HIGHLIGHTED TOPIC | A Physiological Systems Approach to Human and Mammalian Thermoregulation

Heat intolerance: does gene transcription contribute?

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During exertion in the heat, heat-intolerant (HI) subjects have a physiological disability in metabolic heat dissipation. The HI state is either permanent or temporary, depending on whether it stems from transient predisposing factors or inherent thermoregulatory dysfunction. In this investigation, we studied protein levels of heat shock protein (HSP) 70 and HSP72, HSP90, bcl-2, etc., glutathione S-transferase-p, heat shock factor-1, TATA-binding protein-associated factor, and NF-kB transcripts using Western blot and quantitative RT-PCR, respectively, in lymphocytes of HI and tolerant (T) male volunteers of similar anthropometric features. Measurements were made from blood drawn before, during the heat tolerance test (3.5 mph, 40°C, 40% relative humidity, 2 h), and 1 h after recovery at 24°C. Rectal and skin temperatures, as well as heart rate, were continuously recorded. Of 58 subjects, 7 were identified as HI, with a significantly higher physiological strain index than in the T group (6.3 ± 0.9 vs. 3.8 ± 0.6, P < 0.001). The responsiveness of the vasculature to thermal stimuli was decreased in the HI group, as indicated by rectal temperature minus skin temperature. The HSP72 level in the HI group dropped during the recovery session (P < 0.01), whereas that of the T group continued to rise. A significantly increased expression of the transcription factors in the T subjects and significantly decreased expression in the HI group (P < 0.009, 0.013, and 0.005 for heat shock factor-1, NF-kB, and TATA-binding protein-associated factor, respectively) points to impaired transcriptional processes in the HI group. Our data suggest that transcriptional malfunction and sluggishness of the vasculature to thermal stimuli are predisposing factors in the HI group.

skin blood flow; transcription factors; heat shock proteins

AN INDIVIDUAL VARIABILITY in the ability to sustain heat stress exists, whereby certain individuals are more susceptible to heat than others and cannot sustain heat stress. When exercising in the heat, this group of “heat-intolerant” (HI) individuals is characterized by an earlier and greater rise in body temperature, a greater storage of metabolic heat, and a higher physiological strain than found in heat-tolerant (T) individuals. Such differences are apparent even during exercise of moderate intensity. HI is considered life threatening, because it can lead to heat exhaustion or heatstroke and eventually to death in sportsmen, soldiers, or others engaged in heavy exertion, usually in predominantly hot climates (6, 8, 25). The HI state is either permanent or temporary, depending on whether it stems from subject to transient predisposing factors, such as insufficient heat acclimatization, dehydration, infectious diseases, medications or drug abuse, or from an inherent dysfunction of thermoregulation (7). The latter includes individuals with a history of heatstroke episodes, as these could reflect an underlying tendency for heat susceptibility. The results of a large number of physiological studies on subjects predisposed to developing heatstroke corroborate the assumption that HI evolves from a failure of the thermoregulatory effectors for heat dissipation, possibly due to decreased heat conductance from the core to the periphery (28). Whether this failure is centrally initiated is not clear, and whether factors, other than integrative physiological responses, are involved is unknown. Documentation by Shiloh et al. (29) on the greater susceptibility of mental patients to thermoregulatory disorders favors a contribution of the central nervous system to HI.

The abundance of studies on the protective role of heat shock proteins (HSP) and the heat shock response (HSR), the ubiquitous nature of this response, and its phylogenetic conservation all suggest that HSPs are universal components of cell survival (2, 13, 30, 33). Thus the accumulating findings promote the hypothesis that perturbations in HSP-mediated cytoprotection contribute to the development of HI. This hypothesis is further supported by the association between an aberrant expression of stress proteins and disease states (21, 35). In certain disorders, the proteins are presented to the immune system as self-antigens, resulting in the production of auto-antibodies. Within this context, Wu et al. (36, 37) reported a higher incidence of antibodies to HSP71 in heatstroke cases than in controls, with higher titers of anti-HSP71 found in the severely affected victims. Although the differences between the control and heat-injured patients were not significant, this finding raises the question of whether HSP antibodies have the ability to downregulate HSP protection. Xiao et al. (38) reported that, in a group of patients suffering from heat-induced illnesses in the spring, basal levels of HSP70 were significantly higher than those in the control group. The author suggested...
that individuals with increased levels of HSP70 could be more sensitive or could respond differently to heat and exercise in harsh environments. Both studies leave us without conclusive results as to the role of HSP in heatstroke patients. Nevertheless, such findings lead us to hypothesize that impairment in the HSR is linked to HI.

The works of Sonna et al. (30, 32) and others (e.g., Refs. 9, 13) about genes linked with the HSR increased our awareness of the need to explore molecular perturbations as a potential underlying cause of HI. Sonna et al. examined the global genomic profiles of human lymphocytes subjected to heat stress (31) and that of lymphocytes of blood drawn from heat illness victims (32). These reports confirmed that heat shock causes extensive changes in gene expression of all functional categories of the HSR, showing that the HSR is far more extensive than previously recognized from studies in cultured cells. The time-dependent cascade of changes has at least three components: 1) a HSR that involves elements such as HSPs (similar to that described in a variety of cell lines in vitro); 2) a response that includes a substantial number of interferon-inducible genes; and 3) a small nonspecific stress response that is shared by other cell lines and stressors. An additional notable finding in these studies was the upregulation of genes encoding transcription factors, e.g., heat shock factor-1 (HSF-1), TATA-binding protein-associated factor (TAF), and NF-κB, implying a transcriptional regulation of various pathways associated with cellular maintenance and defense (30–32, 34).

Given the findings described above, the purpose of this investigation was to determine whether the HI state coincides with impairments in gene transcription. Based on the genomic profile described for lymphocytes obtained from heat-stressed subjects, and the transcriptome maps of stress-associated genes in the heart (9) and liver (41) of sedentary heat-stressed animals, we studied the transcription profiles of several representative genes from T and HI volunteers before, during, and after a heat tolerance test (HTT). Concomitantly, the integrative physiological responses characterizing strain indexes were monitored. Our data provided evidence that the HI group showed perturbations in the levels of transcription factors and that their basal hsp70 and hsp90 mRNA levels were significantly higher than those of the T group. A decreased vasomotor responsiveness of the HI subjects was recorded. Providing that the maintenance of molecular and cellular integrity under stressful conditions can “guarantee” a persistent performance of the system, our data suggest that combined sluggish vasomotor cardiovascular responses and maltranscription of genes linked to cytoprotection contribute to the evolvement of HI.

**MATERIALS AND METHODS**

**Subjects.** Fifty-one healthy male volunteers, of which 11 were suspected (up to 1 yr earlier than the present examination) as postexertional heatstroke or dehydrated individuals, participated in the study. All subjects signed informed consent forms before participation and underwent a thorough medical examination. The study was approved by the Israel Health Ministry Committee of human genetic studies.

**Anthropometric and physiological measurements.** The study was performed in the winter to prevent the effects of natural heat acclimatization. The medical examination and anthropometric measurements included weight, height, and percentage of body fat. Body fat was assessed using multiple skinfold thickness measurements performed by an experienced researcher. The mean of three skinfold thickness measurements made with calipers at each of three sites (triceps, waist, and subscapular) (18) was calculated to assess percentage of body fat. Body mass index was calculated as weight (kg) divided by height squared (m²). Dubois body surface area (A₅₋) was calculated as: A₅₋ = 0.007184·W⁰.⁴²₅·H₀.₇₂₅ (m²), where W is weight (kg) and H is height (cm) (5). To evaluate the aerobic fitness of the volunteers, we measured maximal oxygen consumption (VO₂max), using online computer-assisted open-circuit spirometry (Sensor Medics) during incremental exercise on a motorized treadmill. Each subject ran on the treadmill (5.5–6 mph), with the grade increased by 2% every 2 min until exhaustion.

**HTT.** To evaluate the physiological response to exercise and heat stress, all subjects performed a HTT. The HTT (23) included walking on a treadmill for 2 h at a speed of 3.5 mph in a climatic chamber (40°C, 40% relative humidity). These conditions are considered to be a “heavy heat load.” During the HTT, rectal temperature (Tₑₑ) was continuously measured using a rectal thermistor (YSI-401, Yellow Springs Instruments USA) inserted 10 cm beyond the anil sphincter. Skin temperature (Tₛₚₚ) was continuously measured at three sites (arm, chest, and leg) using skin thermistors (YSI-409). Mean skin temperature (Tₛₚₚₑₑ) was calculated according to Burton’s equation (1):

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Tₛₚₑₑ = 0.5Tₑₑₑₑ + 0.34Tₑₑₑₑ + 0.16Tₑₑₑₑ
\]

All measurements were continuously recorded and displayed by using a computerized system (Envidas, Envitech, Israel). Heart rate (HR) was continuously monitored by using a hearth watch with a data logger (Polar, Stamford, CT). The Physiological Strain Index (PSI), based on Tₑₑ and HR, was calculated at 10-min intervals to assess the relative level of heat strain as follows (24):

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PSI = 5(Tₑₑₑₑ - Tₑₑₑₑ)(39.5 - Tₑₑₑₑ)⁻¹ + 5(180 - HRₒₒ)(HRₑₑ - HRₒₒ)⁻¹
\]

where Tₑₑₑₑ and HR, are simultaneous measurements taken at any time during heat exposure, Tₑₑₑₑₒₒ and HRₒₒ are the initial resting values, 39.5 is maximal core temperature (°C), and 180 is HR (beats/min). Sweat rate (in g/h) was calculated according to the difference between pretest and posttest nude body weight, with the posttest weight corrected for fluid input and urine output, divided by the time of exposure.

**Exclusion criteria.** Volunteers matching any of the following criteria were removed from the heat exposure: resting Tₑₑₑₑ > 39°C, HR > 170 beats/min, nausea, weakness, dizziness, subject’s request, or the decision of the physician in charge. The volunteers were instructed to rest for at least 3 days before the experiment and to drink 0.5 liter of noncaffeinated beverages on the night before and on each morning of the experiment to ensure body euhydration. Throughout the HTT period, the subjects were allowed and encouraged to drink ad libitum.

**Gene expression and protein analyses in blood lymphocyte.** For gene expression and protein analyses in lymphocytes, peripheral blood samples, 3–5 ml each, were drawn (into EDTA-containing tubes) before, at the end of the HTT, and following 1-h recovery at a constant environment (40°C, 40% relative humidity). These conditions are considered to be more severe (10x) than previously recognized from studies in cultured cells. The time-dependent cascade of changes has at least three components: 1) a HSR that involves elements such as HSPs (similar to that described in a variety of cell lines in vitro); 2) a response that includes a substantial number of interferon-inducible genes; and 3) a small nonspecific stress response that is shared by other cell lines and stressors. An additional notable finding in these studies was the upregulation of genes encoding transcription factors, e.g., heat shock factor-1 (HSF-1), TATA-binding protein-associated factor (TAF), and NF-κB, implying a transcriptional regulation of various pathways associated with cellular maintenance and defense (30–32, 34).

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containing 0.5 μg of oligo(dT)15 primer, together with 400 units of Moloney murine leukemia virus reverse transcriptase, according to the manufacturer’s instructions (USB, United States Biochemical, Cleveland, OH). For the PCR, 5 μl of the cDNA mixture were added to 50 μl of a master mix containing 200 μM of each dNTP, 100 pM of each specific primer, and 1.5 units of Vent polymerase (USB). We synthesized DNA oligonucleotide primers for human inducible HSP70, HSF-1, NF-κB, TAF, HSP90, bcl-2, and glutathione S-transferase (GST). The choice of this list of genes was based on current published knowledge of gene transcription during heat stress and following heatstroke (9, 13, 16, 30–32, 34, 37). Inducible HSP70 plays a universal role in stress response; HSP90, an essential component of the HSR, bcl-2, and GST-p, are also considered part of the heat stress-induced response. HSF-1, a key factor in HSP transcription, and NF-κB, which upregulates in several HSP-mediated protective cascades, were chosen in accordance with our hypothesis that impairment in gene transcription predisposes to HI. Given that heat stress induces the transcription of a large number of genes, the general transcription factor TAF, which is involved in initiating transcriptional process in general (including that of HSF-1), was also chosen.

The oligonucleotide sequence and RT-PCR protocols were obtained from the published literature (Clontech Laboratory, Palo Alto, CA). To ensure equal amounts of initial mRNA, we performed parallel actin amplification (annealing temperature 62°C, 28 cycles, Ref. 9). The PCR products were resolved on 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. Band density was analyzed by using TINA software (Raytest, Straubenhardt, Germany). HSF-1, TAF, and NF-κB mRNAs were also measured using quantitative real-time RT-PCR (ABI Prism 7000 Sequence Detection System, Applied Biosystems). The reaction was carried out in a 20-μl reaction volume containing 10 μl of SYBRgreen Master Mix (Applied Biosystems), 500 nM each of the forward and reverse primer, and 5 μl of diluted cDNA. The appropriate cDNA dilution was determined from the calibration curves established for each primer pair. The thermal profile for SYBRgreen real-time RT-PCR was 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min. The primers for real-time RT-PCR were designed by using Prime Express software (Applied Biosystems). Sense sequences were as follows: HSF-1: GTGCAGTCAAACCGGATCCT; NF-κB: CCATAC-TTCTGGGCAATCTGATG; TAF: AGAACATGATGAGCTTCGG-GAG; and the antisense HSF-1: CGGGAGCCTATCTCAGTGAAA; NF-κB: GGGAGGCTATCTCAGGTTA; and TAF: GAATTCCC-ATAACCAAGCTGAAG. The results were analyzed by the comparative threshold cycle method, which reflects the difference in target gene threshold relative to that of β-actin in each sample (17).

**Western blot analysis.** Total protein (50 μg/lane) was fractionated by electrophoresis on 12% polyacrylamide gels under denaturing conditions (14) and transferred onto nitrocellulose membranes. The membranes were blocked for 2 h in PBS containing 5% dried skimmed milk powder and then probed overnight at 4°C with primary antibody, diluted 1:1,000. After repeated washings, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Jackson) diluted 1:1,000. The antibodies used were mouse polyclonal anti-HSP70 (Stressgen). Specific antibody binding was detected by using enhanced chemiluminescence (Amersham) and visualized by exposing X-ray film to the membrane (for further details, see Refs. 9 and 16). The density of the scanned protein bands was calculated using TINA software (Raytest, Straubenhardt, Germany).

**Statistical analysis.** One- and two-way ANOVA were carried out using commercially available computer software (Sigmastat, SPPS). Treatments were taken as the fixed effects, and the individual lymphocyte gene samples were assumed to be random samples from the population. Student’s unpaired t-test was used for individual matched-group comparisons. The data are expressed as means ± SE, unless otherwise stated. P < 0.05 was considered statistically significant.

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**RESULTS**

**Physiological responses to heat stress.** Collectively, the physical characteristics of all volunteers were as follows (means ± SD): age 22 yr (SD 1), height 174 cm (SD 7), weight 73 kg (SD 11), body mass index 24 kg/m² (SD 3), body surface area 1.8 m² (SD 0.15), body fat 16.5% (SD 5), and VO2max 48.6 ml·kg⁻¹·min⁻¹ (SD 1.8). Based on the HTT, seven subjects were labeled as HI. The values of the HI group compared with the collective values of all subjects were characterized with significantly lower (P < 0.05) VO2max [42.03 (SD 24.7)] and higher (P > 0.05) values of height [177.62 cm (SD 7.3)], weight [79 kg (SD 13.78)], body surface area [1.92 m² (SD 0.17)], and body fat [18.8% (SD 10)]. Of the seven HI subjects, all but two had suffered from heat exhaustion or heatstroke in the past. The physiological heat-stress responses are presented in Fig. 1. The differences in HR and Tre, as well as the physiological strain between the two
groups, are clear from the onset of heat stress and throughout the entire exposure period. Both Tre and HR for the HI subjects were markedly higher than in the T group, resulting in a threefold increase in their PSI. The data agree with published results (23). Given that the Tre - Tsk value is indicative of body shell thermal conductivity (12), and in turn blood flow, we calculated this value as well (Fig. 2). Notably, the slope of the curve delineating Tre - Tsk vs. Tre in the T group is 48% steeper than that of the HI group, suggesting greater core temperature-to-Tsk gradients to allow heat dissipation (Fig. 2A). Interestingly, the Tsk flow is oscillatory, and the Tre - Tsk oscillation frequency is faster in the T group (Fig. 2A), suggesting an enhanced cardiovascular responsiveness.

Genomic responses to heat stress. The level of the stress biomarker HSP70 was measured to determine whether the HSR is different in T and HI subjects. Figure 3 shows that no difference was found between the T and the HI groups in basal HSP levels and levels following the HTT. After 1 h of recovery at room temperature, however, T subjects continued to synthesize HSP70, whereas HI subjects failed to do so, and the HSP70 levels in the latter group were significantly lower than those of the matched T sample (P < 0.002, Fig. 3). Similar differences were observed in the expression profile of gene transcripts encoding the cytoprotective proteins and transcription factors involved with cellular maintenance and defense. The results of this experiment are presented in Figs. 4 and 5. Both the HSP70 and HSP90 transcripts were markedly higher in HI subjects than in the T group. Whereas HSP70 mRNA did not show a significant change upon recovery at room temperature in the HI group, HSP90 continued to rise in both groups. The anti-apoptotic bcl-2 was upregulated at the end of the exercise bout in the T group but showed a drop among the HI subjects at that point (Fig. 4). No change was detected in the ROS scavenger GST-p (data not shown). Interestingly, all transcription factors, HSF-1, NF-κB, and TAF, were markedly upregulated in the T group but downregulated in the HI group (Fig. 5). A significant drop in transcription factors was shown only after the 1-h recovery at room temperature.

**DISCUSSION**

HI is a potentially life-threatening condition, particularly in hot climates. Nevertheless, few studies have been designed to gain an understanding of the origin of HI as a congenital disorder and are confined to physiological responses. In the absence of known predisposing pathological factors, inefficient thermoregulation, possibly due to decreased heat conductance from the body core to periphery (11, 28), and greater metabolic heat production, usually in a hot environment, are considered the major causes for this phenomenon. The recent advancements in our knowledge of heat-tolerance-linked genes (9, 13, 30–32) indicate a need to revisit our current concept of HI and to consider gene transcription malfunction as a pivotal constitutive predisposing factor. In the present investigation, HI subjects with a history of heatstroke and T subjects with similar anthropometric features were studied. Taken together, we discovered that the HI phenotype is characterized by an attenuated
transcription of genes known as cytoprotective and by a sluggish vasomotor response, leading to impairments in the responsiveness of skin blood flow (SkBF) compared with that of T subjects. We, therefore, suggest that the HI phenotype demonstrates concomitant physiological and molecular altered functions. In the thermoregulatory hierarchy, during acute heat stress, the outcome of the inducible molecular response lags behind that of the integrative response. Therefore, the inability to upregulate transcription of cytoprotective-associated mechanisms (leading to hastening of cellular thermal injuries) probably “predisposes” to HI.

**Physiological responses.** Differences between the groups were apparent from the onset of heat stress. Both T re and HR were elevated in the HI group. Although the HI group was somewhat heavier (8.2%) and had a lower \( V_{O2 \, max} \) (8.9%) than the averaged values of all subjects, the differences in the physiological responses observed in our tests were related to vasomotor features, independent (and markedly greater than) of these differences. Furthermore, in a previous investigation (23) in which T and HI subjects were compared, no difference in \( V_{O2 \, max} \) between the two groups was observed. However, HI subjects elevated their HR markedly faster than did T subjects during the onset of the comfort tolerance test, implying cardiovascular vasomotor impairments as well. In the present investigation, the acquisition of \( T_{sk} \) allowed us to calculate the core-to-surface temperature gradient, a value controlled by blood flow. Two aspects of the blood flow of the HI subjects were different from those of T subjects: 1) slower oscillations, and 2) lower \( T_{r} \) increments per unit rise in core temperature. Assuming that the increase in peripheral blood
flow is autonominously controlled, the data suggest that, in HI subjects, the peripheral response is attenuated. However, our data are insufficient to even begin to suggest that this change is due to a pre- or postsynaptic response. Nevertheless, similar attenuation in SKBF was reported for aged rats and humans in the heat (11, 12, 20, 22). In older individuals, this transformation is associated with collapse, disorganization, and, in some cases, a total disappearance of the vessels of the microcirculation in the dermal papillary and superficial vascular plexus (22), i.e., structural alterations leading to diminished maximal SKBF capacities in this group. Mechanistic changes underlying this age-related decrement in SKBF (e.g., nitric oxide and axon reflexes, and inhibition of active vasodilatation) have been revealed only recently (11). In accordance with these findings is the discovery that impairment in orthostatic tolerance during heat exposure in diabetic patients stems from endothelial dysfunction rather than neuropathy (27). Similarly, no change was found in old subjects in the baroreflex control of the orthostatic response (39). The possible mechanisms for attenuated blood flow in HI have not yet been studied. Our data, unequivocally, support the notion that HI involves a decreased responsiveness of the peripheral vasculature. In analogy to aging, this phenomenon might be a consequence of altered endothelial function.

Genomic responses. The cellular reserves of cytoprotective proteins play a pivotal predisposing factor in the development of thermal injury. Among these proteins, the universal HSP70 is the most extensively studied with respect to injuries induced by heat. HSPs are protective yet under certain conditions can behave as “danger biomarkers” and can be cytotoxic (26). Here, our finding of no difference in HSP72 levels between the groups, neither before nor during the HTT, suggests that the basal HSP level of lymphocytes per se cannot serve as an indicator of HI. In contrast to the consensus profile of the HSR, namely an elevation of the inducible HSP70 for 24–48 h in T populations, HI subjects studied here failed to elevate their HSP 72-kDa levels following the HTT. Furthermore, the HSP70 transcript-to-protein ratio was markedly higher in the HI than in the T group (see Fig. 4A, inset). Hence, such individuals may not be protected when exposed to subsequent acute heat stress via the HSR. Accordingly, HI subjects maintained relatively stable HSP72 transcripts, markedly higher than those of the T group, who upregulated their transcript levels over time as the experiment progressed. HSP90 mRNA, an additional component in the HSR, showed a similar profile (Fig. 4). In thermotolerant cells, protein synthesis is rapidly inhibited by heat stress but recovers faster than in naive heat-stressed cells, a phenomenon known as translational thermotolerance (3). Hence, the drop in HSP for HI subjects following the HTT might be associated with disrupted synthesis. In contrast to our results in the HI subjects, exertional heatstroke victims studied in China during the early spring (38) showed significantly elevated inducible HSP70 levels in their lymphocytes compared with the healthy matched group. Unfortunately, the lymphocyte HSP70 levels of the subjects were not measured before the onset of exercise.

Along with the observed decrease in HSP70 synthesis, HSF-1, which initiates HSP transcription by binding to the nuclear heat shock element (19, 26), was downregulated in the HI group. This phenomenon concurs with decreased HSP levels and the abrogation of the HSR (19), but is hard to reconcile with the elevated HSP70 transcript level detected in this group. Given the negative feedback loop in the control of HSP70 translation, we can hypothesize that constitutively elevated HSP70 mRNA reflects disrupted translational processes, whereas the inability of its overexpression, triggered by exercise in the heat (during the HTT), stems from HSF-1 down-regulation. This explanation agrees with the relative stability of HSP90 mRNA, which is also controlled by HSF-1. The cellular induction of HSP is one successful strategy for surviving the damaging effect of stress (2, 9, 13, 19, 30, 31). Except for its protective role against proteins misfolding or denaturation and inflammation, several indications of its involvement in the maintenance of synaptic transmission have been reported (15). Nevertheless, the awareness that heat shock causes extensive changes in the gene expression of all functional categories of the HSR (13, 30, 31) led us to believe that key signaling networks, other than those activated by the HSR, play a role. We, therefore, measured NF-κB and TAF profiles in our experimental setup. In the HI subjects, both transcript profiles resembled that of HSF-1 in this group, namely a drop during the HTT and recovery period (Fig. 5).

Both NF-κB and TAF are transcription factors involved with a plethora of cellular processes and stress responses. Although detailed signaling pathways are beyond the scope of this investigation, we can hypothesize that certain consequences result from the progressive decrease in their transcripts seen here. NF-κB belongs to a family of ubiquitously expressed transcription factors that are critical regulators of mammalian immune and inflammatory responses (3). This factor plays an essential role in the immediate-early activation of a multitude of genes encoding signaling and defense proteins and, therefore, appears to be a general mediator of cellular responses to stress (16). The DNA binding activity of this transcription factor and its activation, which are linked to the oxidative stress and redox state of the cell, can be modulated by environmental stressors in two ways. 1) Induction of the HSR before a proinflammatory signal inhibits NF-κB activation and NF-κB-dependent proinflammatory gene expression; under such conditions, inhibition of this transcription factor abrogates cell survival (3, 40). 2) In direct contrast, induction of the HSR after a proinflammatory signal can lead to apoptosis (16). We postulate that the attenuated HSR in HI subjects can lead to accelerated proinflammatory processes, which are destructive if or when the heatstroke syndrome develops. We characterized the TAF family transcription factors to identify potential targets in the transcriptional preinitiation complex, including that of HSF-1. Such contacts represent one of the final steps in the signal transfer/translation of heat stress to the transcriptional apparatus (10). Sonna et al. (31), in their study on peripheral mononuclear heat-stressed cells, note its significant upregulation as well.

Future perspectives. Collectively, the changes observed in the expression of the transcription factors studied here lead us to conclude that HI subjects suffer from a variety of cellular and molecular perturbations, which are seen (like the physiological responses) only when challenged by exertional heat stress. The development of molecular changes takes time to become apparent in the physiological outcome. We, therefore, hypothesize that, during heat stress, the manifestation of molecular changes lags behind physiological responses. Nevertheless, the molecular contribution to delayed thermal injury is
certain. The combination of rapid physiological responses and molecular responses is likely to determine thermal tolerance. An impaired molecular response accompanies HI and hastens the development of the heatstroke syndrome. Abrupt transcription of genes encoding proteins linked with neural activation (e.g., K currents and ion transporters) was measured in the rat hypothalamus under stressful thermal conditions (Schwimmer H and Horowitz M, GEO Series accession number GSE2890, http://www.ncbi.nlm.nih.gov/geo/). It is likely that such genes perform differently in the HI and T phenotypes, thereby effecting dissimilarly physiological responses.

REFERENCES