Thirst and Fluid Intake following Graded Hypohydration Levels in Humans

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ABSTRACT

Engell, D.B. et al. Thirst and fluid intake following graded hypohydration levels in humans. The relationship among changes in thirst sensations, blood variables, and dirrefential fluid intake in hypohydrated humans was examined. Seven subjects were hypohydrated by 0%, 3%, 5%, and 7% of their body weight on four separate trials which were systematically randomized between subjects. Hypohydration levels were achieved with a regimen of restricted food and fluid intake and moderate heat-exercise stress. Statistically significant linear and quadratic trends were found for the intensity of several sensations with progressive hypohydration levels. In general plasma osmolality and renin activity increased and plasma volume decreased with increasing hypohydration levels. During a one hour period of ad-libitum drinking, all subjects consumed insufficient fluid to rehydrate back to baseline body weights. Using regression analyses, fluid intake was predicted by the magnitude of subjective and physiological indices of hypohydration. Results demonstrate that both hypovolemia and plasma osmolality contribute significantly to fluid intake in hypohydrated humans. The results also indicate that thirst sensations are not epiphenomena but make a substantial contribution to differential fluid intake in humans,

Index Terms: Thirst, Fluid Intake, Hypohydration, Drinking Behavior, Water Regulation Most contemporary research on drinking behavior has focused on the elucidation of the neurophysiology of drinking in subhuman species. While hundreds of studies have addressed the detection and correction of cellular and extracellular fluid losses in laboratory animals <see 10, 12, 21>, few have investigated thirst and drinking behavior in humans. Although this emphasis has increased our understanding of neurophysiological concomitants of fluid loss and drinking in laboratory animals, very little is known about thirst, the subjective experience evoked by fluid deficits, which can only strictly be studied in humans. The contemporary theory of drinking reflects this research bias and holds that sensations are epiphenomenal to stimuli such as plasma osmolality, plasma volume, and angiotensin II.

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The experimental study of drinking did not always focus on the neurohumoral consequences of fluid changes in subhuman species <11>. Historically, the study of thirst and drinking behavior parallels the study of hunger and eating behavior <5>. During the nineteenth through the middle of the twentieth centuries, sensations were considered by some theorists to be important contributors to the control of eating and drinking. However, the

development of neurophysiological techniques such as stereotaxic surgery in which electrodes and cannulae are implanted in precise brain regions in subhuman species, revolutionized the study of eating and drinking behaviors during the 1940's and 1950's <11>. As a result of the implementation of these techniques, the influence of behaviorism, and also perhaps the widely quoted assertion by Adolph that thirst does not increase in intensity with increasing water deficits <1>, investigations of the intensity, frequency, and duration of thirst sensations were abandoned.

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Recently, however, several investigators <e.g. 18, 22, 24> have shown that thirst sensations may contribute to the initiation, maintenance, and termination of drinking in humans. Their work suggests that thirst sensations should not be considered epiphenomenal to neurophysiological stimuli and should not be minimized, dismissed, or ignored in studies on the mechanisms of drinking behavior in humans. In light of the paucity of information on thirst and recent investigations of drinking behavior in humans <6, 13, 15, 18, 22, 24> the present study was designed to 1) quantify the nomothetic sensations associated with progressive hypohydration levels and rehydration, 2) determine the relationship among thirst sensations, physiological

variables (plasma osmolality, volume, and renin activity), and fluid intake, and 3) determine the relationship between graded hypohydration levels and rehydration in humans. The present study is the first systematic investigation of both thirst sensations and blood variables associated with progressive hypohydration levels in humans.

METHOD

Subjects

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Seven moderately fit males participated in these studies; they had a mean age (\pm SE) of 23 (\pm 0.3) years, height of 179.0 (\pm 3.0) cm, and body fat of 15.5% (\pm 4.3%). Prior to participation, all subjects were medically evaluated.

Protocol

During the three weeks prior to the experiment, nude body weights were recorded on an electronic balance (±10g) each morning after subjects voided and before they ate breakfast to establish baseline body weights which represented the euhydration level for each subject. To preclude the effects of partial heat acclimation occurring during the course of the study, subjects were heat acclimated for nine days preceding the experiment. The

acclimation program consisted of two hours per day of treadmill walking in a hot dry climatic chamber (Ta=49°C, rh=20%).

To achieve the 3%, 5%, and 7% levels of hypohydration, a regimen of limited food and fluid intake was initiated approximately 24 to 48 hours prior to each of the three hypohydration trials. Also in the afternoon prior to each trial, all subjects performed light exercise in a hot dry environment(Ta=38°C, rh=20%). All subjects' weights were carefully monitored during this time so that target weights were achieved. In some cases, subjects lost slightly more weight than required to achieve their target weights. For the euhydration trial subjects performed light exercise in the hot dry environment, but with adequate rehydration to maintain weights close to baseline weights. The order of presentaion of the euhydration, 3%, 5%, and 7% hypohydration trials was systematically varied between subjects. Each trial was separated by two or three days of rest.

Immediately after losing at least the required amount of weight to achieve target weights, subjects filled out the Thirst Sensation Scale (TSS), and then were escorted to a comfortable environment to spend the night under a monitor's supervision. Because some subjects lost more weight than required to achieve their target weights, they were given

either some fruit juice or a small piece of fruit to ingest in order to achieve the target weights. The following morning subjects were awakened (0600), weighed, and if necessary, provided with sufficient beverage to maintain their target weights.

Subjects then participated in a heat stress test in a hot dry environment (Ta=49 $^{\circ}$ C, rh=20%) because they were simultaneously participating in another experiment to assess the effects of hypohydration level on thermoregulatory and vascular responses <25>. Each subject partipated in a heat stress test once when euhydrated and once when hypohydrated by approximately 3%, 5%, and 7% of his baseline body weight.

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Each of the four heat stress tests lasted 140 minutes (four repeats of a ten minute rest and 25 minute exercise period) unless terminated by predetermined end-points (HR > 180 bpm or Tco > 39.5° C). Body weights (including instruments and clothing) were measured when subjects entered the chamber and after each rest period. Water loss as calculated by change in body weight was replaced by fluid intake after each exercise period except the last.

Before entering a climatic chamber to participate in each heat stress test, subjects were instrumented and catheterized in a comfortable environment (Ta= 20° C, rh=40%). Instrumentation included chest electrodes to obtain telemetered electrocardiograms, a three-point thermocouple

skin harness (chest, calf, and forearm), and a rectal thermister. Following cathetization of a superficial arm vein, subjects completed the TSS while standing (20 minutes) before resting blood samples were taken in the comfortable environment.

Following the last exercise period (or earlier if a subject's test was terminated by the predetermined criteria) of each heat stress test, subjects left the chamber and retired to the comfortable environment. After each subject was weighed on an electronic balance $(\pm 10g)$, he was allowed to drink ad libitum for an hour. Only one beverage (a sugar-based fruit flavored beverage, approximately 5°C) was available for drinking. This beverage was found to be palatable and preferred over water by all subjects. Subjects were reweighed following one hour of ad-libitum drinking. The difference between this weight and the weight recorded immediately prior to beverage access was calculated to determine fluid intake. Subjects completed the TSS for the final time after the one hour rehydration period, but not during rehydration in order to minimize interference with natural drinking.

Thirst Sensations

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The Thirst Sensation Scale (TSS) was used to assess the subjective sensations associated with graded

hypohydration levels. The TSS is a booklet containing 37 graded category scales paired either with sensations and symptoms reported to be associated with thirst in the scientific and anecdotal literature and with a few sensations and symptoms unrelated to thirst. Each sensation, paired with a ten point category scale marked "not at all" (0) at one end and "severe" (9) at the other, appears on a separate page in the booklet. On half of the pages "not at all" appears on the right end of the scale, and on the other half of the pages it appears on the left. Separate pages for each sensation and scale pair, random order of sensations, and random position of "not at all" and "severe" on each scale were used to minimize possible order effects. Similar scales have been used successfully in recent investigations of thirst <e.g. 18, 22, 24>. The instructions on each booklet were as follows: "Circle the number on the scale following each statement that corresponds to HOW YOU FEEL AT THIS MOMENT. The numbers on each line scale represent levels of each symptom ranging from not having the symptom at all ("not at all") to having the symptom at an extreme level ("severe"). Please answer every item."

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The TSS was completed three times by all subjects at each target weight which was achieved during separate trials: 1. immediately following the achievement of each

target weight, 2. the following morning when blood samples were withdrawn (12-15 hours following time 1), and 3. following the one hour rehydration period. All TSS were completed in a temperate environment (Ta=20 $^{\circ}$ C, rh=40%).

Blood Measures

Venous blood samples were collected from an indwelling teflon catheter placed within a superficial arm vein. A catheter was used to obtain several blood samples during the heat stress experiment. Patency was maintained with heparinized saline. The catheter (2ml) was flushed with 4 ml of blood before the 5 ml samples were obtained. The resting blood samples were obtained after a 20 minute period during which subjects completed the TSS while standing. A commercial kit was used to measure hemoglobin (Hycel, Cat #116C). Plasma protein concentrations were measured with a refractometer (American Optical) while plasma osmolality was measured by freezing point depression (Precision Systems, Inc., Osmette A). Total plasma protein was calculated as a product of plasma volume and protein concentration. Plasma volumes when euhydrated were estimated from the equation of Allen et al <3>, and remaining plasma volumes were calculated from percent changes in the appropriate hemoglobin and hematocrit values <9>. Plasma renin activity was measured with the use of a commercial kit (New England

Nuclear); the assay was performed according to meth s described in the technical bulletin.

RESULTS

During the euhydration trials subjects had a mean $(\pm SE)$ body weight of 81.7 ± 4.0 kg. During the three hypohydration trials, the subjects' mean $(\pm SE)$ body weights were 79.2 ± 3.9 kg, 77.6 ± 3.8 kg, and 76.2 ± 3.7 kg which correspond to 3.1 ± 0.04 %, 5.0 ± 0.04 %, and 6.7 ± 0.08 % hypohydration levels.

Thirst Sensations

The median frequency and intensity of thirst sensations at each hypohydration level are shown in Table 1. The medians were calculated from subjects' responses on the TSS which was completed at the second sampling time (immediately prior to the heat stress test).

(Table 1 goes here)

At 0% hypohydration fewer sensations were reported than at 3% hypohydration, and at 3% hypohydration fewer sensations at all intensities were reported than at 5% hypohydration. Subjects reported fewer sensations at low and intermediate intensities but more at high intensities at 7% hypohydration than at the lower hypohydration levels.

A test of linear and guadratic trends of sensation intensity was conducted by generating orthogonal polynomial linear and quadratic composite variables. A test of significance was performed by dividing the mean of the composite variable by its standard error. Sensations and symptoms showing statistically significant (p<.05) linear trends in intensity were: dry and irritated mouth; bad and chalk-like taste in the mouth; dry, scratchy, and warm throat; chapped lips; feeling weary, dizzy, lightheaded, sleepy, tired, irritable, thirsty; having a headache and loss of appetite; thinking of drinking. The following sensations had statistically significant quadratic components (p<.05): irritated mouth, chapped lips, swollen tongue, and feeling hungry. At the 7% hypohydration level, more than half of the subjects experienced trembling in either their hands or legs and said that they had trouble sleeping at night. The sensations and symptoms that did not show significant linear or quadratic trends in intensity across hypohydration levels were: dry lips; sore and tickling-feeling in the throat; having difficulty swallowing; thermal sensations such as feeling warm and sweaty; feeling weak and a lack of energy; sensations

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localized in the stomach such as the stomach feeling empty and aching. Trends in intensities of representative local and general sensations at graded hypohydration levels are shown in Figures 1 and 2, respectively.

(Figures 1 and 2 go .re)

Blood Measures

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With increasing hypohydration levels there was an increase in plasma osmolality, renin activity, and a decrease in plasma volume. While the 3% hypohydration level primarily reduced plasma volume and had little effect on plasma osmolality, the 7% hypohydration level resulted in no further plasma volume reduction but a significant increment in plasma osmolality relative to the 5% hypohydration level. These data and the effects of heat and exercise stress on blood responses to hypohydration levels are presented elsewhere <25>.

Fluid Intake at Graded Hypohydration Levels

Beverage was available following the completion of each heat stress test or removal of the subject from the heat because of predetermined criteria. All subjects completed the 140 minute heat stress test during the euhydration and 3% hypohydration trials. One subject at the 5% level was withdrawn after 134 minutes because of an arrhythmia and the appearance of several preventricular contractions (PVC's). Five subjects did not complete the 7% trial: one subject was removed because of PVC frequency, and four subjects were removed because of physical exhaustion.

Following the last exercise period of the heat stress test, subjects were below the 0%, 3%, 5%, and 7% target weights because they were not rehydrated following the last exercise periods. The final mean (\pm SE) body weights prior to the rehydration period were $0.9(\pm 0.18)$ %, $4.0(\pm 0.13)$ %, $5.9(\pm 0.14)$ %, and $7.3(\pm 0.18)$ % below baseline for the euhydration and three hypohydration trials, respectively.

Fluid intake during rehydration was directly correlated with hypohydration level (see Figure 3). Regression analysis revealed a very strong linear trend in fluid intake with graded hypohydration levels, (F(1,26) = 76.52, p<.001). A significant quadratic trend (F(2,25) = 40.08, p<.001) was also evidenced in the intake data.

(Figure 3 goes here)

Subjects ingested a mean of about 52%, 19%, 22%, and 38% of their fluid deficits following the euhydration and

three hypohydration trials, respectively. Figure 4 illustrates the relationship between the mean percent of deficit ingested and the mean percent of deficit accrued before access to fluid. Regression analyses show significant linear (F(1,19) = 20.6, p<.001) and quadratic (F(2,18) = 13.4, p<.001) components for the three hypohydration trials.

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(Figure 4 goes here)

Relationship among blood measures, thirst sensations, and fluid intake

Stepwise multiple linear regression was used to determine the extent to which fluid intake could be predicted from vascular indices and eleven of the subjective sensations we considered to be most sensitive to hypohydration level and rehydration. Plasma renin activity had to be withdrawn from this analysis because of missing data. Approximately 65% of the variance associated with fluid intake was predicted by the regression equation relating physiological factors to intake: INTAKE = -16.5 + 0.06(osmolality, mosmol/kg) + 0.14(plasma volume,1). The multiple R² for plasma osmolality was 0.58, and plasma volume added 0.07 to this statistic. Thus, a multiple R² of 0.65 was found for the physiological factors. When intensities of sensations were used to predict intake, 68% of the variance was accounted for by the following regression equation: INTAKE = 0.49 + 0.21(tiredness) + 0.07(lack energy) - 0.45(feeling hungry) + .14(thinking of drinking) + 0.09(chalk-like taste in mouth). However, when both physiological and subjective indices of hypohydration were regressed on intake, the following equation accounted for 78% of the variance associated with fluid intake: INTAKE = -14.1 + 0.06(tiredness) + 0.03(lack energy) -0.13(irritability) + 0.07(chalk-like taste in mouth) + 0.05(osmolality, mosmol/kg) + 0.14(plasma volume, 1).

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To assess the effects of fluid intake on the intensity of some of the thirst sensations, a two-way repeated measures ANOVA (hypohydration level X fluid intake) was used <27>. The results of the ANOVA are shown in Table 2. Post hoc multiple comparisons were conducted using the Tukey test. Adjustments were made to calculate the critical difference for the interaction effects <7>. Because of missing data points one subject had to be omitted from the ANOVA and post hoc analyses.

(Table 2 goes here)

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Fluid intake significantly affected the sensations of mouth dryness and chalkiness, and a very strong trend was observed for feeling "thirsty". A comparison of the means of pre-drinking and post-drinking scores on the TSS illustrates these effects. For example, the mean intensity of mouth dryness changed from 1.1 to 0.3, 1.9 to 0.9, 4.3 to 2.1, and 6.9 to 2.3, and the mean intensity of mouth chalkiness changed from 0.0 to 0.0, 1.6 to 0.7, 3.1 to 0.3, and 6.0 to 1.7 at the 0.9%, 4.0%, 5.9%, and 7.3% hypohydration levels, respectively. The changes in the mean intensity of feeling "thirsty" were from 1.5 to 1.1 at the 0.9% level, 3.3 to 1.9 at the 4.0% level, 5.7 to 2.3 at the 5.9% level, and 7.9 to 3.8 at the 7.3% hypohydration level.

A Tukey test revealed that stomach fullness was affected by fluid intake only at the highest hypohydration level which was associated with the highest volume of fluid intake. At the most severe hypohydration level, fluid intake resulted in a change in the mean intensity of stomach fullness from 0.0 to 4.5, while at the lower hypohydration levels the changes in the intensity of stomach fullness were insignificant.

DISCUSSION

Adolph reported that "thirst is noticeable very early, but does not increase much in intensity as the water deficit continues to increase" <1>, and Wolf in his classic monograph concurred with this assertion <28>. In contrast to this earlier work we found that the frequency and intensity of several thirst sensations increased as hypohydration level increased. Statistically significant linear trends were found for several sensations, including local sensations such as mouth dryness and throat scratchiness and general symptoms such as tiredness and dizziness. Significant quadratic trends were also found for several sensations that were salient only at higher hypohydration levels.

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Most of Adolph's data on the sensations associated with dehydration were collected by observers of men walking in the desert. At the completion of the walk, subjects were asked whether or not they experienced certain sensations and to rate their thirst on a 0 to 3 scale. This limited scale probably restricted the full expression of thirst and sensations. It may be difficult to demonstrate graded levels of intensity on a scale with only four points <8>.

Although there are many studies that have reported

that thirst sensations do not play an integral role in the control of drinking <see 21>, the results from the present experiment suggest that changes in sensations contribute to differential fluid intake in hypohydrated humans. If the frequency or intensity of sensations did not change as hypohydration level increased, one might conclude that sensations do not play a significant role in the control of drinking. However, statistically significant linear and quadratic trends were demonstrated for the intensity and frequency of several sensations with progressive hypohydration levels.

Furthermore, when plasma osmolality and volume were used to predict fluid intake, we could account for only 65% of the variance associated with intake. But 78% of the variance in fluid intake could be accounted for when both blood and subjective indices of hypohydration were regressed on intake. Comparison of these regression equations suggests that thirst sensations are not epiphenomena but function as independent discriminative cues that work in consort with the sodium-osmotic-vasopressin pathway and the renin-angiotensin system to control fluid intake in hypohydrated humans. The former regression equation also indicated that while both hypovolemia and plasma osmolality are significant stimuli to fluid intake following

hypohydration in humans, hypovolemia contributes minimally to fluid intake. This finding does not support recent work that has demonstrated that hypovolemia is a major contributing factor to fluid intake following deprivation-induced thirst in humans <20>.

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The role of thirst sensations in drinking behavior was recently illustrated in a study of water deprivation in elderly and young men <18>. Although an equivalent weight loss occurred in both groups following a 24-hour period of restricted water intake, only the young group reported a dry, unpleasant-tasting mouth and a general feeling of thirst. While plasma osmolality and sodium and vasopressin concentrations were greater in the elderly group than in the young group following water restriction, in the former there was a deficit in the awareness or interpretation of thirst and fluid intake during a rehydration period. The young group sufficiently rehydrated their body fluids back to predeprivation levels, but the elderly subjects did not. The authors suggested several explanations for this observation including cortical dysfunction, reduced osmoreceptor and baroreceptor sensitivity, hypothalamic disturbance, and reduced or altered oropharyngeal factors such as mouth dryness. Evidence for only the last explanation was presented suggesting a prominent role for

sensations in the control of drinking.

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Although a very strong linear trend in fluid intake following graded hypohydraton levels was evidenced in our study, drinking during a one-hour period was insufficient to rehydrate to baseline body weights. Incomplete rehydration by hypohydrated individuals in the presence of adequate fluid has been termed "voluntary dehydration" <23> or "involuntary dehydration" <14>. While the mechanism responsible for involuntary dehydration is not known, there are several possibilities.

Subjects may have lost lean tissue mass and electrolytes in addition to water loss, especially during the highest hypohydration trial. At least one author <26> has argued that deficits measured after fluid intake following water or food and water deprivation are "pseudodeficits" that appear because of parallel loss of cellular potassium or lean tissue mass. Clearly, such deficits could not be replaced during a one-hour rehydration period. Unfortunately, measures of body fluid composition and distribution during and following fluid intake were not obtained, and fluid deficits were estimated only from body weight changes.

An alternative explanation for incomplete rehydration is that the fluid was extremely satiating and thus inhibited

sufficient intake. Several investigators <6, 16, 17, 19> have suggested that significantly less cool than warm water is consumed because of the greater satiating effects of cooler water. It is possible that the cool flavored fluid consumed by subjects in our study was especially satiating. It would be interesting to determine the relative effects of fluid temperature and flavor on the sensations associated with hypohydration.

It is also plausible that gastric distension inhibits sufficient drinking during a relatively brief period. In a classic study gastric distension by balloon or water preload was shown to attenuate fluid intake in several species <2>. Stomach distension has also been incorporated into a model of drinking that involves hydrational, orogastric, and behavioral controls <4>. More recently, gastric distension was suggested as an important factor in the termination of drinking in humans <22>. However, in our study the sensation of stomach fullness was significantly affected only in the group that consumed the most fluid. One might suspect that the subjects in the former study <22> were about 7% hypohydrated. However, plasma osmolality, protein, and renin activity data indicate that the subjects in the earlier study were more physiologically comparable to subjects at the 3% hypohydration level of the present study.

Differences in experimental design such as method for inducing fluid loss, type of fluid available for rehydration, presence/absence of questionnaires during rehydration, and demographics of test subject population, may account for the difference in experimental results. Despite differences in results between studies, the data do suggest that stomach distension may contribute to the temination of drinking. Human and subhuman work support the concept that a temporal contiguity among oropharyngeal, gastric, and systemic cues probably terminates drinking <e.g. 4, 22>. This system may work differentially at graded hypohydration levels depending upon the salience of various cues.

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In conclusion, this experiment demonstrates that several oropharyngeal sensations and general symptoms are prominent in the experience of thirst in humans. Statistically significant linear and quadratic trends in the intensity of sensations and symptoms were observed as hypohydration level increased, thus suggesting that changes in the sensations and symptoms contribute substantially to both the detection and correction of body fluid deficits in humans. Results from regression analyses demonstrate that while both changes in plasma osmolality and volume significanly contribute to fluid intake in hypohydrated

humans, changes in sensations and symptoms make an independent contribution to the experience of thirst and differential fluid intake in hypohydrated humans. It is hypothesized that the sensations of discomfort associated with hypohydration and rehydration function as discriminative cues evoking differential drinking behavior.

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The views, opinions, and findings contained in this report are those of the authors and should not be construed as an official department of the Army position, policy, or decision, unless so designated by other official documentations. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

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Table 1. Median Frequency and Intensity of Thirst Sensations at Each Hypohydration Level -

Approximate Hypohydration Level

	0%	3%	5%	7%
Median Frequency	5	. 1 3	19	20
Median Intensity	1	2	3	7

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Sensation	Hypohydration Effect	Fluid Intake Effect	- Interaction
l feel tired	F(3,15)=13.9 p<.001	NS	NS
I feel irritable	F(3,15)=3.8 p<.05	NS	NS
I feel thirsty	F(3,15)=5.2 p<.02	NS, however F(1,5)= 6.0, p<.06	NS
My mouth feels dry	NS, however F(3,15)= 2.5, p<.10	F(1,5)=8.5 p<.05	NS
l have a chalk⊶like taste in my mouth	F(3,15)=6.5 p<.01	F(1,5)=15.6 p<.02	NS
I feel like having a drink	F(3,15)=5.0 p<.01	NS	NS
My stomach feels full	F(3,15)=3.5 p<.05	NS	F(3,15)=9.08 p<.001

Table 2. Effect of Hypohydration Level and Fluid Intake on Sensations

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FIGURES

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Figure 1. Intensities (mean<u>+</u>SE) of representative local sensations reported by subjects at approximately 0%, 3%, 5%, and 7% hypohydration levels are illustrated. Intensity of each sensation was expressed by subjects on a category scale marked "not at all" (0) at one end and "severe" (9) at the other.

Figure 2. Intensities (mean<u>+SE</u>) of representative general symptoms reported by subjects at approximately 0%, 3%, 5%, and 7% hypohydration levels. Intensity of each sensation was expressed by subjects on a category scale marked "not at all" (0) at one end and "severe" (9) at the other.

Figure 3. Ad-libitum fluid intake (liters, mean<u>+</u>SE) by seven male subjects at approximately 0.9%, 4.0%, 5.9%, and 7.3% hypohydration levels during a one-hour rehydration period.

Figure 4. Relationship between per cent of deficit ingested (mean<u>+SE</u>) and per cent of deficit accrued before access to fluid (mean<u>+SE</u>) in seven subjects at approximately 0.9%, 4.0%, 5.9%, and 7.3% hypohydration.



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Fig 3



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MEAN PERCENT WATER DEFICIT (±SE)

Fig4