



Biochemical Effects of Heat Stress and Acclimation

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ABSTRACT

The illness due to heat is widespread with the rise of global warming. It is necessary to understand the mechanism of illness due to heat, in order to take measures to alleviate this heat stress. To understand this, some stress related biochemical parameters were measured in the serum/tissue samples collected from models of heat stress (humans with exertional heat stress and hypohydration) and acclimation. The heat stress increased the core body temperature in human volunteers. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) activity were measured in serum sample using spectrophotometric methods. The other stress related biochemical parameters like, Heat shock proteins Hsp 40, Hsp 47, hormones like thyroxine (T₄), 3,3',5-Triiodo-L-thyronine (T₃), Norepinephrine (NE), Epinephrine (E), enzymes like Lactate Dehydrogenase (LDH) and Creatine phosphokinase (CPK) were measured by ELISA in plasma samples. The transaminase SGOT and SGPT showed an increase. In the hormones, the heat stress decreased the level of T₃ and T₄. Whereas, the increase in concentration levels of Hsp 47, 40, NE, E, LDH and CPK in heat stress samples were significant. In acclimatized plasma samples the concentration of these parameters was seen to decrease and reach close to control value. This study suggests that T₄/ SGPT/ NE/ E /LDH/ CPK levels along with chaperones, can be used as prognostic proteins in heat stress related disease. Their validation in human samples will help them to be established as biomarkers of heat stress and acclimation.

Keywords: Acclimation, Biomarkers, Heat Stress, Hormones, Stress Proteins

INTRODUCTION

Global warming has affected the ambient temperature to a great extent. We are therefore facing extremes of hot and cold temperatures, both extremes thus affecting the quality of life and sustainability of life forms, decreasing the physical efficiency of the individuals who are working in the open field conditions like farmers, soldiers, laborers etc. thereby making them vulnerable to heat stress related problems. They are important people whose efficiency should be maintained in the interest of the nation. To meet this challenge, there is a need to understand the effects of hot and cold environment on the human body. This understanding will facilitate the design of diagnostic and therapeutic measures to combat the morbidity and mortality associated with heat stress. Earlier studies have shown that heat stress increases the protein unfolding which affects their functioning. These proteins can be folded back with the help of proteins called chaperones. Chaperones have a role in post translational modification of protein as well as increased unfolding associated with different kinds of stress. Chaperones (like Heat Shock Proteins Hsp 70, 72, 90, 105 etc) levels have already been associated with heat stress in humans, rats, cattle, plants (3,6, 9, 11, 13, 15, 16, 17, 22, 23, 24) The induction of chaperones is not only found in heat stress but also with other conditions like viral fever, specific drug use, ageing (1, 4) etc. The chaperones being general indicator of an entire spectrum of stresses cannot be classified as the biomarkers for heat stress per se. There is need to explore other biomarkers which can specifically mark heat stress and acquisition of acclimation along with chaperones. Thus, the present study was designed to explore the biochemical effects of heat stress to identify additional biomarkers and to understand the etiology of heat stress and acclimation to facilitate design of detection and treatment approaches. Here the concentrations of stress markers have been evaluated in these conditions.

METHODOLOGY

Exertional Heat Stress Model (Human): To assess the effects of exertional heat stress induced hypohydration on human physiology and biochemistry, six participants (each group) were made to perform sub-maximal exercise (Ramanathan 1964) in Human Climatic Chamber (HCC) simulated at 45 °C and 30% RH. The exercise was performed at two separate occasions to attain two different hydration states: 2% body weight reduction or 2% hypohydration and 4% body weight reduction or 4% hypohydration (HY2 and HY4 respectively). Oral temperature was recorded using YSI electrodes from which the core body temperature was deduced. These were used as acute heat stress models.

For the acclimation (AC) study: To attain a state of heat acclimation, standardized step test (submaximal exercise) was performed by the participants at 45 °C, 30 % RH simulated in human climatic chamber for 100 minutes for eight days. The sample collected before exercise on first day served as control: after exercise on first day served as acute heat stress sample: post exercise on eighth day served as post acclimation sample. These plasma samples were used for biochemical studies.

Collection of serum and tissues samples: The serum/plasma samples were collected from human volunteers. These were kept at -80°C till use.

Estimation using ELISA kits (Indian make):T3,T4, Heat Shock Protein (Hsp) 40, 47, Epinephrine, Norepinephrine, Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CPK) assay in heat stressed human plasma by ELISA . Sandwich/competitive ELISA were set up for proteins and hormones. The readings were read in ELISA reader.

Colorimetric study: The estimation of activity of Transaminases: The activity of transaminases SGPT and SGOT was estimated using colorimetric estimation kits of Span diagnostics.

Statistical Analysis: The mean, SD and SE were calculated for all the results. The significance was calculated by one way analysis of variance between controls and experimental. Values with P<0.05 were considered as significant are marked as *.

RESULTS

The changes in core body and skin temperature in Human: The exertional heat stress and hypohydration (T1: 2 % hypohydration and T2: 4% hypohydration) increase the core body temperature (Figure 1) in humans.

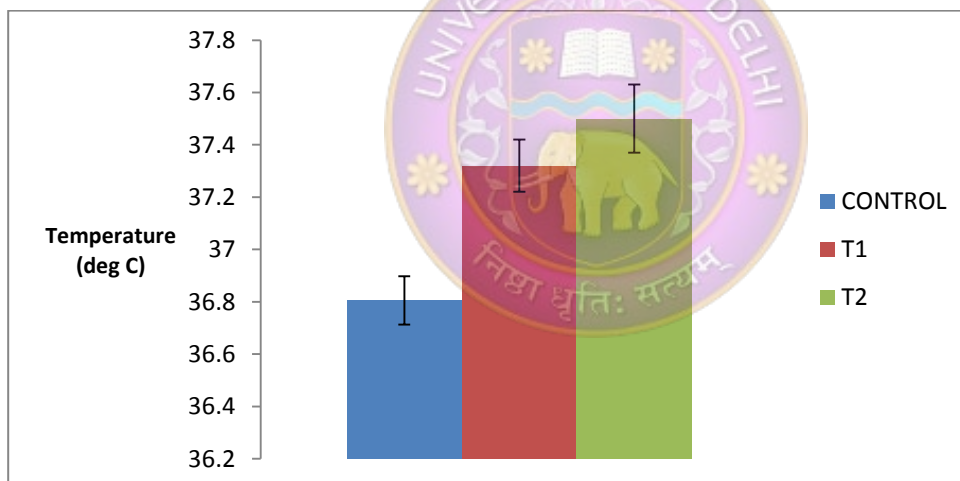


Figure-I. Core Body Temperature in Humans

The results of biochemical assay show insignificant decrease in T3 and significant decrease in T4. The effect is more in samples with HY4 (Table 1, Figure II and III). The transaminases show non significant increase in SGOT values whereas SGPT show a significant increase

in HY4 samples (Table-I, Figure-IV). Similar trend was observed in the serum samples of heat stressed rat.

Table-I. Biochemical levels in heat stressed human serum samples

Assay	Type	Activity in UI/L			Significance
		Control	Heat stressed 2%	Heat stressed % Hypohydration	

			Hypohydration		
Thyroid hormone (ng/ml)	3	67± 0.01	66±0.007	65±0.004	>0.05,NS
	4	68.04±0.46	65.48±0.706	65.34±0.503	<0.05,S
Transaminase (U/)	GPT	68.44±6.12	68.03±5.74	67.49±6.82	<0.05, S
	GOT	65.72±6.24	65.13±4.72	65.16±3.66	>0.05, NS

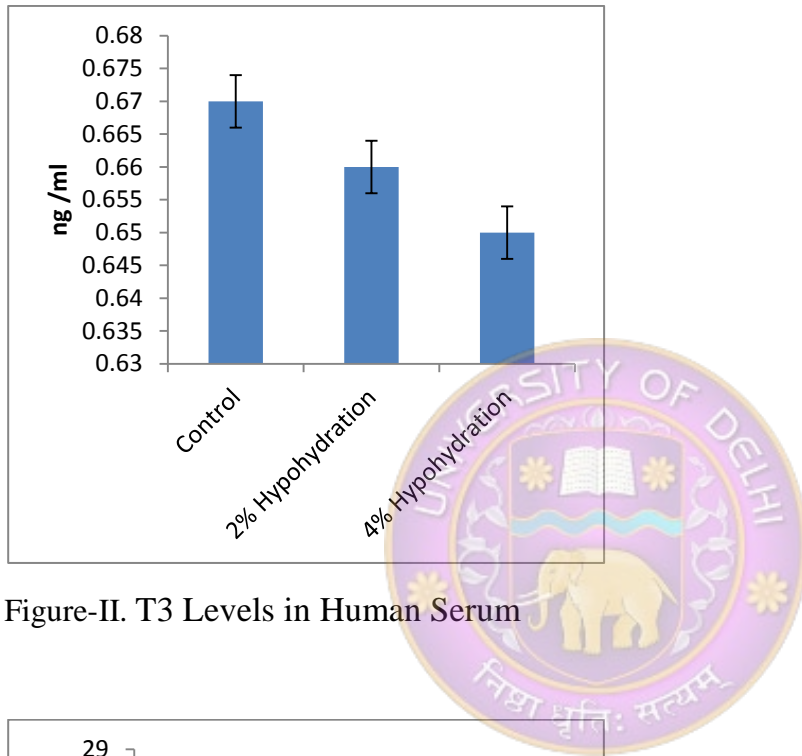


Figure-II. T3 Levels in Human Serum

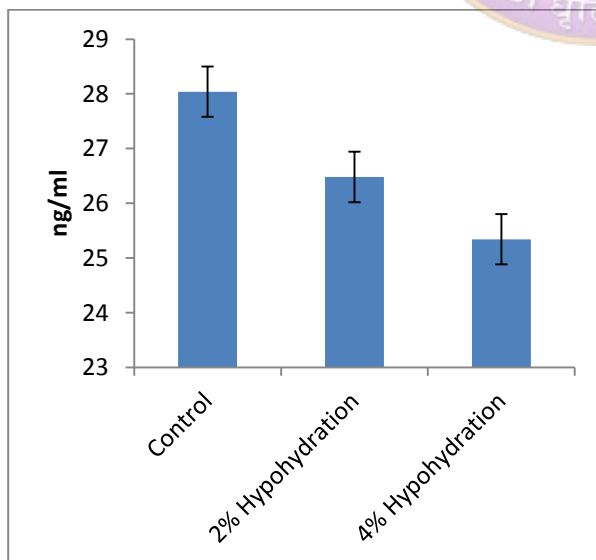


Figure-III. T4 Levels in Human Serum

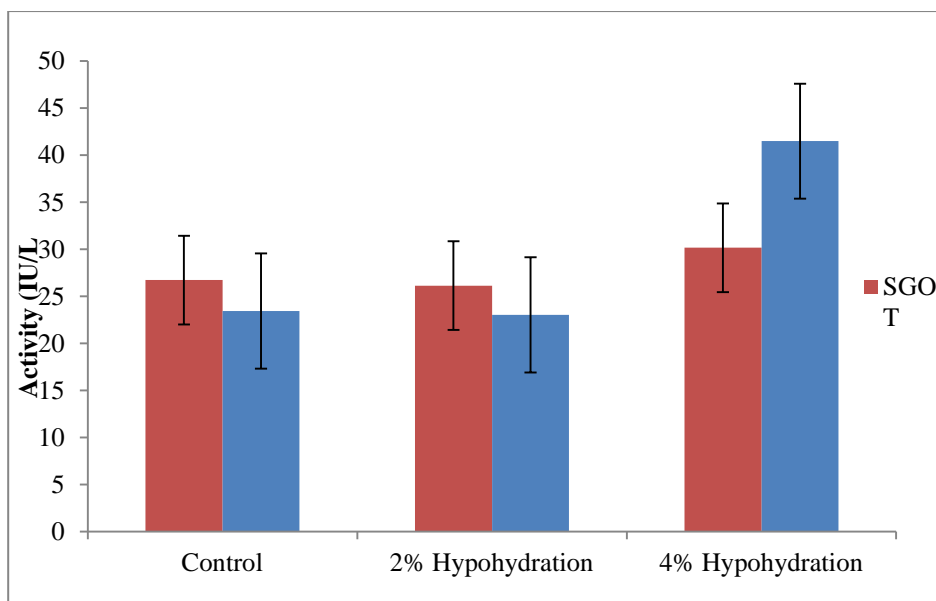


Figure-IV. SGPT & SGOT Levels in Human Serum

The concentrations of stress related proteins/hormones measured in human serum are reported in Table II. The increase in all of them in heat stressed samples is significantly different than controls ($P < 0.05$)

Table-II: Concentration of various proteins/hormones after heat stress and acclimation in human plasma estimated by ELISA.

Parameter (units)	Control Mean \pm SE	Acute Heat Stress Mean \pm SE	Post Acclimatization Mean \pm SE
Hsp 40 (ng/ml)	84.9 \pm 2.8	123.4 \pm 4.0 [~]	68.3 \pm 3.0 [¥]
Hsp 47 (ng/ml)	0.066 \pm 0.004	0.124 \pm 0.005 [~]	0.087 \pm 0.001 [¥]
Norepinephrine (pg/ml)	42.85 \pm 0.7645	55.09 \pm 1.272 [~]	43.40 \pm 0.8119 [¥]
Epinephrine (pg/ml)	32.41 \pm 0.916	46.53 \pm 0.923 [*]	31.18 \pm 0.877 [¥]
LDH (ng/ml)	6.124 \pm 0.985	9.278 \pm 0.270 [*]	7.756 \pm 0.285 [¥]
Creatine Kinase (ng/ml)	1.565 \pm 0.046	3.067 \pm 0.140 [*]	1.692 \pm 0.103 [¥]

The significance test between control and experimental is done using one way analysis of variance. (* depicts $P < 0.05$, significant and ¥ depicts $P > 0.05$).

DISCUSSION

The results of acute heat stress and acclimation show that humans when exposed to exertional heat stress show dehydration and increase in core body temperature (Figure-I) above the normal physiological temperature. The heat stress manifests as increase in levels of transaminases like SGPT and SGOT, LDH, CPK. This is indicative of tissue injury to liver, heart, muscle which is part of multiple organ dysfunctions during heat stress (7, 8). SGPT and CPK have been reported to be prognostic enzymes for heat stroke in humans (10) and cellular damage (1). The acute heat stress also affects the hormonal balance of the body. There are several hormones which assist in thermoregulation (2, 5, 7, 8, 12, 14). To observe this effect the concentration of some of the hormones like Thyroid Hormones, Catecholamines were investigated. The levels of Thyroid Hormone T3 and T4 show reduction under heat stress. This may be to help the body to bring down the basal metabolic rate which may reduce the body heat. The stress hormones like Epinephrine and Norepinephrine show an increase in concentrations. These hormones are likely to help in change in circulation to bring about thermoregulation. The concentrations of Hsp 40, 47 also are increased in AHS plasma samples. The chaperones have been implicated in heat stress. And the increase in chaperone levels is indicative of heat stress response. This response is shown in stress condition wherein excess protein unfolds due to the increase in core body temperature. The proteins then use chaperones to fold the proteins back into correct conformation. To maintain proper conformation of protein the body tries to increase the transcriptional and translational levels of chaperones.

Acclimation is known to improve physiological functions in presence of the stressor (18, 19). The stressor can be environmental change in temperature, hypoxia etc. Our study shows that the biochemical levels of hormone and proteins return to the levels when the human is relaxed and not under any stress. In the present study the levels of chaperones Hsp 40, 47, Norepinephrine, Epinephrine, LDH, CPK return to near control levels. As compared to the controls the levels are slightly higher but the difference is not significantly different. This may be conferring them greater ability in thermoregulation.

CONCLUSION AND RECOMMENDATIONS

We would like to propose that T4/ SGPT/ Epinephrine / Norepinephrine/ LDH/ CPK can be used as biomarkers of heat stress in addition to chaperones. We also suggest that if one maintains good hydration level, one can combat heat stress. Our traditional method of drinking lemonade in hot summers had both these components, the water and the antioxidant (lemon). That is why they are the popular summer drinks.

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