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**EFFECT OF RATE OF HEAT GAIN ON SELECTED
SERUM ENZYMES IN RHESUS MONKEYS**

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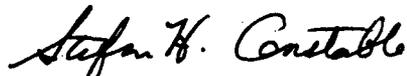
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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.


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13. ABSTRACT (Maximum 200 words) It has been demonstrated in rats that the rate of body heat storage contributes to increased cellular membrane permeability during the prodromal phase of heatstroke. The purpose of the present research was to document this effect in primates. On separate occasions, unacclimatized, female rhesus monkeys (N=7) were exposed to two different environmental heat loads while seated in a primate restraint chair. The two heat loads, 50 °C, p _{H₂O} >50 torr (high stress; HS) vs. 45 °C, p _{H₂O} <10 torr (low stress; LS), resulted in dissimilar rates of body heat storage. Heat exposure was terminated when core temperature (Tre) reached a pre-selected end-point of 41.0 °C. Serum levels of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and blood lactate (La) were determined immediately postexposure and at 6 h and 24 h following termination of the exposure. Body weight was also determined pre- and immediately postexposure. Total exposure time (mean ±SE) differed significantly between trials (38±2 vs. 83±9 min; HS vs. LS). Because the absolute increase in Tre was similar in both the HS and LS trials (2.23±0.1 vs. 2.27±0.1 °C, respectively), the rate of heat gain was significantly greater during the HS vs. the LS trial. Analysis of variance indicated no differences in any of the marker enzyme responses between HS and LS trials. However, AST levels did rise significantly over time from immediately postexposure to both the 6 and 24 h samples. CPK and LDH tended to rise over time, but not significantly. No significant differences were found in ALT and La over time. The HS condition allowed for little, if any, evaporative heat loss (0.07±0.05 vs. 0.50±0.05 gmin ⁻¹ ; HS vs. LS). These findings indicate that acute heat exposure, at these levels of body heat storage, does not appear to provide an adequate thermal stress in rhesus monkeys for meaningful elevations of the serum enzymes, indicative of increased tissue permeability.				
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EFFECT OF RATE OF HEAT GAIN ON SELECTED SERUM ENZYMES IN RHESUS MONKEYS

INTRODUCTION

Cell membrane leakage of sodium and potassium ions results in an energy drain at the cellular level as active transport activity must increase to maintain the membrane potential. This membrane permeability increases as body temperature rises, a result of an increase in the kinetic energy of the ions (4). Thermal stress and the resultant increased cell membrane permeability poses a challenge to the cell to maintain homeostasis and may contribute to fatigue during physical exertion. Ultimately, the work capacity of an individual may be limited by this cellular energy drain. Hubbard (3) suggests that the *rate* of heat gain plays a significant role in the energy drain at the cellular level as membrane permeability increases dramatically with faster rates of heat storage. This relationship is described as part of the Energy Depletion Model developed by Hubbard et al. (4) and has been examined in the rat model by both Hubbard et al. (5) and Manjoo et al. (8).

Increased membrane permeability can be assessed by elevations in specific serum enzymes including creatine phosphokinase (CPK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The greatest elevation in these enzymes has been documented in cases of heatstroke during physical exertion. Enzyme levels increase less dramatically following nonsymptomatic physical exertion in the heat and are even less elevated following hyperthermia alone (6).

Previous research efforts have explored this area using the rat model. Rats do not sweat, a major route of heat dissipation in humans. Thus, the rhesus monkey may be a more appropriate human analogue as the rhesus does have the ability to modestly thermoregulate via sweating. The purpose in the present investigation was to characterize the response of a nonhuman primate, the rhesus monkey, to different rates of body heat storage on the selected serum enzymes.

METHODS

Primates. Seven female rhesus monkeys, weighing 3.6-5.4 kg, were used for this study. The monkeys were housed in individual cages in a room maintained at an ambient temperature (T_a) of 22 ± 2 °C, 50% relative humidity and a 12:12 h light:dark cycle. The typical diet of the primates consisted of Purina Monkey Chow supplemented with fruit. Water was available ad libitum.

Experimental procedures. Prior to each experimental session, monkeys were fasted for 14-16 h with water available ad libitum. The study was conducted in a balanced, randomized design and all testing was performed in an environmental chamber. On separate occasions, unacclimatized monkeys were exposed to two different environmental heat loads while seated in a primate restraint chair. The two heat loads, 50 °C, $pH_2O > 50$ torr (high stress; HS) vs. 45 °C, $pH_2O < 10$ torr (low stress; LS), were intended to result in dissimilar rates of body heat storage.

Initially, the monkey was placed in a Plexiglas restraining chair in an animal preparation room separate from the environmental chamber where the primate was instrumented with ECG electrodes, a rectal probe (model 701, Yellow Springs Instruments (YSI), Yellow Springs, OH) inserted 10 cm beyond the anal sphincter, and a skin

thermistor (model 709B, Yellow Springs Instruments) at the inner thigh. Body weight (Weightmeter 533, ElectroScale Co., Santa Rosa, CA) was obtained immediately prior to heat exposure. The monkey was then transferred to the environmental chamber and the heat exposure was initiated.

During heat stress, core temperature (T_{re}), thigh skin temperature (T_{sk}), and heart rate (HR) were collected every 30 s. Oxygen consumption (VO_2) was determined by drawing room air (Kurz 565-7A Mass Flowmeter, Monterey, CA) at a constant rate (3-4 L/min) through a Plexiglas hood enclosing the head of the monkey. Aliquots of expired air were sampled for oxygen (O_2) and carbon dioxide (CO_2) content (Perkin Elmer Medical 1100 Gas Analyzer, Pomona, CA) from a mixing chamber connected to the hood. The outputs of the O_2 and CO_2 analyzers, the flowmeter, YSI probes and ECG monitor (Hewlett-Packard 43100A), were interfaced with a Macintosh IIx computer with analog-to-digital conversions handled by a program written in LabVIEW graphical programming language, Version 2.0.6 (National Instruments, Austin, TX).

Heat exposure was terminated when T_{re} reached a preselected end-point of 41.0 °C. The monkey was then promptly removed from the chamber and returned to the animal prep room. Post body weight (BW) was obtained within 1 min of the termination of the heat stress. The control blood sample was obtained within 5 min following the termination of the heat exposure. The recovery of T_{re} was monitored carefully to ensure the safety of the animal. Water only was made available for the initial 6 h postexposure. Six hours following the termination of the heat stress, a second blood sample was obtained. Animals were then returned to their housing cages and fed. Animals were returned to the animal prep room the next morning, again 14-16 h fasted to minimize any effects from nutritional or hormonal status on plasma enzymes, for the 24 h blood sample.

Changes in body water. During the heat stress, all body waste was captured in a pan beneath the chair and weighed. Thus, the change in body weight was considered representative of evaporative sweat loss. Body water lost as respiration was not measured but was assumed to be minimal. The rate of evaporative sweat loss during the two conditions was calculated as the body weight lost over the duration of the exposure.

Blood collection and analysis. Venous blood (3-3.5 ml) was obtained with a 22 gauge hypodermic needle from the saphenous vein of the leg within 5 min following the termination of heat exposure, and at 6 h and 24 h postexposure. The blood sample was transferred from the syringe to a plain test tube. A 100- μ l aliquot of this blood was then deproteinized in 200- μ l cold 8% perchloric acid, centrifuged and the supernatant stored at -70 °C until analysis for blood lactate (La) concentration (1). The remaining blood was centrifuged and the serum separated for subsequent analysis of creatine phosphokinase (CPK; Sigma Kit No. 661), lactate dehydrogenase (LDH; Sigma Kit No. 500), and aspartate aminotransferase and alanine aminotransferase (AST and ALT, respectively; Sigma Kit No. 505). All assays were performed using a Gilford spectrophotometer.

Statistical analysis. Comparisons of the two experimental trials (LS and HS) were made using a repeated measures analysis of variance (ANOVA; monkey = random factor, condition and time = fixed factors). The level of significance was set at $P \leq 0.05$ and significant differences in change over time (deltas) between trials were identified using post-hoc Duncan's Multiple Range Tests. The body heat gain, evaporative sweat loss, T_{sk} , HR and VO_2 were analyzed for differences between conditions using paired t-tests.

RESULTS

Heat gain. Comparison of the HS vs. the LS trial (mean \pm SE; 0.059 ± 0.002 vs. 0.029 ± 0.003 °C·min⁻¹, respectively) indicated that the rate of gain was significantly greater in the HS trial. These rates are represented in Figure 1.

Evaporative sweat loss. As illustrated in Figure 2, rate of evaporative sweat loss was significantly greater in the LS than in the HS trial (0.50 ± 0.05 vs. 0.07 ± 0.05 g·min⁻¹, respectively).

Skin temperature. No significant difference existed between the mean skin temperature of the thigh over time during the HS and LS trials. Mean values over the time of exposure for this measure were 39.51 ± 0.1 and 39.54 ± 0.2 °C in the HS and LS conditions, respectively.

Heart rate. Mean HR during the two exposures was not significantly different. HR averaged 199 ± 5 and 192 ± 8 during the HS and LS conditions, respectively.

Oxygen consumption. The mean VO₂ during the HS exposure tended to be greater than during the LS trial, however, no significant differences were identified (52 ± 4 vs. 43 ± 2 ml·min⁻¹; HS vs. LS).

CPK and LDH. No significant differences existed in either the CPK (Figure 3) or LDH (Figure 4) responses during the two exposures. However, both enzymes did tend to rise over time in both the HS and LS trials.

AST and ALT. AST increased significantly from 0 h post at both the 6 h and 24 h post time points (Figure 5). No significant differences existed among the ALT values. The ALT response is depicted in Figure 6.

Blood lactate. No significant differences existed among lactate values. The lactate response is illustrated in Figure 7.

DISCUSSION

Rats exposed to heat stress while at rest demonstrate increased membrane permeability as indicated by the elevation of selected serum enzymes. Manjoo et al. (8) exposed anesthetized rats to two environmental heat loads which resulted in different rates of body heat gain. Plasma was assayed for CPK, LDH, AST, and ALT immediately post-exposure, 6 h and 24 h postexposure. Enzyme levels were not different from control levels at termination of the heat stress; however, all enzymes were significantly elevated at 6 h postexposure in both environmental conditions with the greatest increase in CPK, LDH, and AST following the higher rate of heat gain. All enzyme levels had returned to control values by the 24 h sample. This response pattern of the measured enzymes indicates that the animals did not suffer heatstroke. In heatstroke, where tissue damage is severe and widespread, the selected serum enzymes elevate to very high levels and remain elevated above normal limits after 96 h (7). When heatstroke is not induced, the increase in these enzyme levels is much less extensive and transient.

Hubbard et al. (5, 6) exposed rats to hyperthermic, environmental conditions while at rest or during exercise to exhaustion until core temperature reached a preselected

endpoint between 41.0-43.3 °C. Serum levels of CPK, AST, and ALT were determined at 30 min, 24, 48, 72 and 96 h postexposure. Peak enzyme responses occurred at 24 h post-exposure for the transaminases and at the 30 min sample for CPK. Increases in CPK appeared most responsive to the exercise stress whereas both AST and ALT were most elevated following hyperthermia alone. With hyperthermia alone, the ALT and AST respond similarly. However, when work is combined with heat stress, there was a greater incidence of elevated AST at lower core temperatures. Hubbard et al. (6) hypothesize that AST reflects generalized cellular damage better than ALT.

Although the mean maximum core temperature attained in both the Manjoo et al. (8) and Hubbard et al. (5) studies was similar, the difference in blood sampling times makes comparison of the two studies difficult. Both studies passively heated groups of rats to a maximum core temperature at 42.3 °C; however, the ALT and AST levels at 24 h in Hubbard et al. (5) were much higher than the enzyme values at the same sample time in the Manjoo study. Differences in rates of heat gain in the two investigations or the fact that Manjoo's rats were anesthetized and Hubbard's rats were restrained may have contributed to this discrepancy in the enzyme response.

Physiological responses to heat stress. Because literature on the response of the rhesus monkey to hyperthermic conditions is limited, we were uncertain as to the maximum core temperature these primates could endure without suffering irreversible heat damage. Our laboratory has previously attained core temperatures of approximately 41 °C in the rhesus with no injury to the primate (2). Therefore, all exposures during this investigation were terminated when core temperature reached 41.0 °C. Because of the different rates of heat gain, the time of exposure for the two trials also differed significantly (Figure 1). The duration that these animals remained in the restraint chair may have influenced the tendency for a greater serum enzyme response (NS) during the LS trial than the HS trial as the primates became more active in the restraint chair as time progressed.

The significantly greater body weight loss (Figure 2), representative of evaporative sweat loss during the LS trial occurred as environmental conditions allowed for evaporation of sweat whereas during the HS trial, the high relative humidity of the environmental chamber did not allow for evaporation and thus very little change in BW occurred.

Serum enzyme response. During the present investigation, all enzyme levels found immediately postexposure were similar to resting, control values for female rhesus monkeys found in the literature (9) and obtained in our laboratory on another group of rhesus monkeys.

CPK is found in high concentrations in skeletal muscle, cardiac muscle, and the brain. Different isoenzymes are located in each of these organs; however, we measured total CPK rather than distinguishing between isoenzymes. Increases in CPK levels will occur with thermal stress, however, are rarely elevated above 1000 IU/L without physical effort (5). Manjoo et al. (8) found values up to 800 IU/L at 6 h postexposure when exposing anesthetized rats to hyperthermic conditions. These values are much higher than those found in the present investigation and are probably the result of the higher core temperature attained in the rats and perhaps differences between the animal models.

LDH is indicative of generalized tissue damage as it is located in all body tissues. Manjoo et al. (8) found levels up to 850 IU/L when exposing anesthetized rats to hyperthermic conditions. In the present investigation, we found levels up to only 275 IU/L. This enzyme remained elevated, although not significantly, at the 24 h sample.

ALT did not respond at all over time to either heat exposure. This enzyme is usually indicative of generalized tissue damage and typically responds to hyperthermia alone. The addition of physical effort does not increase ALT to higher levels than hyperthermia alone. Hubbard et al. (5) suggest that the elevation of this enzyme is perhaps indicative of hyperthermic injury to the liver. AST is sensitive to hyperthermic stress alone; however, the addition of physical exertion results in even greater enzyme levels than hyperthermia alone. Both AST and ALT appear more sensitive to heat stress without physical effort than CPK (6).

Blood lactate levels (Figure 7) immediately postexposure tended to be higher following the LS exposure than the HS exposure. Again, we relate this finding to the observation that the monkeys became more active in the primate restraint chair during the LS trial as time progressed. The higher lactate level (NS) during the LS trial supports our belief that the monkey was more physically active during the LS and this low level of physical exertion may have resulted in slightly elevated (NS) enzyme patterns during the LS trial.

Our findings indicate that the levels of body heat storage attained in this study did not provide an adequate thermal stress to obtain significant increases in the selected serum enzyme levels. Because of the possible danger of increasing the monkeys core temperature to values higher than those attained here, we suggest future research in this area to include physical exertion in a hyperthermic environment in an effort to induce elevations in these enzymes to further our knowledge in this area.

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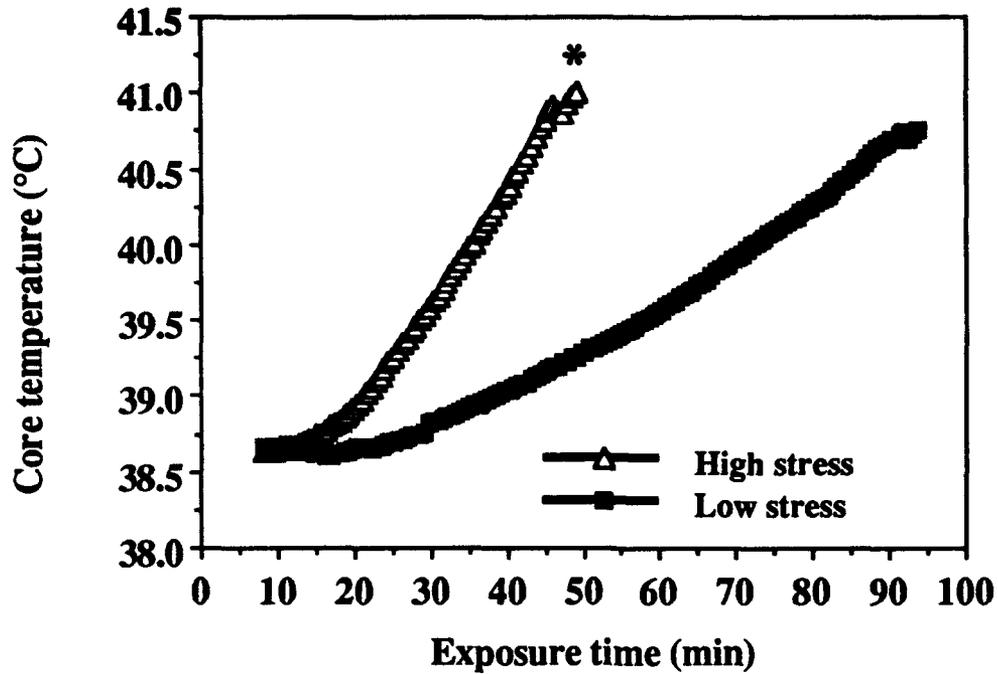


Figure 1. Core temperature over time in both the HS and LS exposures. Values were obtained every 30 s throughout exposure. *Significantly greater rate of heat gain than LS trial ($P \leq 0.05$).

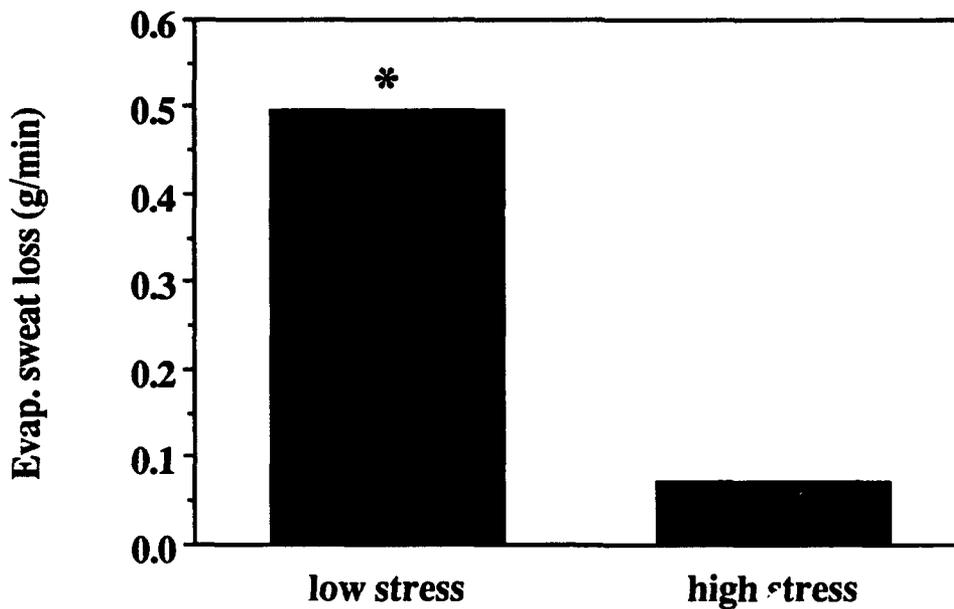


Figure 2. Rate of evaporative sweat loss as determined by body weight loss over the duration of the exposure. Values are means; $P \leq 0.05$. *Significantly greater than HS.

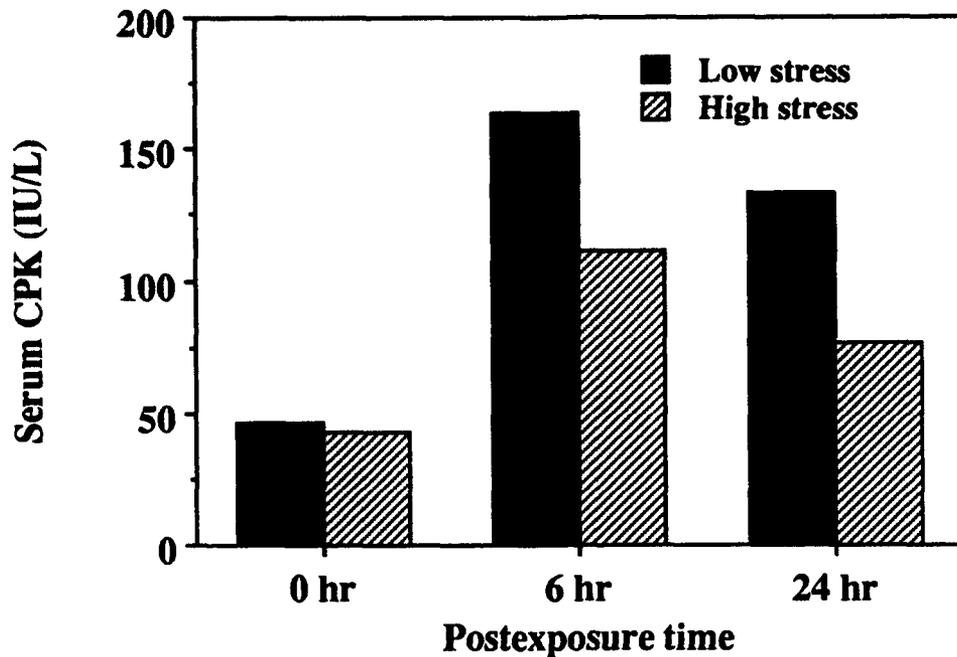


Figure 3. Serum CPK response at 0, 6, 24 h following heat exposure in both the LS and HS trials. No significant differences.

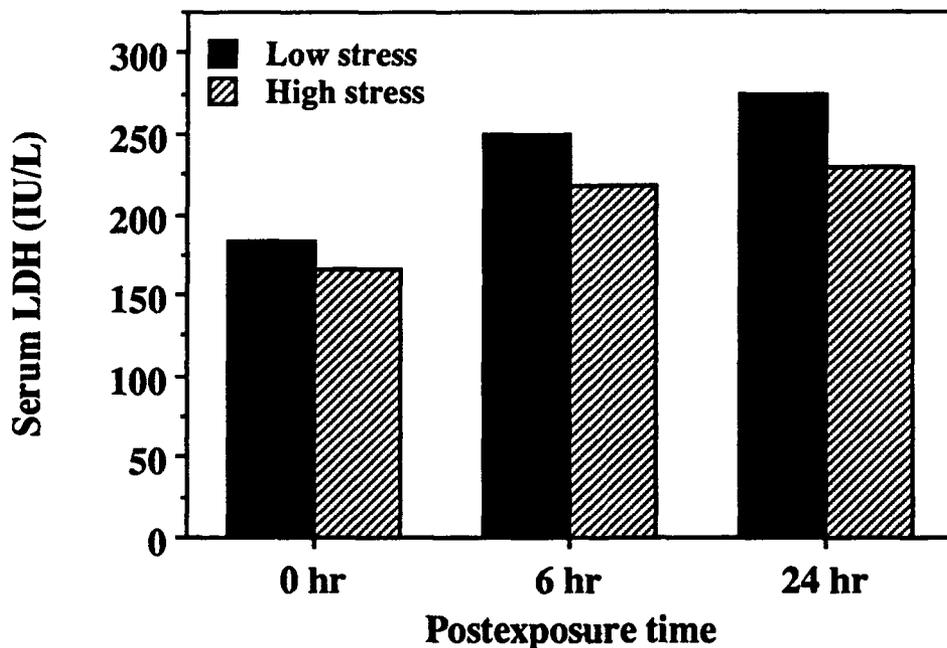


Figure 4. Serum LDH response at 0, 6, 24 h following heat exposure in both the LS and HS trials. No significant differences.

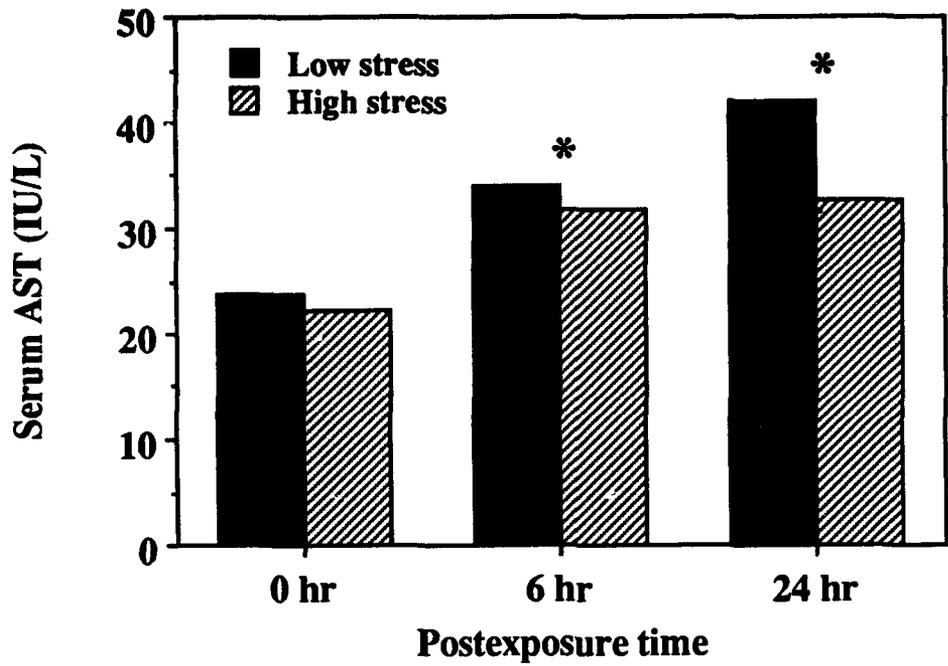


Figure 5. Serum AST response at 0, 6, 24 h following heat exposure in both the LS and HS trials. *Significantly greater than 0 h post.

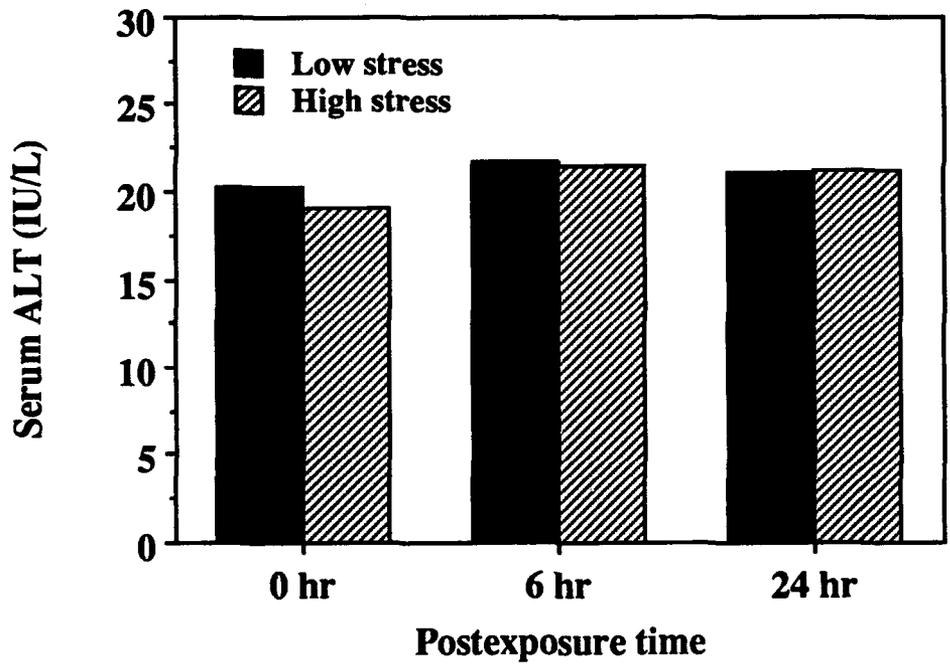


Figure 6. Serum ALT response at 0, 6, 24 h following heat exposure in both the LS and HS trials. No significant differences.

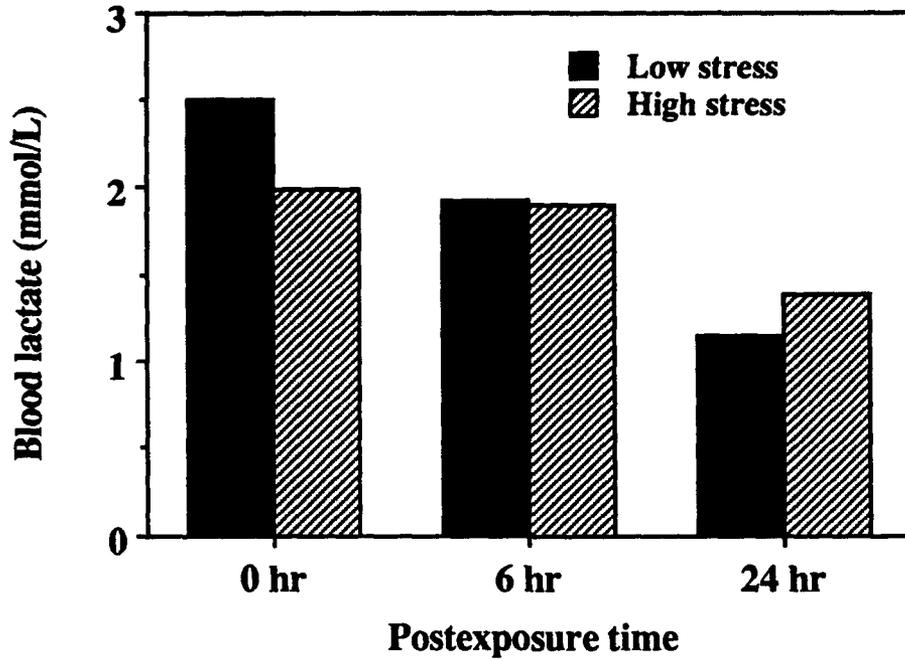


Figure 7. Blood lactate response at 0, 6, 24 h following heat exposure in both the LS and HS trials. No significant differences.