 ± 0	42.8 ± 0.3	13.4 ± 0.1	1.99 ± 0.07	164 ± 19
55 min after HE	7.41 ± 0.01	35.8 ± 1.1†	75 ± 3*	95 ± 1	43.0 ± 0.7	13.4 ± 0.3	2.50 ± 0.13*	165 ± 16
60 min after HE	7.42 ± 0.02†	32.1 ± 1.5*†	71 ± 2*	92 ± 1*	43.3 ± 0.8	13.6 ± 0.3	2.59 ± 0.21*†	151 ± 13†
3wk10D rats kept at 43°C								
0 min before HE	7.44 ± 0.01	35.1 ± 1.2	97 ± 2	98 ± 0	41.8 ± 0.2	13.5 ± 0.2	1.89 ± 0.09	168 ± 17
55 min after HE	7.41 ± 0.03	29.0 ± 2.8*	79 ± 4*	95 ± 1	41.2 ± 0.9	13.7 ± 0.2	2.64 ± 0.25*	139 ± 18
60 min after HE	7.34 ± 0.02*	23.1 ± 1.2*	73 ± 2*	93 ± 1*	41.1 ± 1.2	13.6 ± 0.2	3.89 ± 0.43*	99 ± 11*

Values are means ± SEM of eight rats per group.
*$P < 0.05$ compared with SED rats kept at 24°C at the same time point (ANOVA, followed by Duncan's test).
†$P < 0.05$ compared with SED rats kept at 43°C at the same time point (ANOVA, followed by Duncan's test).

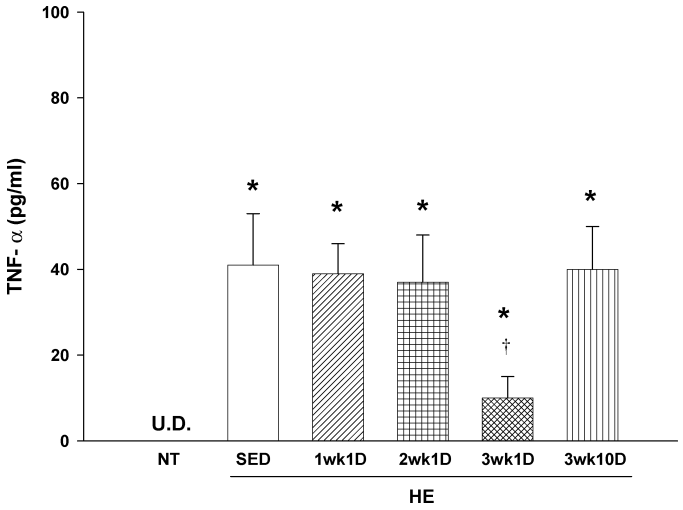

FIG. 3. **The serum TNF-α levels in different groups of rats: normothermia (NT), sedentary rats kept at normothermia (24° C); HE; SED; 1wk1D; 2wk1D; 3wk1D; 3wk10D; and U.D., undetectable.** Bars represent means ± SEM of eight rats per group obtained 60 min after initiation of HE. *$P < 0.05$ compared with NT group (ANOVA, followed by Duncan's test). †$P < 0.05$ compared with the corresponding control values of SED + HE or 3wk10D + HE (ANOVA followed by Duncan's test).

attributed in part to attenuation of hyperthermia, circulatory shock, and overproduction of TNF-α during heatstroke by exercise-induced HSP72. In the present results, we realize a negative correlation between the circulating or tissues (in particular, the heart, liver, and adrenal gland) TNF-α during heatstroke and HSP72 expression in rats sacrificed without HE to the acute experiment. In contrast, we observed a positive correlation between HSP72 expression in hearts, livers, and adrenal glands of rats sacrificed without HE and MAP and survival time during heatstroke. In addition, tolerance of the 3wk1D group to heat stress had a positive relationship to the tissue levels of HSP 72, strongly suggesting, but not providing, an implication of this protein in the observed protection. The present results further demonstrated that a 3-week, but not a 1- or 2-week, exercise training regimen conferred significant protection against the hyperthermia, arterial hypotension, decreased CO, and increased serum or tissue levels of TNF-α, and improved survival during heatstroke. A 3-week exercise training regimen is able to maintain a high level of HSP72 in several vital organs for only 3 to 4 days. If exercise is maintained for a longer period of time, HSP72 expression would remain elevated (compared with nonexercised animals) and afford the protective effects described in the more acute experiments.

The present results are, in part, consistent with several previous findings in different models. For example, Trudell et al. (20) demonstrated that accumulation of lipid in the liver resulting from chronic ethanol consumption in rats was attenuated by repeated exercise. This protective effect of exercise was strongly associated with the expression of HSP72 after repeated exercise. A exercise training-induced increase of HSP72 in myocardium (14) or adrenal glands (15) is also associated with myocardial protection against ischemia-reperfusion or the adaptive mechanisms of the adrenals to cope with a stress.

The present results are in good agreement with those of many previous findings showing that the cardiovascular dysfunctions were associated with elevated circulating endotoxins and cytokines in heatstroke patients (7, 25, 26) or animals (21, 27–29). Increases in the plasma levels of pyrogenic cytokines can raise the hypothalamic set point to induce hyperthermia (30).

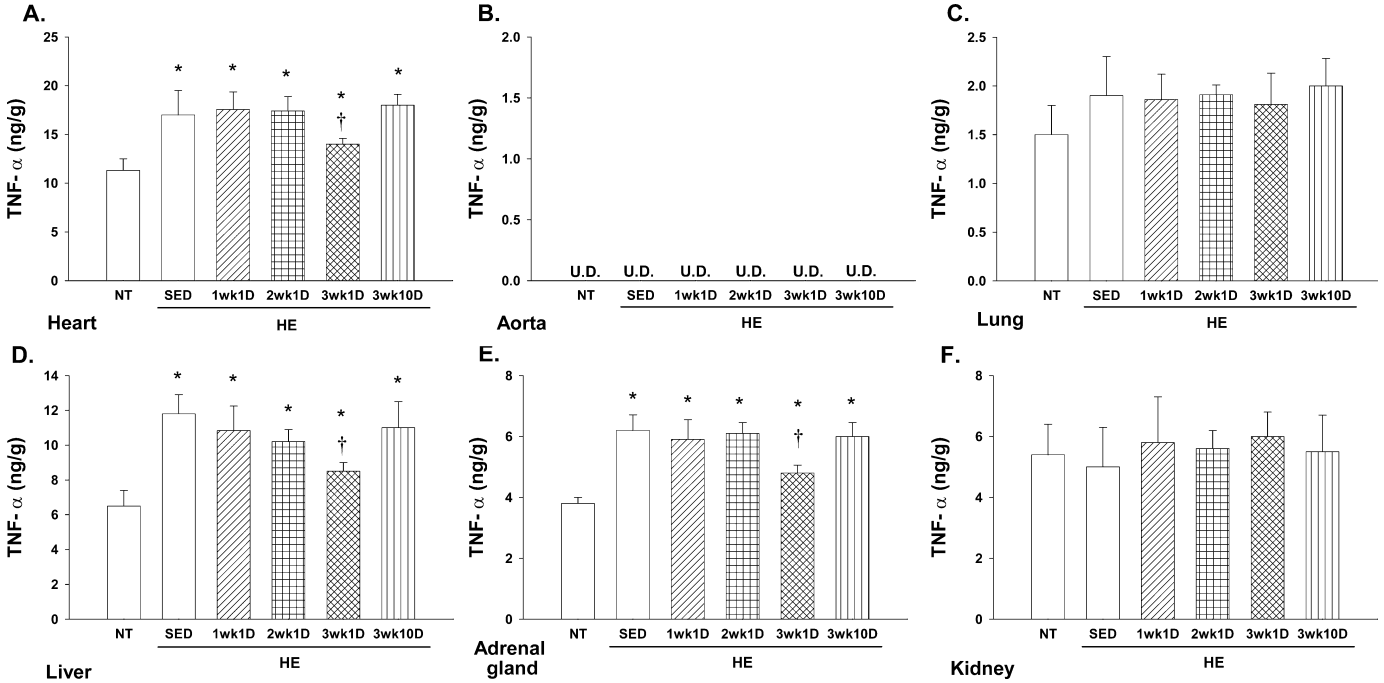

FIG. 4. **The tissue TNF-α levels in different groups of rats: NT, HE, SED, 1wk1D, 2wk1D, 3wk1D, 3wk10D, and U.D.** Bars represent means ± SEM of eight rats per group obtained 60 min after initiation of HE. *$P < 0.05$ compared with NT group; †$P < 0.05$ compared with the corresponding control values of SED + HE or 3wk10D + HE (ANOVA followed by Duncan's test).

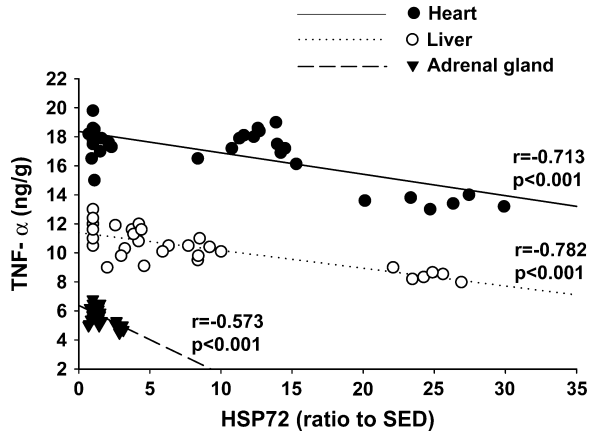

FIG. 5. **Correlation of TNF-α with HSP72 levels in heart, liver, and adrenal gland.**

Animals injected intravenously with an interleukin 1 receptor antagonist at the time of heatstroke onset were protected from some of the cardiovascular dysfunctions during heatstroke, such as decreased ventricular depolarization, decreased SV, decreased CO, and arterial hypotension. Furthermore, the hemodynamic changes associated with heatstroke can be mimicked by interleukin 1β administration (27). The plasma levels of TNF-α and interleukin 6 have also be found to be correlated with the severity of heatstroke in patients (31). As shown in the present results, progressive exercise training for 3 weeks, in addition to reducing overproducing of serum or tissue TNF-α, ameliorated circulatory shock (due to decreased SV and decreased peripheral vascular resistance) during heatstroke. In fact, heatstroke syndromes resemble sepsis in many of their characteristics (7). Any of the responses observed during septic shock can be mimicked by systemic administration of TNF-α (32–34). It was also found that previous exercise suppressed the plasma TNF-α response to bacterial lipopolysaccharide (35). Putting these observations together, progressive exercise training for at least 3 weeks may induce HSP 72 overexpression in many vital organs and attenuate overproduction of tissue cytokines, including TNF-α and arterial hypotension during heatstroke. However, we are not sure that TNF-α is the main cause of this heat shock and death, as TNF-α levels at that point are only 50 to 60 pg/mL in the serum of Wistar rats used in the present study. Nevertheless, our unpublished data showed that TNF-α levels in serum of Sprague-Dawley rats were about 800 to 1000 pg/mL during heatstroke.

Physical training is able to improve endothelial function (35–37). In the present results, the exercise training

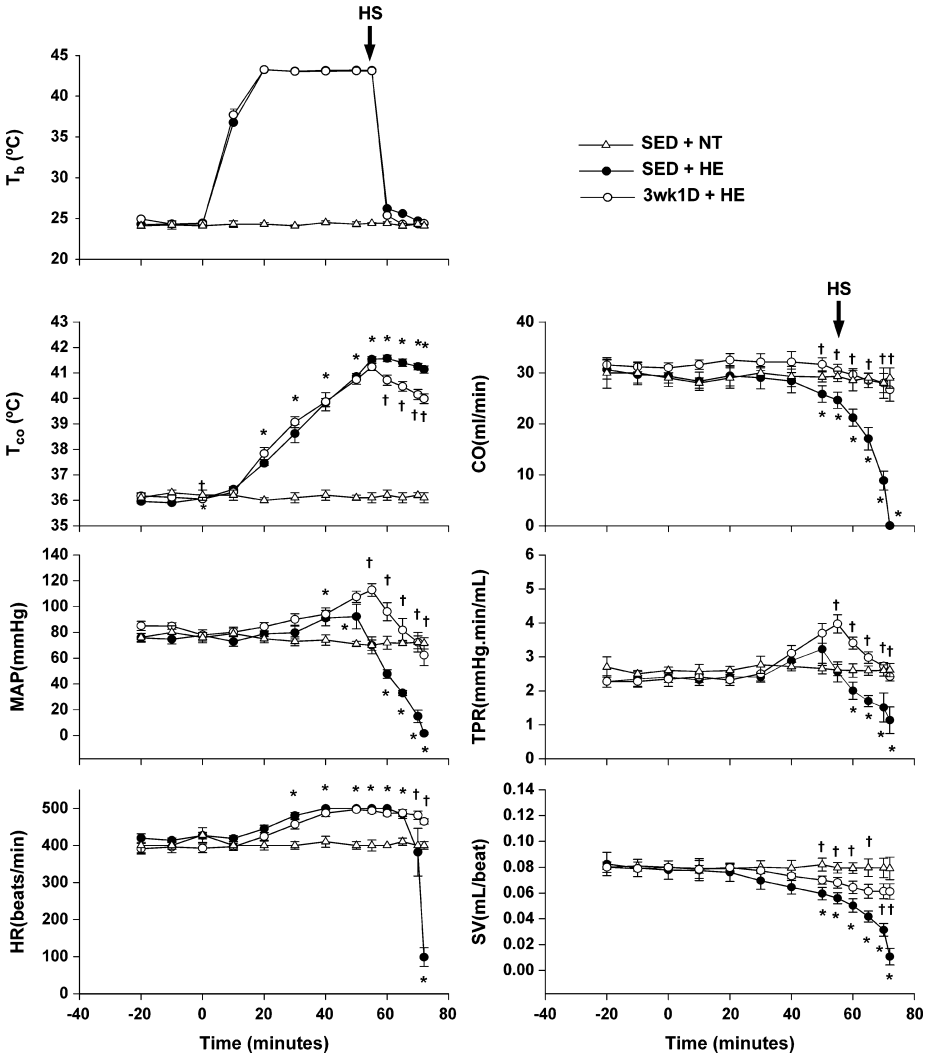

FIG. 6. **Effects of heat stress (43°C for 55 min) on T_{CO}, MAP, HR, CO, TPR, and SV in eight rats of SED (•), 3wk1D (○), and NT rats kept at 24° C (△).** Points represent means ± SEM. $^{*}P < 0.05$ compared with the SED + NT group (ANOVA for repeated measures, followed by Duncan's test). $^{\dagger}P < 0.05$ compared with the SED + HE group (ANOVA for repeated measures, followed by Duncan's test). The arrow denotes the time that HE was started and the onset of heatstroke (HS). ANOVA for repeated measures was used for the analysis of data.

preconditioning, in addition to inducing HSP72 overexpression in aorta, reversed the decreased total peripheral vascular resistance exhibited during heatstroke. In the present study, we assessed solely the aorta HSP72 expression. The same HSP72 overexpression may occur after exercise training in other vascular systems.

Furthermore, as shown in the present results, when we exposed the animals to heat stress, hyperthermia, increased $PaCO_2$ and blood lactate, and decreased PaO_2 and pH were noted. Human volunteers engaged in exhaustive treadmill running also displayed increases in minute ventilation and venous lactate levels (38). It is likely that an increase in lactate in rats with heatstroke may be due to ischemia (decreased MAP values) as well as hypermetabolism (increased body temperature). Three-week physical training significantly ameliorated the arterial hypotension, hyperthermia, and lactacidemia, and resulted in reduction of tissue acidification as demonstrated in the present results. These beneficial effects exerted by physical training may be related to overexpression of HSP72 in multiple organs, including lung and heart.

Hall and Colleagues (39) have proposed that hyperthermia during heatstroke stimulates xanthine oxidase production of reactive oxygen species and limits heat tolerance by promoting circulatory and intestinal barrier dysfunction. Increased oxygen species are able to augment the tissue injury by operating with the regulation of cytokine gene expression (40). Furthermore, our recent results demonstrated that the heatstroke-induced arterial hypotension was associated with an increased production of free radicals, higher lipid peroxidation, and lower enzymatic antioxidant defenses in multiple organs, including the heart and brain (28, 41). Pretreatment with hydroxyl radical scavengers significantly attenuated heatstroke-induced arterial hypotension. In fact, exercise training has also been found to protect against myocardial injury by reducing lipid peroxidation (14) and increasing the cardiac antioxidant defense-system (42). Therefore, exercise training may improve survival during heatstroke by reducing hyperthermia and reactive oxygen species overproduction.

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