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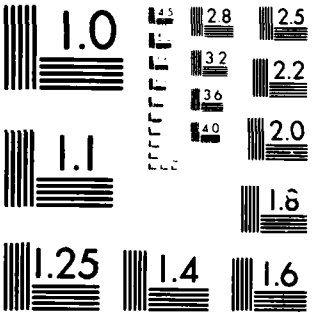

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As part of a large scale field feeding system test we had the unique opportunity to collect and study hundreds of overnight urine aliquots which were obtained immediately prior to a fasting blood sample on days 1, 20, and 44 of the field test. To evaluate the hydrational status of test subjects and criteria of hypohydration, urine aliquots were categorized by specific gravity $\geq$ 1.03 (n=124) or $<$1.03 (n=540). Creatinine levels were elevated (p .001) in the concentrated urine samples, but a decreased trend in Na^+/K^+ ratios in these samples failed to achieve statistical significance (p=0.1). However, when individuals with

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high specific gravity urine were further subclassified by a criterion of weight loss > 3% from original body weight, then creatinine concentrations were elevated ($p=.05$) while Na^+/K^+ ratios were decreased ($p=.05$) when compared with levels in subjects also with high specific gravity but weight loss < 3%. Because of the moderate altitude (2000m) of the field site, there occurred a slight, but significant ($p < .001$), erythropoietic response over time; thus, hematological variables were evaluated by study day. Hematocrit and serum osmolality were not significantly different when classified and examined by the criteria of high or low specific gravity urine and weight loss > or < 3% original body weight. However, serum urea nitrogen/creatinine ratios were significantly increased (days 1 and 44, $p=.02$) in test subjects whose urine samples exceeded 1.03 in specific gravity. The results of this study indicated that prodromal hypohydration, indicated by concomitant elevations in urinary specific gravity and creatinine, was not reflected in the common indices of circulatory hypohydration - hematocrit and osmolality. Alternatively, urea nitrogen/creatinine ratio may be a sensitive circulatory index of imminent hypohydration.

Urinary and Hematological Indices of Hypohydration

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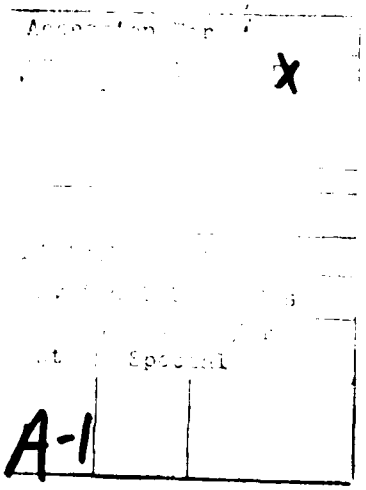

Abstract

As part of a large scale field feeding system test we had the unique opportunity to collect and study hundreds of overnight urine aliquots which were obtained immediately prior to a fasting blood sample on days 1, 20, and 44 of the field test. To evaluate the hydrational status of test subjects and criteria of hypohydration, urine aliquots were categorized by specific gravity $\geq$ 1.03 (n=124) or < 1.03 (n=540). Creatinine levels were elevated ($p<.001$) in the concentrated urine samples, but a decreased trend in Na^+/K^+ ratios in these samples failed to achieve statistical significance ($p=0.1$). However, when individuals with high specific gravity urine were further subclassified by a criterion of weight loss > 3% from original body weight, then creatinine concentrations were elevated ($p=.05$) while Na^+/K^+ ratios were decreased ($p=.05$) when compared with levels in subjects also with high specific gravity but weight loss < 3%. Because of the moderate altitude (2000m) of the field site, there occurred a slight, but significant ($p<.001$), erythropoietic response over time; thus, hematological variables were evaluated by study day. Hematocrit and serum osmolality were not significantly different when classified and examined by the criteria of high or low specific gravity urine and weight loss > or < 3% original body weight. However, serum urea nitrogen/creatinine ratios were significantly increased (days 1 and 44, $p=.02$) in test subjects whose urine samples exceeded 1.03 in specific gravity. The results of this study indicated that prodromal hypohydration, indicated by concomitant elevations in urinary specific gravity and creatinine, was not reflected in the common indices of circulatory hypohydration- hematocrit and osmolality. Alternatively, urea nitrogen/creatinine ratio may be a sensitive circulatory index of imminent hypohydration.

Key words: specific gravity, creatinine, hematocrit, osmolality, urea nitrogen, sodium-potassium ratio.

Introduction

In most studies of acute hypohydration water deficits are effected during a prior interval of exercise in the heat until the targeted weight loss is achieved (4,20,21). Additionally, several investigators have withheld rehydration solutions during an experimental heat stress and measured body weight prior and subsequent to the experimental interval (18,25) to assess hypohydration level. During these experimental scenarios serial blood samples have been analyzed for hematocrit, hemoglobin, sodium, osmolality, and protein to document and quantitate several of the clinical indices of hypohydration. Further, the intensity of hypohydration has been correlated not only with plasma indices, but also with an increased physiological cost of work in the heat (21) and an elevated response pattern of stress and fluid regulatory hormones (9). The comparative value of electrolyte replacement solutions has been assessed during several of these experimental protocols, and, generally, reports indicate that water is equally efficacious in replacing body fluids and reducing the physiological cost of work in the heat (4,5,10).

Alternatively, we are unaware of any longitudinal studies which have assessed and correlated urinary indices of hypohydration with body weight changes or circulating indices of hypohydration. Minard et al. (17) studied 1500 men in a field setting and related the degree of hypohydration to reduced urinary volume and electrolytes and increased specific gravity. Strydom et al. (24) reported that during a 6.5hour, 18 mile road march urine volume was reduced to an average of 134 ml while sweat losses increased to approximately 4.5 liters, and the mean body weight loss was 2.9%. Leithead and Pallister (15) studied men undergoing heat acclimation in a field setting and reported that 24h urine samples of less than 500ml ordinarily manifested specific

gravities in excess of 1.03. However, none of these studies attempted to correlate the degree of hypohydration with other urinary or circulating indices.

In a recently completed, large-scale field test of newly developed rations, we had the opportunity to collect and examine overnight urine aliquots which were obtained just prior to a fasting blood sample. Further, the large number of test subjects (initially 230) and three sampling times during the field scenario afforded us the opportunity to examine several of the more subtle circulating as well as urinary indices of hypohydration. Close monitoring of body weight, simultaneous blood and urine samples, and large numbers of test subjects permitted us to identify variables in both urine and plasma or serum which may be indicative of hypohydration intensity.

Methods

Test subjects (n=230) were adult male and female members of US Army units which were deploying to a field site as part of their routine annual training cycles. All test subjects were fully informed of the testing procedures and potential risks and voluntarily agreed to participate. Test subjects reserved the right to withdraw without retribution.

Prior to deployment a baseline body weight was obtained while each test subject was clad in fatigue trousers, t-shirt, boots, socks, and undergarments. All subsequent weights were taken identically and a correction factor (2.7kg) was subtracted to approximate closely nude body weight. Remaining measurements were made in the field setting on the first (T1), twentieth (T20), and forty-fourth (T44) days of field deployment.

On the evening prior to an experimental day prelabelled urine containers were delivered to each test subject, and he or she was instructed to collect

at least 25 mls of the first-void urine of the subsequent morning. Urine containers were then hand-carried to investigators between 0500 and 0700 h. A small aliquot of the fresh urine was immediately assayed for specific gravity in a field laboratory by refractometry (10400A TS Meter, AO Reichert Scientific Instruments, Buffalo, NY). Further aliquots were prepared and deep frozen for analysis of creatinine, sodium, and potassium. These latter assays were performed at a hospital laboratory where the samples were air transported while frozen. Creatinine was quantitated on a Gilford Stasar IV semi-automated spectrophotometer by methods outlined in their technical bulletin (Gilford Diagnostics, Cleveland, OH). Sodium (Na^+) and potassium (K^+) were quantitated using an FLM3 flame photometer (Radiometer, Copenhagen) also by generally described methods.

Blood was taken without stasis from a superficial arm vein by trained phlebotomists after test subjects remained in an upright posture for at least 20 min. This blood sample was taken approximately 0.5-2.5h after the urine sample. Vacutainers (10ml, Becton-Dickinson, Rutherford, NJ) either without additives or pretreated with heparin were used for this purpose. The blood samples were either processed immediately for hematocrit determination or allowed to clot (no additive) for 30-45 min, after which the sample was centrifuged (2000g, 4°C, 30 min) and the serum layer removed. Hematocrit was measured in the heparinized sample after centrifugation at approximately 12000g in a microhematocrit centrifuge (IEC Model MB). A small serum aliquot was assayed expeditiously at the field site for osmolality by freezing point depression (uOsmette, Precision Systems, Natick, MA). The remaining serum was aliquotted, deep frozen, and air-transported for the subsequent analysis of urea nitrogen (Gilford Stasar IV) and creatinine.

Appropriate group means were compared for statistical significance using Student's non-paired t test for independent data (16). The null hypothesis was rejected at $p \leq .05$. A urinary specific gravity (SG) of 1.03 or greater was used as an initial criterion of hypohydration, and this group was generally compared to the remainder of test subjects (and trials) manifesting a SG less than 1.03. Test subjects with a SG of 1.03 or greater were subdivided further into two groups: > or < 3% body weight loss (from predeployment level) determined immediately prior to phlebotomy on each experimental day. Because the field exercise was executed at an elevation of approximately 2000m, there occurred a slight, but consistent, hematopoietic response over the prolonged experimental interval. Consequently, circulatory variables are reported by test day.

Results

Table 1 illustrates mean values of urinary variables when the calculations were based and separated initially on specific gravity (left-side). The data indicate that of 664 samples taken on three days (T1, T20, T44), 124 (18.7%) manifested SG $\geq$ 1.03, an initial criterion of hypohydration. However, when this group (SG $\geq$ 1.03) was further subdivided and examined by weight loss (< or > 3% of predeployment body weight), mean specific gravities were virtually identical (SG=1.0318, $p > .05$) in the two groups. Using the same criteria, we observed that creatinine concentrations were analogously greatly increased ($p < .001$) in the concentrated urine samples (SG $\geq$ 1.03). Further, when creatinine concentrations were compared by body weight criteria in all samples having SG $\geq$ 1.03, individuals losing < 3% body weight had significantly ($p = .05$) reduced concentrations (< 3% body wt loss, $\bar{X}$ = 3.10g/l and > 3% body weight loss, $\bar{X}$ = 3.41 g/l). Urinary sodium/potassium ratios were not

significantly different in dilute and concentrated urines ($\bar{X}$ = 3.99 and 3.61, respectively, p=0.10). However, it is noteworthy that when urines displaying SG > 1.03 were examined by weight loss of the test subject, the Na+/K+ was significantly (p=.05) reduced in test subjects having a weight loss greater than 3% of predeployment body weight.

Data for hematocrit ratios are illustrated in Table 2. The slight hemoconcentration noted generally by test date irrespective of other criteria was indicative of a slight erythropoietic response due to prolonged exposure (44d) at the moderate attitude (2000m) (e.g. SG < 1.03, $\bar{X}$ = 47.7 at T1 and $\bar{X}$ = 49.2 at T44, t=4.69, p < .001). Interestingly, hematocrit ratios were not significantly different on any of the test days when subjects were separated and compared by the urinary specific gravity criterion. Likewise, in test subjects with urinary SG $\geq$ 1.03, weight loss of > or < 3% of body weight had no effect on hematocrit ratios (p > .05).

Analogous results were noted for serum osmolality as illustrated in Table 3, although sojourn at the moderate altitude of this experiment apparently had no effects on serum osmolality under all criteria. Examination of values for serum osmolality on all test days indicated that test subjects having a urine SG $\geq$ 1.03 manifested no significant differences (p >.05) when compared to subjects with SG < 1.03. Likewise, no differences (p >.05) were obseved in subjects with > or < 3% body weight loss and SG $\geq$ 1.03.

The data in Table 4 demonstrated that prolonged altitude exposure had no effects on serum urea nitrogen (UN)/creatinine ratios. However, on days T1 and T44 subjects with urine SG $\geq$ 1.03 also had significantly (p=.02) elevated UN/creat ratios. On T20, while the trend was maintained, statistical signficance was not achieved (p=.10). When test subjects with SG $\geq$ 1.03 were

categorized in terms of body weight loss, no significant differences were noted in UN/creatinine ratios.

Discussion

Increasing body water deficit or hypohydration has long been associated with an increased risk of heat illness (2,13,14,22). Thus, it is not unexpected that during times when fluid consumption may be inadequate to meet thermoregulatory, metabolic, and cardiovascular requirements, homeostatic mechanisms will be activated to conserve body fluids; one of the very early manifestations of this conservation will be the production of a concentrated urine. For example, acute exposure to heat or exercise stress or fluid restriction usually elicits marked increases in circulating levels of hormones such as vasopressin (11,12), angiotensin I (3,6), and aldosterone (7,8), all of which function to retain body fluids, increase urine concentration, and decrease urine volume. Thus, it may be concluded that a urinary specific gravity of 1.03 or greater is indicative of hypohydration, impending hypohydration, or may be simply reflective of a homeostatic adaptation to prevent debilitating hypohydration. Generally, the results of the present investigation indicate that in the vast majority of cases a highly concentrated urine sample did not reflect frank hypohydration since two of the generally accepted criteria of reduced plasma volume, hematocrit (18,25) and osmolality (1,5), were unaffected when test populations were categorized by urinary specific gravity.

It is noteworthy that while mean urinary specific gravity was identical when individuals with high specific gravity were subclassified by weight losses either greater or less than 3% of predeployment body weight, urinary creatinine concentration was signficantly increased in individuals with the

greater weight loss. It is possible that this increment in the higher weight loss subjects may be associated with a slight reduction in muscle mass which could give rise to an elevated urinary creatinine content. Of course, the greatly increased urinary creatinine in samples having high specific gravity was as expected in these concentrated samples. However, a decreased sodium/potassium ratio was also anticipated in high specific gravity urine specimens (4,17) since the aforementioned endocrinological adaptations would potentially promote sodium conservation and potassium excretion. The actual data indicate that the logical trend was apparent, but statistical significance was not achieved. However, when individuals with high specific gravity urine were examined by weight loss, individuals with the greater weight loss did manifest a significantly depressed Na+/K+ ratio. This could be reflective of increased reabsorption and decreased excretion of sodium in this subgroup or increased potassium excretion related to the aforementioned speculation on muscle mass. Since circulating indices of hypohydration were not markedly different when these were compared for individuals with high and low specific gravity urine, it is difficult to attribute the differing urinary creatinine concentrations and sodium/potassium ratios to variable intensities of hypohydration. This conclusion is strengthened by the observation that when the high specific gravities were compared by the body weight loss criterion, mean specific gravities were virtually identical. These observations are consonant with the hypothesis that plasma volume is defended to maintain cardiovascular stability, and plasma indices are unaffected until a threshold level of total body water loss has been achieved.

As noted earlier, hematological criteria were considered by experimental day due to the ability of prolonged, albeit moderate, altitude exposure to

induce an erythropoietic response (23). In fact, comparison of hematocrit levels in subjects having urine specific gravities < 1.03 on T1 vs T44 demonstrated small (3.14%), but highly significant ($p<.001$), increments in hematocrit ratio. However, comparison of hematocrit ratios by the criterion of urinary specific gravity or specific gravity and body weight loss indicated that the frequency of concentrated urines did not correlate with hemoconcentration. Again, we interpret this as an adaptive response among a large group of young healthy test subjects to prevent circulatory hypohydration during an interval of marginally adequate fluid intake (approx. 3.5-4L/day from all sources) despite moderate environmental conditions (20-25^{o}C WBGT).

Analogous results were noted for serum osmolality in terms of the specific gravity and body weight loss criteria. Unlike hematocrit, however, there occurred no effect of altitude on this variable. Serum osmolalities were remarkably consistent on each test day between specific gravity or weight loss groups; this consistency did not change when all test days were considered collectively. In fact, of the hematological variables examined only serum urea nitrogen/creatinine ratios provided statistically significant data when analyzed subsequent to specific gravity categorization.

In the absence of kidney malfunction the reabsorption of urea is closely associated with the reabsorption of water (19). Thus, when urine volume is reduced, urea reabsorption is increased. Since creatinine clearance is independent of urine volume, the ratio of circulating urea nitrogen/creatinine has been utilized as a clinical index of hypohydration. In individuals with urine SG > 1.03 this variable was a more sensitive index of prodromal hypohydration than either hematocrit or osmolality since on days T1 and T44

this ratio was significantly increased and on day T20 the same trend was evident in the absence of statistical significance ($p=0.1$).

We have concluded from these data that occurrence of highly concentrated urine specimens, determined by marked elevations in specific gravity and creatinine concentrations, did not indicate concomitant increases in hematocrit or plasma osmolality. Further, when body weight loss in excess of 3% was combined as a criterion with high specific gravity, then urinary creatinine levels were increased and sodium/potassium ratios were attenuated when compared with subjects losing less than 3% of body weight. Additionally, the ratio of circulating urea nitrogen/creatinine may be a sensitive index of prodromal hypohydration. Further studies are planned utilizing stable isotope technology to quantitate hypohydration and to compare body water deficits with urinary and circulating indices.

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The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense. 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 in Use of Volunteers in Research.

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Table 1

MEAN URINARY SPECIFIC GRAVITY, CREATININE, AND SODIUM/POTASSIUM RATIOS. LEFT SIDE COLUMNS COMPARE VALUES IN SUBJECTS HAVING SG > AND < 1.03 WHILE RIGHT SIDE COLUMNS COMPARE VALUES ONLY IN SUBJECTS HAVING SG > 1.03 AND FURTHER CLASSIFIED BY WEIGHT LOSS.

SPECIFIC GRAVITY

	ALL SUBJECTS — ALL TRIALS SG < 1.03		SG > 1.03	SUBJECTS WITH SG > 1.03 AND WT. LOSS < 3%		SUBJECTS WITH SG > 1.03 AND WT. LOSS > 3%
n	540		124	93		31
$\bar{X}$	1.0215		1.0318	1.0318		1.0318
SD_X	0.005		0.0017	0.002		0.0015
SE_X	0.0002		0.0002	0.0002		0.0003
t		22.629			0	
p		< .001			> .05	

CREATININE (G/L)

n	540		124	93		31
$\bar{X}$	1.88		3.19	3.10		3.41
SD_X	0.73		0.67	0.75		0.55
SE_X	0.03		0.06	0.078		0.099
t		18.30			2.12	
p		< .001			= .05	

NA^+/K^+

n	539		122	92		30
$\bar{X}$	3.99		3.61	3.78		2.985
SD_X	2.23		1.82	1.91		1.26
SE_X	0.10		0.16	0.2		0.23
t		1.75			2.16	
p		> .05			= .05	

Table 2

MEAN HEMATOCRIT LEVELS ON EACH TEST DAY. LEFT SIDE COLUMNS DENOTE VALUES IN SUBJECTS HAVING URINARY SG > AND < 1.03. RIGHT SIDE COLUMNS COMPARE VALUES ONLY IN SUBJECTS HAVING SG > 1.03 AND FURTHER CLASSIFIED BY WEIGHT LOSS.

HEMATOCRIT

	ALL SUBJECTS		SUBJECTS WITH	SUBJECTS WITH
	SG < 1.03	SG > 1.03	SG > 1.03 AND WT. LOSS < 3%	SG > 1.03 AND WT. LOSS > 3%
		T_1		
n	186	49	46	3
$\bar{X}$	47.7	48.1	48.1	47.8
SD_X	3.18	2.2	2.1	4.01
SE_X	0.23	0.31	0.31	2.31
t	.83		—	
p	>.05		—	
		T_{20}		
n	176	43	28	15
$\bar{X}$	48.3	48.3	48.3	48.35
SD_X	2.66	2.39	2.08	2.97
SE_X	0.20	0.36	0.39	0.77
t	0		.06	
p	>.05		>.05	
		T_{44}		
n	171	32	19	13
$\bar{X}$	49.2	49.8	49.4	50.3
SD_X	3.03	2.73	2.71	2.78
SE_X	0.23	0.48	0.62	0.77
t	1.04		.91	
p	>.05		>.05	

Table 3

MEAN SERUM OSMOLALITY ON EACH TEST DAY. LEFT SIDE COLUMNS DENOTE VALUES IN SUBJECTS HAVING URINARY SG > AND < 1.03. RIGHT SIDE COLUMNS COMPARE VALUES ONLY IN SUBJECTS HAVING SG > 1.03 AND FURTHER CLASSIFIED BY WEIGHT LOSS.

SERUM OSMOLALITY (MOSM/KG)

	ALL SUBJECTS		SUBJECTS WITH SG > 1.03 AND WT. LOSS < 3%	SUBJECTS WITH SG > 1.03 AND WT. LOSS > 3%
	SG < 1.03	SG > 1.03		
		T_1		
n	186	48	45	3
$\bar{X}$	290.8	291.3	291.6	287.3
SD_X	6.05	5.48	5.39	6.43
SE_X	0.44	0.79	0.80	3.71
t	.52		—	
p	>.05		—	
		T_{20}		
n	177	43	28	15
$\bar{X}$	289.2	289.6	289.8	289.2
SD_X	4.07	4.05	3.48	5.06
SE_X	0.31	0.62	0.66	1.31
t	.58		.46	
p	>.05		>.05	
		T_{44}		
n	171	32	19	13
$\bar{X}$	288.9	290.4	290.2	290.8
SD_X	4.27	3.22	3.5	2.9
SE_X	0.33	0.57	0.8	0.8
t	1.89		.51	
p	>.05		>.05	

Table 4

MEAN SERUM UREA NITROGEN/CREATININE RATIOS ON EACH TEST DAY. LEFT SIDE COLUMNS DENOTE VALUES IN SUBJECTS HAVING URINARY SG > AND < 1.03, WHILE RIGHT SIDE COLUMNS COMPARE VALUES ONLY IN SUBJECTS WITH SG > 1.03 AND FURTHER CLASSIFIED BY WEIGHT LOSS.

SERUM UREA NITROGEN/CREATININE

	ALL SUBJECTS				SUBJECTS WITH SG>1.03 AND WT. LOSS <3%		SUBJECTS WITH SG>1.03 AND WT. LOSS >3%
	SG<1.03		SG>1.03				
					T_1		
n	185		49		46		3
$\bar{X}$	13.6		14.9		14.7		18.2
SD_X	3.07		3.96		3.91		3.95
SE_X	0.22		0.57		0.58		2.28
t		2.47				—	
p		=.02				—	
					T_{20}		
n	178		42		27		15
$\bar{X}$	13.4		14.5		14.4		14.7
SD_X	3.52		2.93		3.04		2.82
SE_X	0.26		0.45		0.58		0.73
t		1.88				.31	
p		>.05				>.05	
					T_{44}		
n	171		32		19		13
$\bar{X}$	13.3		14.9		14.7		15.1
SD_X	3.23		3.25		2.72		4.0
SE_X	0.25		0.57		0.62		1.11
t		2.57				.34	
p		=.02				>.05	

END

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