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BODY FLUIDS AND TEMPERATURE RESPONSES OF HEAT-EXPOSED WOMEN BEFORE AND AFTER OVULATION WITH AND WITHOUT REHYDRATION

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SUMMARY

1. Four females (21–25 yr) were exposed to 43·3° C dry bulb, 28–29° C wet bulb, for 10 hr both early and late in two menstrual cycles. During the experiments in one cycle, the subjects were rehydrated while during the experiments in another cycle, the subjects were allowed to progressively dehydrate.

2. Rates of weight loss and oral temperatures were determined hourly while venous blood samples were obtained before and then after 1, 2, 3, 4, 6, 8, and 10 hr of heat exposure.

3. Pre-ovulatory results were compared with post-ovulatory results and dehydration experiments with rehydration experiments. In addition, the data on these female subjects were compared with those presented elsewhere for similarly treated male subjects.

4. When compared to males, these resting females did not significantly haemodilute when heat exposed. In addition, the female subjects apparently suffered a decrease in plasma volume and body water at a rate some 1·5 times that of similarly exposed males.

5. In general, % change in total protein/unit volume of plasma for these female subjects was similar to previously published results for males. However, there were significant differences in the manner in which albumin and globulin fractions changed before and after ovulation.

6. The rates of body weight loss for these subjects were similar to those determined for males. No difference was noted between pre- and post-ovulatory rates of weight loss. Dehydration significantly depressed the rates of weight loss during heat exposure.

7. Stimulation of sweating (assessed as weight loss) appeared to require similar amounts of heat storage before and after ovulation though initial post-ovulatory temperatures were generally higher.

8. Progressive dehydration of females before ovulation was accompanied by rates of increase in oral temperatures that were similar to those seen for similarly exposed males.

9. When females progressively dehydrated after ovulation, there was no statistically significant correlation between temperature rise and weight loss as had been noted in pre-ovulatory experiments. In addition, for all subjects, the rise in body temperature with dehydration was less after than before ovulation. Two subjects showed an ability to decrease their body temperature in the face of continued dehydration.

10. Based on these results, the differences in the responses of males and females to heat exposure were ascribed mainly to two causes: *a*, inherent differences such as skin surface: blood volume ratios, and *b*, the inability of females to maintain their vascular volume during heat exposure.

INTRODUCTION

Whenever human male and female subjects are exposed to the same heat stress, differences in responses to the stress are noted and these differences appear to be sex related (McCance, 1938; Hardy & DuBois 1940; Wyndham, Morrison & Williams, 1965; Morimoto, Slabochova, Naman & Sargent, 1967; Weinman, Slabochova, Bernauer, Morimoto & Sargent, 1967; Fox, Löfstedt, Woodward, Eriksson & Werkstrom, 1969). Fox *et al.* (1969) have recently summarized these findings and conclude that for a given heat stress, women sweat less and have higher body temperatures than do men. The cause of these differences remains rather vague being variously ascribed to items such as hormonal differences, behavioural patterns, etc. Such unsatisfactory explanations are based almost entirely in events that can be measured external to the body, i.e. sweating, skin and 'central' body temperature, oxygen consumption, heart rate and blood pressure. Such measurements are the *results* of processes occurring within the body and it seems evident that the search for differences between the sexes in their response to heat stress should be extended toward monitoring changes *within* the body. Accordingly, the study presented here was designed to examine changes which occur in the constituents and contents of the vascular volume of resting heat stressed females before and after ovulation and with and without rehydration. Comparison then could be made with results previously obtained for males who have been similarly heat stressed.

METHODS

General plan. Four unacclimatized females, whose informed consent had been obtained, were the subjects for these experiments. Outside their normal daily activities, none of these subjects participated in any form of exercise. Each subject reported to the laboratory at 7 a.m. having fasted since 11 p.m. the previous evening. Oral temperatures and a venous blood sample were obtained after the subjects had reclined fully clothed at room temperature (25–26° C) for at least 15 min. The subjects then changed to 'bikini' style bathing suits, entered the heat chamber (43·3° C D.B., 28–29° C W.B.), were immediately weighed and, at rest, continued in the chamber for 10 hr.

Blood samples. The subjects always reclined at least 5 min before each blood sample. Each sample was drawn from the same cubital vein during any one experiment. Blood was drawn with minimal stasis into a syringe and needle whose dead space had been filled with 0·9 % saline that contained 250 i.u. heparin/ml. Blood samples were obtained before entry into the heat chamber and at intervals of 1, 2, 3, 4, 6, 8, and 10 hr after heat exposure began. Each sample was initially used to determine microhaematocrits (uncorrected for trapped plasma) and after centrifugation plasma was used for the determination of total protein (Biuret method), $[Na^+]$, $[K^+]$ flame photometer), freezing point depression (Advanced Instruments osmometer), $[Cl^-]$ (Buchler–Cotlove chloridometer) and electrophoretic fractionation of total protein in cellulose acetate (Beckman microzone cell with Gelman membranes). Total protein was used to calculate the water content of each plasma sample (Eisenman, Mackenzie & Peters, 1936); $[Na^+]$, $[K^+]$, and $[Cl^-]$ were expressed as m-equiv/l. plasma water, and freezing point depression as milliosmolar units. The accuracy was: total protein ± 3 %, $[Na^+]$ and $[Cl^-]$ ± 2 %, $[K^+]$ ± 3 %, freezing point depression and haematocrits ± 0·5 %.

Body weights, oral temperatures and fluid ingestion. Body weights (± 10 g, Buffalo beam balance) were obtained hourly as were oral temperatures (T_0). Only one calibrated clinical thermometer was used throughout this study and temperatures were always read 5 min after insertion of the thermometer. During rehydration experiments, between weighings (i.e. at 30, 90, 150 min, etc., after start of heat exposure) the subjects were given 200 ml. of a warm (37° C) sodium chloride solution, 1 g/l., to drink. After the hourly weighing, any deficit from initial body weight was corrected with additional amounts of saline. During dehydration experiments no fluid was given and weight loss was not replaced.

Statistics. Regression coefficients were calculated for each parameter measured for each subject using either changes in body weight, oral temperature (T_0) or time as the independent variable. When expressed as % change from control values, the differences among subjects for each calculated coefficient were not statistically significant ($P > 0{\cdot}05$) and the data were then treated as arising from a single population.

RESULTS

Anthropometric data as well as information concerning length of menstrual cycle and placement of experimental days within an individual cycle are presented in Table 1. For subjects 1, 2, and 4 the rehydration and dehydration experiments were run with 1 complete menstrual cycle interposed between the two cycles used for experimental purposes during November 1969–February 1970. For subject 3, however, 5 months sepa-

TABLE 1. Anthropometric measurements and initial oral temperatures (T_0) for experiments conducted during the menstrual cycle whose length is given

					Pre-ovulatory			Post-ovulatory		
Subject	Age	Height (cm)	Duration of menstrual cycle (days)	Treatment	Body wt. (kg)	Day of cycle	Pre-exposure T_0	Body wt. (kg)	Day of cycle	Pre-exposure T_0
1	25	165	27	Rehydration	58·38	10	37·2	59·59	25	36·7
			28	Dehydration	58·16	7	36·9	58·30	26	37·2
2	22	160	35	Rehydration	46·81	13	36·7	45·71	26	37·3
			35	Dehydration	46·45	9	36·7	46·72	24	36·9
3	24	170·5	33	Rehydration	67·80	11	36·3	66·38	25	36·7
			31	Dehydration	72·48	10	36·4	73·26	26	36·7
4	21	152·5	30	Rehydration	51·27	12	36·5	51·58	24	36·9
			31	Dehydration	51·98	11	36·7	52·49	25	37

rated rehydration from dehydration exposures. During the interval, subject 3 gained 5·3 kg in body weight.

Rehydration. Apart from a fall in osmolarity there were no significant changes in plasma concentration of individual items when related to body-weight loss. In preovulation experiments, osmolarity dropped 0·29 % for every 1 % body weight loss ($r = -0{\cdot}53$) while in post-ovulation the rate of decrease was 0·43 % ($r = -0{\cdot}5$). Although the changes in individual measured ionic concentrations were not significantly related to weight loss their sum was approximately equal to the total loss in osmolarity.

Dehydration. By contrast, all measured plasma concentrations increased in dehydration experiments and these increases were positively and significantly correlated with weight loss, except for potassium concentration which showed a non-significant but positive correlation with weight loss. The regression coefficients upon weight loss are indicated in Table 2. All values are expressed as % changes from control in order to facilitate comparisons with other data (Discussion).

Of the plasma constituents, the plasma proteins owed their increase in concentration to two separate events – the first was water loss from the vascular compartment while the second was an increase in the amount of total circulating protein. Though the regression coefficients for increases in total protein per unit volume of plasma during dehydration did not differ significantly before and after ovulation, examination of the behaviour of albumin and globulin fractions during dehydration experiments did reveal significant differences in fractional protein dynamics. Regression coefficients (Table 2) indicated that before ovulation rates of change in globulin exceeded those of albumin and that after ovulation the rate of increase of albumin was significantly greater and exceeded that of globulin.

Though the regression coefficients for each individual ion (Table 2) during dehydration are not significantly different before and after ovulation, Na^+ and Cl^- both exhibit increased rates of concentration after ovulation. The behaviour of K^+ (Table 2) during dehydration experiments was the opposite, i.e. a lesser rate of concentration after than before ovulation. For one subject only, pre-ovulatory Na^+/K^+ ratios during dehydration were significantly greater than post-ovulatory dehydration ratios.

Haemodilution. Haematocrit ratios did not change appreciably throughout the rehydration experiments or during the initial hr of heat exposure and dehydration.

Body weight loss. Rates of weight loss approximated 0·5 % of the body weight per hr of heat exposure (Table 3). There were no significant differences between pre- and post-ovulatory rates of weight loss during

TABLE 2. Regression equations of the form $Y = mX + b$. In all cases, X is % body weight loss and Y values are % changes in the given parameter. The 95% confidence intervals are given for m and b while P values accompany the correlation coefficient (r). N.s. = $P > 0.05$. Dehydration experiments only

	Pre-ovulation				Post-ovulation			
Parameter (Y)	m	b	r	$P(r)$	m	b	r	$P(r)$
Haematocrit	2·09 ± 0·66	1·62 + 1·06	0·78	< 0·001	2·06 ± 0·64	− 1·14 ± 1·03	0·78	< 0·001
Total protein	3·05 ± 1·01	3·30 ± 1·63	0·76	< 0·001	4·32 ± 2·51	0·48 ± 4·00	0·56	< 0·01
Albumin	1·98 ± 1·48	3·94 ± 2·38	0·46	< 0·02	4·84 ± 2·41	− 3·25 ± 3·84	0·62	< 0·001
Globulin	4·97 ± 2·30	2·11 ± 3·69	0·64	< 0·001	3·44 ± 4·27	7·14 ± 6·82	0·31	n.s.
Osmolarity	1·47 ± 0·27	0·31 ± 0·41	0·91	< 0·001	1·58 ± 0·21	0·35 ± 0·34	0·95	< 0·001
$[Na^+]$	1·06 ± 0·76	1·20 ± 1·21	0·49	< 0·01	1·61 ± 0·89	0·92 ± 1·43	0·57	< 0·01
$[Cl^-]$	0·91 ± 0·30	2·03 ± 0·49	0·49	< 0·01	1·54 ± 0·72	− 0·22 ± 1·07	0·64	< 0·001
$[K^+]$	1·61 ± 2·10	7·10 ± 3·37	0·29	n.s.	1·12 ± 1·60	− 9·01 ± 2·56	0·26	n.s.

either rehydration or dehydration experiments but rates of weight loss were significantly less for dehydrated subjects.

Oral temperatures and body weight loss. Except for an initial increase upon heat exposure (*b*, Table 4) there were only minor changes in T_0 during rehydration experiments. In pre-ovulatory dehydration experiments the rise in T_0 was significantly correlated with body weight loss. Such was not the case when these 4 subjects were dehydrated after ovulation when the regression coefficient of changes in T_0 on % body weight loss was not

TABLE 3. Regression equations of the form $Y = mX + b$ describing the % of body weight loss (Y) as a function of time (hr) in the chamber. Presentation of m, b and r as in Table 2

Time	Treatment	m	b	r	P
Pre-ovulation	Rehydration	$0{\cdot}52 \pm 0{\cdot}04$	$-0{\cdot}11 \pm 0{\cdot}11$	0·97	$< 0{\cdot}001$
	Dehydration	$0{\cdot}47 \pm 0{\cdot}05$	$-0{\cdot}15 \pm 0{\cdot}12$	0·95	$< 0{\cdot}001$
Post-ovulation					
	Rehydration	$0{\cdot}53 \pm 0{\cdot}04$	$-0{\cdot}20 \pm 0{\cdot}10$	0·97	$< 0{\cdot}001$
	Dehydration	$0{\cdot}47 \pm 0{\cdot}02$	$-0{\cdot}15 \pm 0{\cdot}10$	0·99	$< 0{\cdot}001$

TABLE 4. Regression equations of the form $Y = mX + b$ describing the change (ΔT_0) in body temperature, Y, as a function of % of body weight loss. Presentation of m, b, and r as in Table 2

Time	Treatment	m	b	r	P
Pre-ovulation					
	Rehydration	$0{\cdot}01 \pm 0{\cdot}07$	$0{\cdot}59 \pm 0{\cdot}19$	0·04	n.s.
	Dehydration	$0{\cdot}15 \pm 0{\cdot}07$	$0{\cdot}52 \pm 0{\cdot}11$	0·57	$< 0{\cdot}001$
Post-ovulation					
	Rehydration	$0{\cdot}005 \pm 0{\cdot}04$	$0{\cdot}45 \pm 0{\cdot}07$	0·04	n.s.
	Dehydration	$0{\cdot}03 \pm 0{\cdot}07$	$0{\cdot}52 \pm 0{\cdot}11$	0·16	n.s.

significantly different from zero (Table 4). Each subject behaved similarly in that the increase in T_0 during post-ovulatory dehydration was much less than pre-ovulatory increments in T_0. Indeed, two subjects showed an ability to reduce their body temperatures even as weight loss was continuing. The intercepts of all the regression equations (*b*, Table 4) on the ordinate were similar, indicating that though pre-exposure post-ovulatory T_0 were higher than pre-ovulatory (Table 1), T_0 a similar amount of heat was accumulated by the subjects before sweating began in both rehydration and dehydration experiments. Also, for three of the four subjects, final post-ovulatory T_0 was equal to or less than final pre-ovulatory T_0.

DISCUSSION

The results given above allow a partial explanation as to why females exhibit greater strain for a given heat stress than men. It appears that, in contrast to males, unacclimatized females fail to expand their circulating blood volume upon heat exposure (Adolph & Associates, 1947; Bass & Henschel, 1956; Senay & Christensen, 1965; Senay, 1972). The reason for this lack of plasma volume expansion in these female subjects is not directly revealed by the experimental results. However, from events occurring during the rehydration experiments and early in dehydration experiments, the differences between male and female may directly involve the cutaneous vascular bed (Senay, 1972). In resting males, heat-induced haemodilution appears to depend upon dilatation of the cutaneous vascular bed (Senay & Christensen, 1965; Senay, 1972). Cutaneous dilatation also occurs in females upon heat exposure (Hardy, Milhorat & DuBois, 1941; Haslag & Hertzman, 1965; Kenshalo, 1966) and Fox *et al.* (1969) indicate that such dilatation is similar in males and females. However, either fluid is not transferred into the vascular system of heat exposed females, or, once transferred, it cannot be adequately retained. The changes in plasma osmolarity during rehydration experiments appears to favour the latter event. Several explanations are possible with the simplest perhaps being the most plausible, i.e. lack of haemodilution (or loss of fluid) could depend upon the ratio of capillary wall area to plasma volume. In general females have a higher skin surface area : blood volume ratio than do men and when cutaneous vasodilatation occurs during heat exposure a greater proportion of female plasma volume will be in the cutaneous capillary bed. If blood pressure is maintained (Weinman *et al.* 1967) the amount of water and salt in transit in the interstitial spaces will then be proportionally greater in the female than male. Changes in total protein during rehydration experiments support this interpretation, i.e. more protein than water was lost from the vascular volume.

The initial haemodilution deficit does not account for the significant differences ($P < 0{\cdot}05$) between male and female as to the rate of water loss from the vascular volume during dehydration as assessed by changes in haematocrit ratios. The regression coefficient for changes in haematocrit ratios/% body weight loss for females before and after ovulation was approximately 2·1 while similar plots for males consistently yield a regression coefficient of 1·4 or less (Adolph & Associates, 1947; Senay & Christensen, 1965; L. C. Senay, unpublished observations). Whilst lack of haemodilution was ascribed to differences in surface area : blood volume ratios, the difference in rates of water loss from the vascular volume can be ascribed to a smaller ratio of total body water to body weight for

females. The consequences of this can be seen by contrasting a 50 kg female whose total body water equals 50 % of her body weight with a 70 kg male whose total body water is 60 % of his body weight (Scientific Tables, Geigy, 1962) and assuming that both individuals lose 0·5 % of their body weights as sweat in 1 hr of heat exposure (Table 3, Senay & Christensen, 1965). Because of the differences in total body water: body weight, the 0·25 l. of sweat lost by the female equals 1 % of her total body water while for the male, the 0·35 l. equals only 0·83 % of his total body water. If such water losses are equally reflected in all body compartments, then the ratios of rates of reduction in plasma volume (as indicated by haematocrit values) of female to male should equal 1/0·83 = 1·2. Results presented above indicate for the females used in this study and similarly exposed males (Adolph & Associates, 1947; Senay & Christensen, 1965) a ratio of 2·1/1·4 = 1·5. Therefore, based on body weight or surface area, if a female loses water via sweat glands at the same rate as a male, her plasma osmolarity increases at a greater rate than that of the male, thus affecting her sweat rate (Senay, 1968). Indeed, in support of this position the plasma osmolarity of the four female subjects during dehydration increased approximately 1·5 % for each 1 % body weight loss (Table 2), while similarly exposed males exhibited a 1·1 % increase in plasma osmolarity for each 1 % body weight loss (Senay & Christensen, 1965). If both male and female were to concentrate their body fluids at equal rates via sweating, the 70 kg male could lose 0·5 % (0·35 l.) of his body weight while the 50 kg female could lose only 0·42 % (0·20 l.) of her body weight per hour. Given equal heights and weights, the same argument would hold. These arithmetical procedures based on the present results seem to provide a basis for male–female differences in rates of water loss from the vascular volume. Such calculations also may offer some insight into the generally accepted finding that, for a given heat stress, females sweat less than males. The quantity of sweat produced for heat dissipation in both males and females appears to be a compromise situation involving increases in body temperature (Table 4), increases in body fluid osmolarity (Table 2) and decreases in blood volume (Table 2). In the female, this compromise can be reflected in lower absolute amounts of water lost via sweat glands, or an elevated body temperature or a reduction in work capability when compared to the male. During work in heat, the usual finding appears to be a combination of all three factors (Wyndham *et al.* 1965; Weinman *et al.* 1967; Fox *et al.* 1969). The more rapid rates of sweat suppression in females may also be the result of differences in dynamics of plasma (and interstitial fluid) constituents rather than an epithelial response to hormones (Fox *et al.* 1969).

The rate at which water loss occurred in the four heat stressed female

subjects was not affected by the menstrual cycle. Rates of water loss were remarkably similar (Table 3) in pre- and post-ovulatory experiments. Such consistency would imply constancy in the regulation of body temperature. This did not prove to be true. Though post-ovulatory dehydration rates of water loss were similar to pre-ovulatory rates (Table 3) the relationship of changes in body temperature to the rate of weight loss were quite different after ovulation (Table 4). In all subjects the increases in body temperature were less during the post-ovulatory dehydration heat exposures, and for three of the four subjects the final post-ovulatory dehydration temperatures were equal to or less than the final pre-ovulatory dehydration temperatures. Therefore, the role played by central body temperatures in attaining and maintaining equal rates of sweat loss before and after ovulation during dehydration was apparently not the same.

A partial explanation for such results can be obtained from the data of Kenshalo (1966) who recorded an increase in cutaneous blood flow during the post-ovulatory phase of the menstrual cycle. If post-ovulatory sweat rates were similar to pre-ovulatory rates, then an increase in cutaneous blood flow could account for the smaller increments in T_0 observed in the present studies. However, if this is true, the question remains as to why rates of weight loss did not diminish during post-ovulatory dehydration exposures.

Though rates of water loss from the body were similar in pre- and post-ovulatory experiments, the data suggested differences in movement of albumin and globulins into and/or out of the vascular compartment during both dehydration and rehydration experiments (Table 2). Dehydration did not significantly alter the rate at which albumin accumulated in the vascular volume during pre-ovulatory experiments. However, post-ovulatory albumin concentrations during dehydration changed at a rate that was significantly greater than for any of the other dehydration or rehydration experiments (Table 2). As above, these selective changes can be ascribed to capillary permeability and/or availability of translocatable protein (Senay, 1972), but no matter which of these alternatives is chosen, the menstrual cycle appears to play a role in determining protein (and water) movement into and out of the vascular compartment during exposure to heat.

The general behaviour of plasma proteins in heat stressed females is qualitatively similar to that in males. However, maintenance or increase in oncotic pressure due to protein accumulation does not appear to be as effective in maintaining vascular volume in females as it does in resting males (Senay, 1972).

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