Effect of Acute Cold Exposure on ACTH and Zinc Concentrations in Human Plasma

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Summary Effects of acute cold exposure (10°C for 60 min) on the concentrations of adrenocorticotropin (ACTH) and zinc in plasma were investigated in seven healthy male students. There were no significant changes in total zinc, albumin-bound zinc, and α_2 -macroglobulin-bound zinc concentrations throughout the experimental period. On the other hand, ACTH concentration increased markedly during cold exposure. In addition, a statistically significant inverse relationship existed between the changes in ACTH and albumin-bound zinc values during the experiment. These results suggest that acute cold exposure produces elevated plasma ACTH levels, with resulting zinc redistribution in the human body.

Key words: cold exposure, ACTH, zinc.

Zinc is without doubt one of the essential trace elements in the living organism. A number of studies have demonstrated that acute stresses including surgical trauma, burns, myocardial infarction, and a variety of acute infectious illnesses reduce plasma zinc values (BEISEL *et al.*, 1976). Disappearance of zinc from plasma can develop within only a few hours after the initiation of a stressful insult. Our recent study in humans has shown that, despite significantly decreased erythrocyte zinc levels as well as a notable decrease in plasma volume (PV), there are no significant increases in plasma zinc concentration during acute exposure to cold (OHNO *et al.*, 1986). It has been reported that adrenocorticotropin (ACTH) infusion into humans reduces serum zinc from 10 to 60 $\mu\text{g} \cdot 100 \text{ ml}^{-1}$ (FALCHUK, 1977) and that only 15 min of cold exposure leads to a marked increase in ACTH concentration in the plasma of rats (USATEGUI *et al.*, 1977). To our knowledge, however, reports concerning the influence of acute cold exposure on ACTH in human plasma are not available. In the present study we investigated an inter-relationship between the changes in ACTH and zinc concentrations in human

plasma during acute exposure to cold.

The subjects were seven sedentary healthy male students whose mean ($\pm$ S.E.) age, height, and weight were 21.6 years (± 0.7), 174.6 cm (± 1.6), and 67.8 kg (± 2.2), respectively. Informed consent was obtained from all subjects. Through late January to February, the subjects wearing only T shirts and shorts underwent cold exposure in the supine position at 10°C, relative humidity of 40–50%, and wind velocity less than 0.5 m·s⁻¹ for 60 min. They were allowed to rest at an ambient temperature of 25–26°C for 30 min prior to and for 15 min after the exposure to cold. Skin temperatures were continuously recorded with a thermistor thermometer (TER-36, Takara Kogyo, Tokyo) at five different locations (forehead, chest, forearm, thigh, and calf). Rectal temperature was recorded with a thermistor probe inserted 10 cm into the rectum. Mean skin and body temperatures were calculated by the method described elsewhere (DOI *et al.*, 1979). Blood samples were collected from the antecubital vein in a zinc-free heparinized plastic syringe at 0, 15, and 60 min during cold exposure, and at 15 min after cold exposure.

Zinc concentration in plasma was assayed by use of a Shimadzu model AA 640-13 atomic absorption spectrophotometer (Tokyo) (for details see OHNO *et al.*, 1985). The zinc distributions in plasma were determined according to the method described by GIROUX (1975). Total plasma protein concentration was measured by the method of LOWRY *et al.* (1951). The quantitative determinations of albumin and α_2 -macroglobulin (α_2 -MG) were made by a single radial immunodiffusion technique, using Partigen plates (Behringwerke, Marburg). ACTH and cortisol concentrations were determined by commercial kits (a Green Cross ACTH radioimmunoassay kit, Tokyo and an Eiken cortisol radioimmunoassay kit, Tokyo). The plasma concentrations of norepinephrine (NE) and epinephrine (E) were assayed using a BAS model LC-304 catecholamine analyzer (Bioanalytical System, West Lafayette). Hematocrit (Hct) level was measured by a microcapillary tube technique and used to calculate changes in PV as described by VAN BEAUMONT *et al.* (1973). To minimize the effect of hemoconcentration on each plasma solute concentration, the Van Beaumont quotient (VBQ) (VAN BEAUMONT *et al.*, 1973) was also determined.

Scheffe's tests were used to identify significant mean differences across time when significant ($p < 0.05$) *F*-ratios were obtained in a one-way analysis of variance (SNEDECOR and COCHRAN, 1980). Each VBQ was evaluated for significance by the paired *t*-test. In all statistical analyses, the 0.05 level of significance was used.

Table 1 shows the temperature responses. No definite changes were observed in the rectal temperature during cold exposure, whereas both mean skin and body temperatures showed significant decreases. The mean skin temperature at 15 min after cold exposure remained significantly lower compared to the initial control value. Plasma NE concentration increased notably after 60-min cold exposure and 15 min after cold exposure, while E concentration did not change substantially (Table 2), in agreement with the findings of previous studies (JESSEN, 1980; OHNO *et al.*, 1986). These results on the indices of cold exposure indicate that the subjects were under possible effects of cold exposure.

Table 1. Effects of acute exposure to cold on temperature responses.

Temperature	Initial control	During cold exposure		15 min after cold exposure
		15 min	60 min	
Rectal temperature (°C)	37.0 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	36.9 ± 0.1
Mean skin temperature (°C)	33.3 ± 0.1	29.3 ± 0.3*	27.7 ± 0.4***	31.2 ± 0.3***
Mean body temperature (°C)	35.8 ± 0.1	34.5 ± 0.1*	34.0 ± 0.1*	35.0 ± 0.1***

Values are mean ± S.E. Significantly different from * initial control, ** 15-min cold exposure, and *** 60-min cold exposure: $p < 0.05$.

Table 2. Variations in plasma concentrations of ACTH, cortisol, and catecholamines and the Van Beaumont quotient for them under acute exposure to cold.

Parameter	Initial control	During cold exposure		15 min after cold exposure
		15 min	60 min	
ACTH (pg · ml ⁻¹)	32.9 ± 3.1	52.5 ± 6.0* (50.0 ± 15.3*)	49.9 ± 6.3* (34.2 ± 10.8*)	40.4 ± 4.5 (23.0 ± 11.2)
Cortisol (μg · 100 ml ⁻¹)	11.6 ± 1.6	11.7 ± 2.3 (- 4.32 ± 6.86)	10.7 ± 2.2 (- 11.3 ± 5.4)	9.11 ± 2.1 (- 16.0 ± 6.7)
Norepinephrine (pg · ml ⁻¹)	470.4 ± 70.2	695.7 ± 83.7 (44.3 ± 13.6*)	854.4 ± 114.5* (67.5 ± 22.7*)	751.0 ± 108.6* (53.3 ± 16.8*)
Epinephrine (pg · ml ⁻¹)	96.9 ± 32.7	105.7 ± 24.5 (44.5 ± 31.0)	116.6 ± 22.6 (67.2 ± 49.0)	106.9 ± 17.1 (68.6 ± 49.5)

Values are mean ± S.E. ACTH, adrenocorticotrophic hormone. Van Beaumont quotient (%) (VAN BEAUMONT *et al.*, 1973) is given in parentheses. * Significantly higher than initial control: $p < 0.05$.

Plasma ACTH concentration increased significantly during cold exposure and none of the VBQ for ACTH followed the decrease in PV during the experiment (Table 2). Therefore, the result suggested an influx of ACTH into the plasma as indicated by LUKASKI *et al.* (1984) after exercise in men fed a low-zinc diet. On the other hand, cortisol concentration did not change significantly at any time. However, the VBQ for cortisol appeared to decrease after 60-min cold exposure and at 15 min after cold exposure ($p < 0.10$).

As shown in Table 3, there were no significant changes in total zinc, albumin-bound zinc, and α_2 -MG-bound zinc concentrations at any time. While plasma α_2 -MG concentration did not vary significantly during the experiment, there were significant elevations in total protein and albumin after 60-min cold exposure and 15 min after cold exposure. Conversely, the VBQ for total protein and albumin

Table 3. Variations in plasma concentrations of zinc and related proteins and the Van Beaumont quotient for them under acute exposure to cold.

Parameter	Initial control	During cold exposure		15 min after cold exposure
		15 min	60 min	
Total zinc ($\mu\text{g} \cdot 100 \text{ ml}^{-1}$)	78.3 $\pm$ 4.1	82.9 $\pm$ 3.1 (-1.19 $\pm$ 2.84)	86.8 $\pm$ 3.0 (-1.77 $\pm$ 3.76)	80.4 $\pm$ 4.8 (-4.83 $\pm$ 3.04)
Albumin-bound zinc ($\mu\text{g} \cdot 100 \text{ ml}^{-1}$)	56.5 $\pm$ 4.5	63.0 $\pm$ 3.7 (2.54 $\pm$ 5.33)	63.8 $\pm$ 3.8 (-0.329 $\pm$ 7.984)	59.6 $\pm$ 3.3 (-0.857 $\pm$ 6.734)
% of total zinc	72.0 $\pm$ 3.9	76.1 $\pm$ 3.7	73.7 $\pm$ 2.2	74.9 $\pm$ 3.9
α_2 -MG-bound zinc ($\mu\text{g} \cdot 100 \text{ ml}^{-1}$)	21.8 $\pm$ 3.0	19.9 $\pm$ 3.1 (-11.3 $\pm$ 6.1)	23.0 $\pm$ 2.2 (5.51 $\pm$ 19.1)	20.7 $\pm$ 3.8 (-6.84 $\pm$ 18.5)
% of total zinc	28.0 $\pm$ 3.9	23.9 $\pm$ 3.7	26.3 $\pm$ 2.2	25.1 $\pm$ 3.9
Total protein ($\text{g} \cdot 100 \text{ ml}^{-1}$)	7.10 $\pm$ 0.13	7.50 $\pm$ 0.12 (-2.04 $\pm$ 0.61*)	7.76 $\pm$ 0.15* (-3.99 $\pm$ 1.10*)	7.56 $\pm$ 0.14* (0.229 $\pm$ 2.228)
Albumin ($\text{g} \cdot 100 \text{ ml}^{-1}$)	4.40 $\pm$ 0.07	4.64 $\pm$ 0.09 (-1.53 $\pm$ 1.27)	4.80 $\pm$ 0.09* (-4.21 $\pm$ 0.75*)	4.67 $\pm$ 0.09* (-1.99 $\pm$ 1.50)
α_2 -MG ($\text{mg} \cdot 100 \text{ ml}^{-1}$)	246.6 $\pm$ 8.7	249.6 $\pm$ 7.4 (-5.67 $\pm$ 3.40)	264.7 $\pm$ 10.2 (-5.66 $\pm$ 2.39)	262.6 $\pm$ 7.3 (-1.09 $\pm$ 2.52)
Hematocrit (%)	43.3 $\pm$ 0.3	45.3 $\pm$ 0.5*	46.6 $\pm$ 0.5*	45.3 $\pm$ 0.6*
ΔPV (%)		-7.19 $\pm$ 1.27	-12.1 $\pm$ 1.69	-7.27 $\pm$ 1.85

Values are mean $\pm$ S.E. α_2 -MG, α_2 -macroglobulin; ΔPV , plasma volume change. Van Beaumont quotient (%) (VAN BEAUMONT *et al.*, 1973) is given in parentheses. * Significantly different from initial control: $p < 0.05$.

showed significant decreases during cold exposure. Hct level rose significantly throughout the experimental period, resulting in a remarkable decrease in PV. The VBQ for total zinc did not change significantly during the experimental period. From the fact that erythrocyte zinc levels reduced during the experiment (1,140.2 $\pm$ 33.0, 1,109.1 $\pm$ 27.0, 1,113.1 $\pm$ 26.1, and 1,110.8 $\pm$ 45.6 $\mu\text{g} \cdot 100 \text{ ml}^{-1}$ of erythrocytes in the order of blood-collecting), however, the possibility might exist that there was an efflux of zinc from the plasma during cold exposure.

A statistically significant inverse relationship was observed between the changes in ACTH and albumin-bound zinc concentrations in plasma (Fig. 1), but not among ACTH, total zinc, and α_2 -MG-bound zinc. The increase of ACTH release, though less in its extent, resembled that reported by USATEGUI *et al.* (1977) in plasma of cold-exposed rats. USATEGUI *et al.* (1977) found that acute cold exposure leads to an increase in plasma ACTH, but the enhanced release of ACTH is of short duration, irrespective of the length of cold exposure. The present study suggests that ACTH response in human plasma after acute exposure to cold is prolonged compared to that of ACTH in the plasma of rats. It was noted that the elevated ACTH did not cause the increase in plasma cortisol in the present study.

Fig. 1. Correlation between ACTH and albumin-bound zinc concentrations in plasma under acute exposure to cold. ● after 15-min cold exposure, ○ after 60-min cold exposure, ◐ 15 min after cold exposure.

When hemoconcentration was taken into account, there appeared to be rather an efflux of cortisol from the plasma. Under acute exposure to cold, cortisol in human plasma has been reported to be slightly increased (SUZUKI *et al.*, 1967; WILSON *et al.*, 1970), unchanged (WILKERSON *et al.*, 1974), or slightly decreased (GOLSTEIN-GOLAIRE *et al.*, 1970). Such different results may be due to different experimental conditions such as clothing, blood-collecting time, and duration and temperature of cold exposure. The report by TATAI (1964) of markedly increased urinary excretion of 17-hydroxycorticosteroids in man following cold exposure, may point to an elevated clearance rate of cortisol by the cold in man. Glucocorticosteroid hormones seem to be capable of producing depressed plasma zinc values under acute stresses (BEISEL *et al.*, 1976). However, FALCHUK (1977) has speculated that the decrease in serum zinc content that follows ACTH infusion does not appear to be mediated through release of adrenal hormones since the response is also observed in adrenalectomized patients. In the present study there was no relationship between cortisol and zinc concentrations. It was suggested, thus, that cortisol plays no important role in plasma zinc metabolism during acute exposure to cold.

The zinc present in human serum is principally composed of two fractions: one fraction is firmly bound to α_2 -MG and the other is more loosely associated with albumin (GIROUX, 1975). It has been reported that although albumin-bound zinc in plasma fluctuates under various physiological or pathological conditions, α_2 -MG-bound zinc is relatively constant (BOGDEN, 1980). The present study has shown that there is a significant inverse relationship between the changes in ACTH and

albumin-bound zinc concentrations during cold exposure, in approximate agreement with the study of FALCHUCK (1977) in healthy and diseased subjects. Namely, acute cold exposure produces elevated plasma ACTH levels in man, with resultant zinc redistribution within the body. However, the question whether such changes in plasma zinc (especially albumin-bound zinc) during cold exposure are attributed to a direct effect of ACTH cannot be answered at present. It is summarized by BEISEL *et al.* (1976) that initial zinc depression in plasma during acute stresses is explained by its abrupt redistribution within the body and that movement of zinc from plasma especially to liver, rather than loss of zinc from the body, causes the initial response. OH *et al.* (1978) have also demonstrated that the increased synthesis of metallothionein (MT, a zinc-binding protein) in the liver of rats is accompanied by a reduction of plasma zinc during 24-h cold exposure. Zinc ions stabilize lysosomal and microsomal membranes of rat liver, both *in vivo* and *in vitro* (CHVAPIL *et al.*, 1972). In addition, it is known that in rats the redistribution of zinc within the body is accompanied by the release of leukocytic endogenous mediators (LEM) and endogenous pyrogen (EP) (BEISEL *et al.*, 1976). Effects of acute cold exposure on MT, LEM, and EP in humans, however, are still not known. Thus, although it has been demonstrated that cold-exposed zinc-deficient rats show an impairment of homeothermia which increases with the duration of dietary manipulation of zinc (TOPPING *et al.*, 1981), definite physiological significance of zinc redistribution in the human body during cold exposure awaits further study.

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