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Prolonged whole body immersion in cold water: hormonal and metabolic changes

D. J. SMITH, P. A. DEUSTER, C. J. RYAN, and T. J. DOUBT

Diving Medicine Department, Naval Medical Research Institute, Bethesda, Maryland 20814-5055 (D. J. S., T. J. D.),
and Department of Military Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland
20814-4799 (P. A. D., C. J. R.)

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Smith DJ, Deuster PA, Ryan CJ, Doubt TJ. Prolonged whole body immersion in cold water: hormonal and metabolic changes. Undersea Biomed Res 1990; 17(2):139-147. To characterize metabolic and hormonal responses during prolonged whole body immersion, 16 divers wearing dry suits completed four immersions in 5°C water during each of two 5-day air saturation dives at 6.1 meters of sea water. One immersion began in the AM (1000 h) and one began in the PM (2200 h) to evaluate diurnal variations. Venous blood samples were obtained before and after completion of each immersion. Cortisol and ACTH levels demonstrated diurnal variation, with larger increases occurring after PM immersions. A greater than three-fold postimmersion increase occurred in norepinephrine (NE). There were significant increases in triiodothyronone (T₃) uptake and epinephrine, but no change in T₄, thyroxine, thyrotrophic hormone, and dopamine. Postimmersion free fatty acid levels increased 409% from preimmersion levels; glucose levels declined, and lactate increased significantly. Only changes in NE correlated significantly with changes in rectal temperature. In summary, when subjects are immersed in cold water for prolonged periods, with a slow rate of body cooling afforded by thermal protection and intermittent exercise, hormonal and metabolic changes occur that are similar in direction and magnitude to short-duration unprotected exposures. Except for cortisol and ACTH, none of the other measured variables exhibited diurnal alterations.

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Divers must routinely sustain performance while in cold water. Metabolic and hormonal responses have been examined previously in unprotected resting and exercising subjects during head-out cold water immersions for short periods (1-4). Such responses have not been studied under simulated diving conditions with passive thermal protection.

The present study was conducted to characterize the extent of hormonal and metabolic changes in response to long duration whole body immersions in cold water

with ECG leads, heat flux sensors, and temperature thermistor for other research studies. During each immersion the divers pedaled on the cycle ergometer at 50 rpm for 3 min at each of three workloads (50, 70, and 90 W). One group of 8 divers performed the exercise every hour during the immersion and another group of 8 divers exercised only at Hours 3 and 6 of immersion. No significant differences between groups were encountered with respect to measured variables, and thus data were combined for subsequent analysis. It was planned that the immersions be terminated after 6 h, but some were terminated earlier if equipment malfunctioned, medical problems arose, rectal temperature declined to 35°C, or if the diver chose voluntary termination. Upon completion or termination of the immersion, divers were rewarmed passively until their core temperature returned to within 0.5°C of baseline.

Dietary regimens and dietary assessment

Two different diets were served to the divers. On one saturation dive, a high carbohydrate diet (3000 kcal, 73% carbohydrate) was provided and during the second dive a standard mixed American diet (3000 kcal, 37% carbohydrate) was provided. Meals were provided 3 h before the start of each immersion. On AM immersion days subjects consumed only two meals, whereas on PM immersion days three regularly spaced meals were provided. The total caloric intake on each day was similar, however. Subjects were allowed free access to water and noncaffeinated beverages. Ingestion of fluids was encouraged; however, fluid was not available during the immersion.

For 3 days before the dive series, divers were instructed to follow a dietary regimen similar to the one they would be served in the chamber. Dietary records were used to document compliance. Divers were also instructed to minimize consumption of alcoholic beverages for 3 days before the dives.

Blood collections

Blood samples were obtained 30 min before each AM and PM immersion. Postimmersion blood samples were obtained within 15 min after the diver left the water. Blood samples (25 ml) were drawn from an antecubital vein with minimum stasis. Venipuncture was uncomplicated in all cases. Anecdotally, most subjects reported less discomfort with the venipuncture postexposure as compared to preimmersion.

Sample processing and biochemical analysis

Blood samples were immediately placed on ice and taken to the onsite laboratory for initial processing. Each sample was divided into four tubes: a) a chilled tube containing lithium-heparin; b) an EDTA tube for hemoglobin, hematocrit, epinephrine, norepinephrine, dopamine, ACTH, and cortisol; c) a plain tube for serum thyrotrophic hormone (TSH), triiodothyronone (T_3) uptake, free fatty acid (FFA); and d) a lithium fluoride tube for lactate and glucose.

Plasma catecholamine concentrations were determined by a single-isotope radioenzymatic method with commercial kits (Cat-A-Kit, Upjohn Diagnostics). Plasma ACTH and cortisol, and serum TSH, T_3 uptake, T_3 , and thyroxine (T_4) values were

determined by standard radioimmunoassay techniques. Serum FFA determination was made by an enzymatic colorimetric method. Serum glucose was measured with a glucose analyzer, and plasma lactate determined by a lactate peroxidase enzyme with a lactate analyzer. Blood hemoglobin was determined by the cyanomethemoglobin method (Coulter Hemoglobinometer), and hematocrit was determined by centrifugation. All samples were measured in triplicate; the coefficient of variation for all methods was less than 5%.

Calculations

Methods for calculating blood and plasma volumes are described in detail elsewhere (6). Briefly, preimmersion blood volume was estimated from height and weight (7, 8). Postimmersion blood volume was estimated from the fractional change in hemoglobin concentration. Plasma volume before and after immersion was determined from the corresponding blood volume and hematocrit values.

In addition to pre- and postimmersion concentrations, a calculated concentration adjusted for plasma volume loss is presented for each variable and is referred to as the expected concentration. The expected value is what the venous concentration would have been if there had been no net gain or loss in total content of the specific constituent with the observed changes in plasma volume. Differences between the measured postimmersion concentration and the expected concentration indicate the net change in content. Thus, postimmersion measured values (A) greater than expected concentrations (E) in Tables 2 and 3 indicate a relative increase in concentration.

Statistical analyses

The Statistical Analysis System (SAS) computer package was used for all statistical analyses (9). Data were analyzed as a $2 \times 2 \times 2$ factorial with repeated measures by multivariate analysis of variance. Data are expressed as mean $\pm$ standard error (SEM).

TABLE 2
MEAN ($\pm$ SEM) CONCENTRATION BEFORE (B) AND AFTER (A) AM AND PM IMMERSIONS^a

		AM Immersion	PM Immersion
Cortisol, pg/ml	B	14.3 $\pm$ 0.7	4.1 $\pm$ 0.6 ^b
	A	15.4 $\pm$ 1.2	20.4 $\pm$ 1.4 ^{b c}
	E	17.0 $\pm$ 0.9	4.7 $\pm$ 0.6 ^{b c}
ACTH, pg/ml	B	30.4 $\pm$ 3.0	22.5 $\pm$ 2.2 ^b
	A	29.4 $\pm$ 2.8	36.7 $\pm$ 2.7 ^{b d}
	E	36.3 $\pm$ 3.6	26.8 $\pm$ 2.6 ^{b d}

^aExpected values (E) were calculated as described in the methods and represent expected concentration due to plasma volume loss only; ^bsignificant differences between AM and PM value ($P < 0.05$); ^cA and E significantly different ($P < 0.01$); ^dA and E significantly different ($P < 0.02$).

TABLE 3
POOLED AM/PM CONCENTRATIONS (MEAN $\pm$ SEM) BEFORE (B) AND AFTER (A)
IMMERSIONS^a

Norepinephrine, pg $\cdot$ ml ⁻¹	B	278.1 $\pm$ 14.0
	A	1082.8 $\pm$ 71.0 ^b
	E	330.2 $\pm$ 16.4
Epinephrine, pg $\cdot$ ml ⁻¹	B	57.0 $\pm$ 4.0
	A	86.3 $\pm$ 6.9 ^b
	E	67.4 $\pm$ 4.7
Dopamine, pg $\cdot$ ml ⁻¹	B	130.2 $\pm$ 11.2
	A	170.0 $\pm$ 14.5
	E	156.4 $\pm$ 14.3
T ₃ , ng $\cdot$ dl ⁻¹	B	108.3 $\pm$ 2.9
	A	125.9 $\pm$ 3.1
	E	129.2 $\pm$ 3.5
T ₄ , μ g $\cdot$ dl ⁻¹	B	6.2 $\pm$ 0.3
	A	6.9 $\pm$ 0.3
	E	7.3 $\pm$ 0.4
T ₃ uptake, %	B	31.9 $\pm$ 0.5
	A	30.7 $\pm$ 0.5 ^b
	E	38.3 $\pm$ 0.9
TSH, μ IU $\cdot$ liter ⁻¹	B	2.3 $\pm$ 0.2
	A	2.8 $\pm$ 0.2
	E	2.8 $\pm$ 0.2
FFA, mEq $\cdot$ liter ⁻¹	B	0.110 $\pm$ 0.026
	A	0.668 $\pm$ 0.033 ^a
	E	0.131 $\pm$ 0.018
Glucose, mg $\cdot$ dl ⁻¹	B	98.2 $\pm$ 1.9
	A	106.5 $\pm$ 2.5 ^a
	E	117.1 $\pm$ 2.6
Lactate, mg $\cdot$ dl ⁻¹	B	12.1 $\pm$ 0.5
	A	18.0 $\pm$ 1.3 ^a
	E	14.5 $\pm$ 0.5

^aExpected values (E) represent value due to plasma volume loss only; ^bsignificant difference between A and E ($P < 0.05$).

Standard correlation and regression techniques were used to identify associations between variables. The level of significance was set at 0.05.

RESULTS

Of the 64 immersions, 27 lasted 6 h (42% of all immersions). Seven immersions lasted less than 3 h, providing insufficient information, and were therefore excluded

from the data analyses. The data presented are for 57 immersions that lasted over 3 h. Mean exposure times for these immersions were 317 ± 12 min for AM ($n = 29$) and 304 ± 12 min ($n = 28$) for PM immersions. The average reduction in rectal temperature over the course of the dives was $1.17^\circ \pm 0.17^\circ\text{C}$.

Dietary effects

No differences were detected in any of the measured variables with respect to dietary parameters. Therefore, the results that were initially segregated by diet were pooled.

Hormonal constituents

Blood volume decreased from pre- to postimmersion, due almost entirely to a decrease in plasma volume. AM and PM calculated plasma volume decreased $17.3 \pm 1.1\%$ and $16.9 \pm 1.3\%$, respectively (6).

Significant diurnal differences in cortisol and ACTH were found between AM and PM immersions (Table 2). Postimmersion cortisol levels were not significantly different from preimmersion values during the AM exposures (venipuncture 0930, 1600), whereas during the PM immersions cortisol was significantly higher postimmersion ($P < 0.01$). Postimmersion ACTH concentration declined slightly ($P > 0.05$) during AM immersions, whereas ACTH levels were elevated significantly ($P < 0.02$) after PM immersions. The PM measurements occurred at approximately 2130 for preimmersion (cortisol nadir) and 0400 for postimmersion (cortisol peak). No significant diurnal differences were noted in any of the other measured venous constituents, therefore the data initially categorized by AM and PM were pooled and are presented in Table 3.

Postimmersion plasma norepinephrine (NE) and epinephrine (E) concentrations were significantly higher than preimmersion values. A greater than threefold increase in NE concentration occurred after correction for plasma loss ($P < 0.01$), and a small but significant increase was noted in E ($P < 0.02$). The increase in dopamine was not statistically significant.

There was a statistically significant decrease in T_3 uptake ($P < 0.01$) after immersion. When corrected for plasma volume the serum T_3 , T_4 , and TSH concentrations remained unchanged.

Metabolic constituents

Table 3 also presents the results for nonesterified FFA, glucose, and lactate concentrations. Serum FFA levels increased significantly from pre- to postimmersion ($P < 0.001$), and glucose levels declined slightly ($P < 0.001$). Lactate was significantly increased from pre- to postimmersion ($P < 0.02$).

Correlations with rectal temperature and dive time

Pearson rank correlation coefficients were determined for changes in blood constituents and rectal temperature. Only NE levels significantly correlated with changes in rectal temperature ($R = -0.276$, $P = 0.048$). Individual subject immersion times

correlated significantly with changes in NE ($R = 0.464$, $P = 0.0004$), dopamine ($R = 0.385$, $P = 0.004$), and glucose ($R = -0.294$, $P = 0.03$). When the ratio of FFA:glucose postimmersion was compared to immersion time, the correlation was significant ($R = 0.38$, $P = 0.004$).

DISCUSSION

The present study provides evidence that men in passive thermal protective gear who are immersed in cold water for extended periods experience marked increases in FFA and stress hormone concentrations. A small but significant decrease in glucose also occurs. Thyroid hormones remain unchanged except for a slight decrease in T_3 uptake.

Many studies have provided evidence that fat stores are mobilized during cold exposure. Increases in FFA ranging from 38 to 96% with cold exposure have been well documented (1-3, 10-14), but the values were frequently not corrected for changes in plasma volume. Wilson et al. (11) observed no significant change in FFA after correction for changes in plasma volume, but noted their subjects were in a "fasted" state. Other studies (12, 13) concluded that hemoconcentration could not account for the entire elevation in FFA when the percent change in hematocrit (Hct) was compared to the change in FFA concentration. Two recent studies from this laboratory demonstrated a 100% increase in FFA after correction for plasma volume in thermally unprotected subjects during 3-h resting immersions in 25°C water (2) and an increase of similar magnitude in subjects exercising at a moderate workload for 1 h in 18°C water (3). In the present study, the percent change in FFA after correction for plasma volume averaged 409%; markedly higher than previously reported. Significant experimental design differences can account for some of the observed differences, inasmuch as in the present study the subjects cooled slowly and were consistently fed 3 h before immersion.

The mechanism for cold-induced FFA mobilization may be that proposed by Jensen (14), whereby the stimulus is NE released by the sympathetic nervous system rather than catecholamines released from the adrenal medulla. Our findings of an increase in NE and no change in dopamine support this hypothesis.

Immersion in thermoneutral water results in no change in NE or E (15-17). On the other hand, cold stress significantly increases NE and E during cold air exposure (18, 19) and during cold water immersions lasting 42-45 min (4, 20, 21). Peak NE concentrations in these latter short-duration cold water studies (800-1400 pg · ml⁻¹) are comparable to that obtained after our 6-h exposures (1083 pg · ml⁻¹). While one study reported a decline in E in 25°C water (4), others (3, 20) have reported increases in 18-28°C water that were of similar magnitude to present values measured after 5°C water exposure.

The correlations of increases in NE with both declines in core temperature and increasing immersion time suggest that NE may be a good indicator of thermal stress during cold water exposures.

The heavy thermal protection and safety considerations obviated the possibility of using indwelling catheters in our subjects to measure temporal changes in venous metabolites and hormones. Nonetheless, the present venipuncture results compare closely with previous results obtained in this laboratory using indwelling catheters for blood collection at frequent intervals (2, 3).

Serum glucose concentrations decreased on average 10% from pre- to postimmersion. Other investigators have reported increases (22) or no change (1, 11-13) in glucose levels over a wide range of temperatures, but hemoconcentration effects were not accounted for. Studies correcting for changes in plasma volume have noted no change in serum glucose at rest in 25°C water (2) and decreases with exercise (3) in 18-28°C water. Thus, relative changes in glucose may depend on both the magnitude of the thermal challenge and hydration status.

Plasma lactate concentrations increased an average of 28% from pre- to postexposure. This increase in lactate may reflect the effect of exercise just before termination of the immersion, although the blood sample was obtained 15 min after exiting the water. Jacobs et al. (1) found that changes in lactate did not correlate with immersion duration, amount of shivering reported, or changes in rectal temperature. Since lactate is readily metabolized and serial measurements were not obtained, it is difficult to provide an explanation for increased lactate.

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