

Effects of water deprivation on behavioral Changes in BALB/c mice in hot humid climate of north east India

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Abstract

Aim: Abnormalities in water balance are manifested as hyponatremia and hypernatremia and the present investigation shows the effect of water deprivation in hot and humid climatic condition of north eastern part of India. **Methods:** Twenty four BALB/c mice of either sex (weight 30-40 g) were used for a 13-week experiment to determine the effects of water deprivation on behavioral and physiological changes. Mice were divided into control group (I) ($n = 8$), given water *ad libitum*, water-deprivation male group (15 ml/day) (Group II, $n=8$) and water-deprivation female group (15 ml/day) (Group III, $n=8$). **Results:** The water deprivation mice shows significantly ($P<0.05$) deteriorated of reflex, progressive visual placement, emotional whereas body weight, grip strength and rectal temperature were significantly ($P<0.05$) increased as compared to the control group. This information is useful to those living or deployed in crisis situations where the water supply limited in a hot-humid climate.

Key words: Emotional change, grip strength, hot-humid, reflex, water deprivation

INTRODUCTION

There are many life situations when unfavorable circumstances influence the hydration and causes leads to water deprivation physiological impairment of dehydration or water deprivation in humans has been extensively studied and the physiology of this phenomenon is generally well understood.^[1,2] It is generally assumed that unless there is pathology or environmental stress, healthy adults maintain fluid and electrolyte balance. Hydration status has the potential of being critical to the performance of military personnel. An American soldier afflicted by heat illness during World War II was in part the impetus for early research. Yoram Epstein of the Israel Defense Forces Physiological Research Unit and his colleagues assessed

the relative effects of varying heat loads and mission intensities on psychomotor performance.^[3] A landmark study demonstrating the impact of various levels of water intake on marching in the heat was conducted by Pitts and colleagues.^[4] Mental and psychomotor processing deterioration and the dynamics of its onset during dehydration are of great practical importance but are still not completely understood. Deteriorated mental and psychomotor processing could endanger performance of tasks that require high precision, are performed under forced regime and rhythm, permit no mistakes, or involve sophisticated, expensive, or dangerous equipment. In the north eastern part of India the weather is different from throughout the Indian subcontinent. Hot ($>40^{\circ}\text{C}$), humid (above 80% relative humidity) and rainfall is observed throughout the year and the winter season is only for two months. The effect of such climate and water deprivation conditions is still obscure.

As such, very little scientific exploration of the hydration status in normal healthy individuals has been conducted. Because of the paucity of objective data, numerous recommendations—recommendations not substantiated by the scientific process—are being given to the public regarding hydration.

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Extrapolating animal studies to human behavior in water deprivation state in the hot humid climate of north east India could provide a foundation from which to elucidate the underlying basis of behavioral and physiological impairment if any. We have begun to explore this possibility in young, genetically similar BALB/c mice of both sexes, and have determined that processing components of the physiological system in a water deprivation state in a hot-humid climate.

MATERIALS AND METHODS

The study was done for 13 weeks in summer and observation were done in comparing water deprivation and water *ad libitum*. Animals were withdrawn from any deviation from common behaviors at least two weeks before the experimentation. After baseline completion, the mice were assigned to control group where water was *ad libitum* (Group I, n=8), water-deprivation male group (15 ml/day) (Group II, n=8), and water-deprivation female group (15 ml/day) (Group III, n=8). All subjects in a home cage were examined before proceeding on to the task in the sequence and tests were conducted on a blind basis.

Body weight

Prior to the study, the animals were ranked according to body weight and randomly assigned to matching (same body weight) groups. Body weights of mice were taken regularly during the entire study period

Reflexes

The evaluation of reflexes to externally applied stimuli was measured. At the start of the test, each of subject was lifted from the home cage by the experimenter and tested for the pinna (ear twitch) and the eye blink reflexes as described by Irwin.^[5] The subjects were then held by the nape of the neck and the lateral surfaces of the middle toe of each hind leg were compressed with a pair of forceps (ipsilateral flexor reflex). The subjects were grasped by the tail at a point approximately three-fourths of the way a pair of forceps. In all cases, an experienced rater determined whether or not the flaxes were normal or depressed. A cage of animals known to be untreated was used as a reference group to aid in the rating of depressed responses.

Grip strength

Strength of forelimb was evaluated by modified methods of Irwin as described earlier.^[5] Briefly, mice were held by the tail and provoked to grasp a wire ring 50 mm in diameter and the hindquarters of the animal were rotated at 20 revolution per minute. The time was noted until the mice released the wire. The average time length of three consecutive trials in which the mice pulled continuously was noted.

Visual placing

After being examined for reflexes, the subjects were picked up by their tail and lowered nose-first from a height of about 15-20 cm toward a wire-grasping ring. The animal was rated as having made a visual placing response if it extended its head and forelimbs toward the grasping wire before the nose or whiskers contacted the ring.

Rectal temperature

The animals were restrained for a 1-2 min period and rectal temperature was measured by thermometer (Hicks, Mumbai, India)

Emotionality

The number of defecations and urinations occurring during the 3-min observation period in the open field maze was taken as a measure of emotionality.^[6]

Statistics

All values were expressed as mean \pm SD. Differences in mean values were compared by one-way ANOVA and Student-Newman-Keul (SNK) test. $P < 0.05$ was considered statistically significant

Body weight

Body weight was recorded at 0 day to 90 days at 15-day intervals. After 45 days a water-deprivation male mouse (Group II) showed reduced weight but after 60 days weight was increased gradually and at 90 days increasing weight was static. Water-deprivation female mice (Group III) showed increasing weight after 30 days and showed constant weight at 45 days and 90 days respectively. Control group (Group I) did not show any significant changes of weight throughout the experiment [Figure 1].

Reflexes

Male, female and the control group showed no significant changes on depressed reflexes at 45 days. However, at the 90-day test, water deprivation both male and female group shows significant decreased reflexes as compared to control group. The effect of water deprivation on the reflexes of females was more pronounced than that observed in male rats [Figure 2].

Visual placement

Male, female and control group showed no significant changes in visual placement at 30 days. After 30 days, female mice showed significantly low visual placing responses as compared to males and the control group. After 90 days, male mice were observed significantly ($P < 0.05$) changes visual placing responses as compared to control mice [Figure 3].

Emotionality in the open field

After 30 days, female mice significantly ($P < 0.05$) defecated

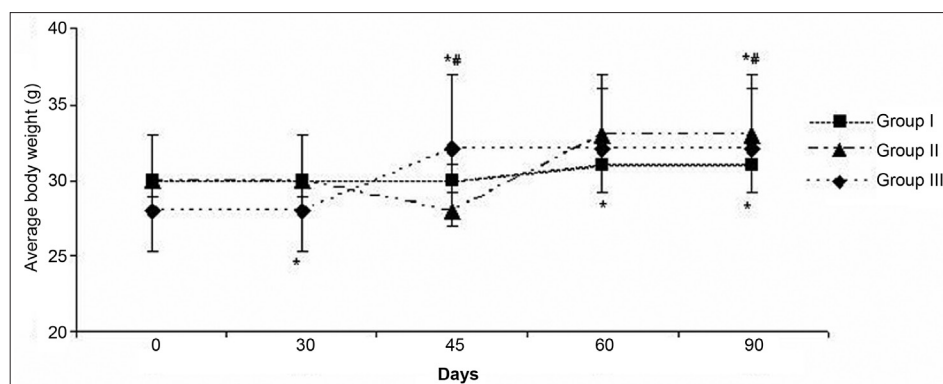


Figure 1: Average body weight of water deprivation group. Results are expressed as mean \pm SD (n=8). Significantly (* $P < 0.05$, # $P < 0.01$) different from control group

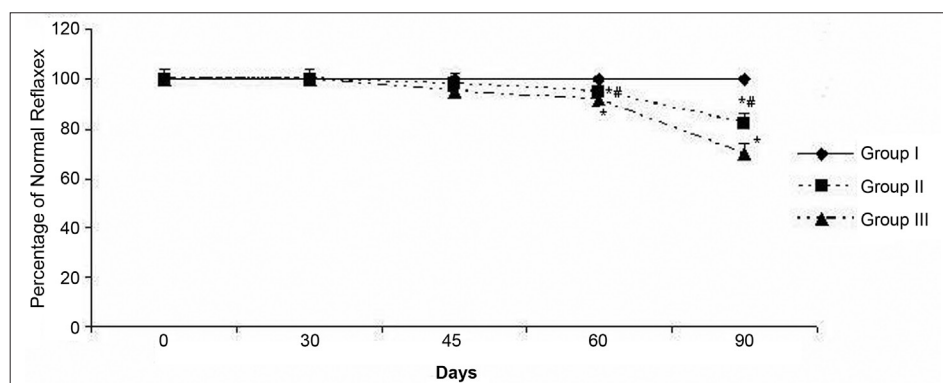


Figure 2: Effect of water deprivation on normal reflexes of mice. Results are expressed as mean \pm SD (n=8). Significantly (* $P < 0.05$) different from control group. Significantly (# $P < 0.05$) different from Group II

and urinated as compared to male mice and the control group during the 5-minutes observation period in the open field. Interestingly, the water-deprived male mice group showed significantly lower urination as compared to the control group, while there were more defecations and urination on Day 45 than on Day 90. These data indicate emotionality, as measured by the number of defecations and urinations in the open field

Rectal Temperature

Group I and Group III showed no alternation in rectal temperature throughout the experiments whereas after 30 days the rectal temperature of Group II was slightly increased as compared to Group III and Group I, and the increased rectal temperature was constant throughout the experiment [Figure 4]

Grip Strength

Water deprivation increases grip strength in male mice. After 30 days of water deprivation, grip strength of Group II mice increased significantly ($P < 0.05$) as compared to Group I and Group III. Interestingly, after 60 days the grip strength of Group I was constant [Figure 5].

DISCUSSION

Our study showed that the water deprivation group showed decreasing of reflex, progressive visual placement and emotionality whereas body weight, grip strength and rectal temperature were increasing as compared to the control group. Another interesting finding was that female mice gained more body weight as compared to male mice and other normal physiological indices, viz. reflex, progressive visual placement, emotionality of female mice were deteriorated as compared to male mice. In the present experiment, the abnormal physiological signs that were persistent after 30 days water deprivation in female mice whereas in male mice were observed after 45 days.

Fluid is essential for life and the mechanisms for maintaining homeostasis of fluids are highly controlled physiologically. Environmental stress is an important factor for healthy adults to maintain fluid and electrolyte balance. India is a large country and variation of climatic change is evident. Extreme hot in deserts and cool in the Himalayan region is common in Indian climate but the weather of the north eastern part of India is typically different where hot, humid, highly moisture content in air and rainfall throughout the

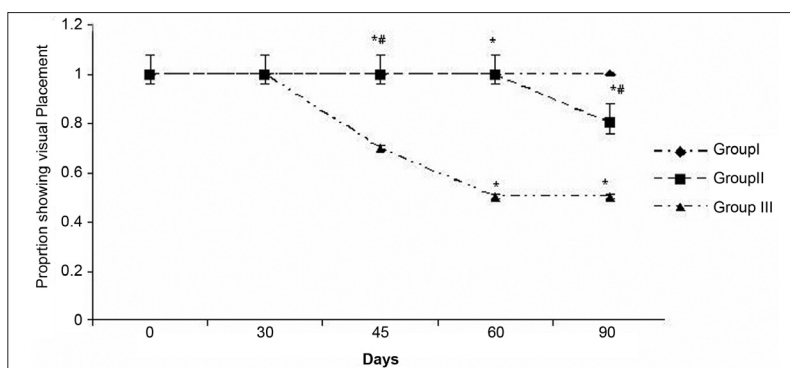


Figure 3: Effect of water deprivation on the visual placement of mice. Results are expressed as mean \pm SD (n=8). Significantly (* $P<0.05$) different from control group. Significantly (# $P<0.05$) different from Group II

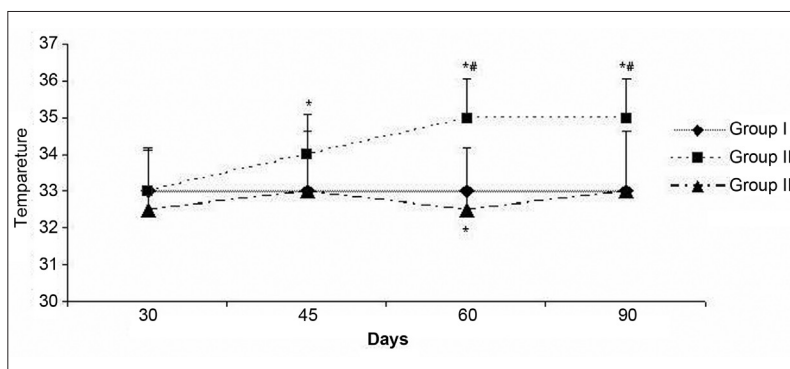


Figure 4: Effect of water deprivation on the rectal temperature of mice. Results are expressed as mean \pm SD (n=8). Significantly (* $P<0.05$) different from control group. Significantly (# $P<0.05$) different from Group II.

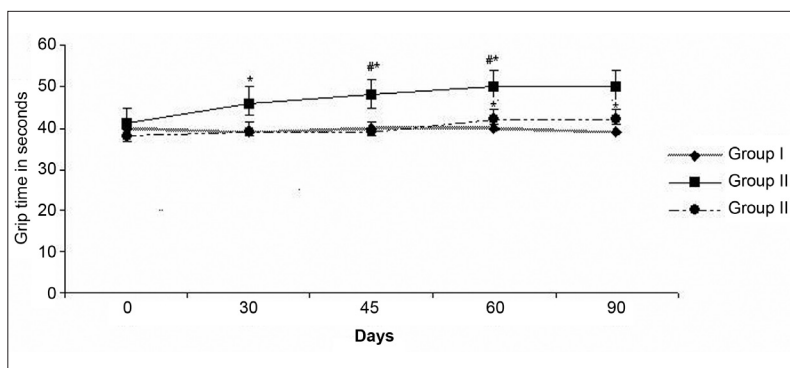


Figure 5: Effect of water deprivation on grip strength of mice. Results are expressed as mean \pm SD (n=8). Significantly (* $P<0.05$) different from control group. Significantly (# $P<0.05$) different from Group II

year is observe As such in this typical weather, very little scientific exploration of water deprivation status in normal healthy individuals has been conducted. Because of the paucity of objective data, numerous recommendations, recommendations not substantiated by the scientific process, and irrational pathophysiological approach are being given to the public regarding water deprivation.

In the present experiment, water deprivation caused increases in body weight in female mice as compared to male mice which is an uncommon finding of other investigators.

Body weight is a sensitive, accurate, straightforward and affordable marker of hydration status crosses disciplines, including medicine, physiology and exercise science.^[7-9] Valtin and Schafer state that body weight is more accurate and simple and can be determined more cheaply than other methods of assessing fluid balance and that its usefulness for measuring body weight in the field of fluid and solute balance cannot be overemphasized^[10] but weight gain in experimental mice in a hot and humid climate due to water deprivation is quite interesting. Thermoregulation is largely accomplished through evaporative heat loss through

sweating and is typically the most effective mechanism for thermoregulation in adverse environmental conditions. When the ambient temperature is hot and humid (i.e. less than skin temperature) the metabolic process goes slow down because produced heat by body is very less and dissipated heat from the body through radiation and convection is similarly very less. Thus, lower metabolic process and less heat production from body which is probable mechanism for gaining body weight.

Water deprivation resulted in an increase in the rectal temperature which is associated with thermoregulation in a humid climate. A previous study concluded that fluid loss as little as 1% of body weight has been associated with a significant increase in rectal temperature in normal hydration^[11] but increasing rectal temperature followed by weight gain is an interesting observation

Another interesting observation was that water deprivation in a hot and humid climate increases motor function as grip strength increased but the reflex action was decreased. The second observation is in accord with the previous study of Jackson^[12] where it was showed that water deprivation condition leads to losses the reflex action. Changes in skeletomuscular function in water deprivation are common findings but increase of grip strength is an important finding.

In the hot, humid and rainy climate of north eastern India, physiological fluid compartments are constantly fluctuating, an evaluation of physiological changes provides new information such as increasing grip strength.

The visual response decreased in the water-deprivation groups may be resulted deficit-sensory –perceptual functioning. However, water deprivation on dysfunction of sensory-perceptual provides a clue for skeletomuscular dysfunction and further experiments using more sensitive methods are required for investigation.

Our experiments show that decrease of defecation and urination of the water-deprivation group indicates that water influences emotionality. However, future data can be generated through the more precise experiment of emotionality and irritability.

In summary, the results herein are preliminary, but suggest that deprivation of water compared to drinking water in the diet for 90 days leads to decrease sensory–perceptual function and skeletomuscular dysfunction. The investigators also found major differences in cognitive-motor function or in neuropsychological function between water deprivation and control conditions in 90 days’ trial. The authors

observed a gender difference, with females more susceptible as compared to male mice when water was restricted.

This information is useful to those in crisis situations when no safe water supply is available, those who are deployed in hot-humid climatic conditions. It is not the intent of the investigators to imply that one need not drink water as such. Consumption of water, as well as other beverages, will support hydration. It is our expectation that the data reported here serves as a catalyst and is useful for future research.

AUTHOR'S STATEMENTS

Competing Interests

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