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SHORT COMMUNICATION

Changes in Plasma Leptin and Zinc Status of Women Mountaineers at High Altitude

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ABSTRACT

The loss of body weight due to suppressed appetite at high altitude is very common. The hormone leptin and trace metal zinc have key roles in appetite regulation. In the present study, changes in leptin and zinc status of female mountaineers with and without supplementation with zinc have been investigated. Plasma leptin, zinc and enzyme activities of alkaline phosphatase (EC 3.1.3.5) and 5′ nucleotidase (EC 3.1.3.1) of 25 women mountaineers between age group 17–25 yr were studied. The women mountaineers were divided into two groups, i.e., placebo control (n = 7) and supplemented with zinc at dose 15 mg/day for 21 days (n = 18) and all variables were analysed initially at 1981 m, at high altitude (4572–5182 m) and on return to initial altitude. The basal leptin level for both the groups combined was 3.12 ± 0.57 ng/ml (mean ± SD). Plasma leptin levels were found increased by 24 per cent and 58 per cent, respectively at high altitude and after return in case of control group, whereas in case of zinc-supplemented group, there was a statistically non-significant decrease of 20 per cent at high altitude in comparison to basal values. Thereafter, levels were maintained at baseline. There was no significant change in zinc status of control group as indicated by plasma zinc as well as maintained activities of alkaline phosphatase and 5′ nucleotidase in the present study. On the other hand, zinc-supplemented group showed increase in zinc status at high altitude, which was evident by increased plasma zinc levels. On return from high altitude, plasma zinc levels were low in comparison to high altitude but still higher than that of basal. Results indicate beneficial effects of zinc supplementation by decreasing leptin levels at high altitude.

Keywords: Leptin, zinc, high altitude, women mountaineers, alkaline phosphatase, 5′ nucleotidase

1. INTRODUCTION

Anorexia and weight loss, particularly at extreme high altitude, and acute mountain sickness, are the major problems faced by sojourners at high altitude[1-4]. The primary cause of weight loss is anorexia which is more pronounced during early phase of exposure. It has been reported that energy and protein intakes at high altitude are consistently decreased by 30 per cent and 40 per cent, respectively resulting in negative energy and nitrogen balance even when best possible food is available[5-7]. Reduction in meal quantity with rapid onset of satiety and increase in meal frequency have also been reported at high altitude[7]. Biochemical basis of anorexia at high altitude is not fully understood. It has been reported that leptin levels are increased at high altitude[8]. Leptin is a protein encoded by obese gene and acts as key mediator for neuroendocrine regulation of food intake and energy expenditure[9] and is being considered to be responsible for weight loss at high altitude. Similarly zinc also plays important role in modulation of eating

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Table 1 . Physical characteristics of women mountaineers (mean ± SD)

Group	No.	Age (yr)	Weight (kg)		Height (cm)	Body mass index
			Initial	After 21 days		
Placebo control	7	23.71 ± 6.07	51.28 ± 8.78	48.18 ± 8.50	157.71 ± 7.43	20.54 ± 2.58
Zinc supplementation	18	19.72 ± 2.73	49.27 ± 5.95	48.80 ± 5.30	155.00 ± 5.59	20.67 ± 2.97

behaviour[10-12]. Increased urinary losses of zinc are reported during exposure to high altitude[13]. Leptin and zinc levels are also influenced by exercise[14-15]. Keeping these two factors (exercise and high altitude) in mind, attempt has been made to evaluate changes in leptin and zinc levels in women mountaineers with and without zinc supplementations.

2. MATERIALS & METHODS

2.1 Subject Selection

The study was conducted on 25 young women mountaineers trainees. The physical characteristics of these volunteers are given in Table 1.

2.2 Mountaineering Exercise Protocol & Sample Collection

During training the women mountaineers learned basic mountaineering skills and were subjected to physical conditioning at the Himalayan Mountaineering Institute, Darjeeling situated at 1981 m for one week and then were transported to 1524 m by road, the starting point for trekking. The approximate time taken to reach there was 6 hr. They trekked to the base camp at 4511 m. They reached to this height in 5 days after one-day stopovers at 2743 m and 3658 m. Practical training was imparted to them at the base camp for 10 days during which they were exposed to higher altitudes of 4572–5182 m. After training, they trekked back to 1524 m. in 3 days, and finally were transported back to the training institute.

The women mountaineers were explained the procedure and an informed written consent was also taken. The women mountaineers were randomly allocated into two groups, i.e., control (n = 7) and experimental (n = 18). The experimental group was supplemented with zinc (15 mg) in the form of commercially available multivitamins, multimineral capsules daily for the entire period of the study, i.e., 21 days (5 days to reach base camp, 10-day training at heights 4572–5182 m, 4 days to reach back to the training institute and 3-day stay thereafter). Control group received almost similar multivitamin, multi-mineral capsules except zinc (placebo control). Initial blood samples (basal) were drawn at the Himalayan Mountaineering Institute. The second set of blood samples (high altitude) was drawn at the base camp on the last day of training. By this time, the women mountaineers had been exposed for 10 days to altitudes ranging from 4572–5182 m The third set of samples was drawn at the training institute on the 3rd day (de-induction from high altitude) after the trainees had returned back from base camp to the Himalayan Mountaineering Institute.

The heparinised blood samples were allowed to settle and plasma was separated by centrifugation at 1000 g x 10 min at 4 °C and transported to laboratory in ice and salt mixture under frozen condition. Thereafter, plasma samples were stored in aliquots at –70 °C until assayed for various variables.

2.3 Biochemical Estimations

Plasma zinc levels were estimated using atomic absorption spectrophotometer, (Avanta Ver 1.31, GBC Scientific Equipment Pvt. Ltd., Australia) after digestion with nitric acid. Alkaline phosphatase (EC 3.1.3.5) and 5′ nucleotidase (EC 3.1.3.1) were estimated colorimetrically[16-17]. Leptin levels were measured using ELISA kit (EIA 1863), product of DRG International Inc., USA.

Food intake was recorded using 24 hr dietary recall method to find energy and zinc intakes. The energy and zinc intakes were calculated using standard tables[18].

Paired Student t-test was used for statistical comparison between the groups at various altitudes viz., basal, , high altitude and de-induction from high altitude, and the p value less than 0.05 was considered significant.

3. RESULTS

The energy intake at basal condition was 2354 kcal of which major portion was contributed by carbohydrates (62 %), followed by fat (23.5 %). Zinc content was found to be 14.45 mg/day.

At high altitude, subjective reports (self-reports) of a decrease in appetite were noted in control group and account for somewhat lesser food intake (~ 10-15 %) whereas in case of zinc-supplemented group none of the women mountaineers had complaint of appetite loss.

Basal leptin levels of all the 25 women mountaineers combined were 3.12 ± 0.57 ng/ml (mean ± SD). Plasma leptin levels of control group were found increased by 24 per cent and 58 per cent, respectively ($p < 0.05$) at high altitude and after de-induction from high altitude when compared with respective basal values. On the other hand, in case of zinc-supplemented group, a statistically non-significant decrease of 20 per cent in leptin at high altitude was recorded (Fig. 1).

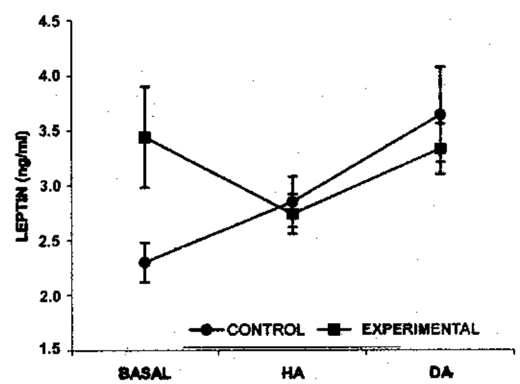

Figure 1. Changes in leptin levels of women mountaineers with and without zinc supplementation. HA = High attitude, DA = De-inducted from high altitude (*$p < 0.05$ in comparison with basal leptin level).

There was no change in plasma zinc levels of control group at high altitude whereas in zinc-supplemented group, significant increase (69.6 %) was observed < 0.05). The zinc levels at de-induction from

Table 2. Plasma zinc levels (mg/l) of women mountaineers

	Control	Experimental
Basal	0.73 ± 0.09	0.56 ± 0.04
High altitude	0.73 ± 0.19	0.95 ± 0.13*
De-inducted from high altitude	0.81 ± 0.11	0.64 ± 0.04*

VALUES are mean ± SEM
* $p < 0.05$ in comparison with basal plasma zinc levels

high altitude though tend to normalise but were still higher than basal (Table 2). The plasma-alkaline phosphatase activity was increased at de-induction in both the groups (Fig. 2). Activity of 5' nucleotidase was increased at high altitude and remained higher even after de-induction (Fig. 3).

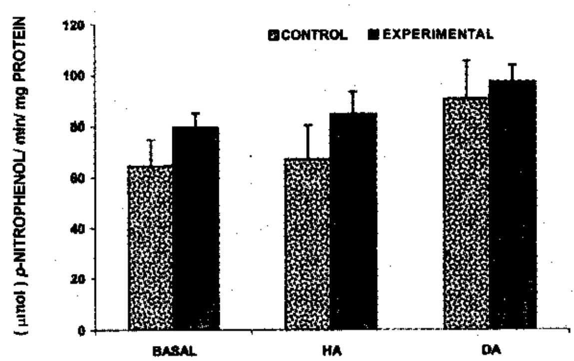

Figure 2. Changes in alkaline phosphatase activity of women mountaineers with and without zinc supplementation. HA= High altitude, DA= De-inducted from altitude (*$p < 0.05$ in comparison with basal leptin level).

4. DISCUSSION

Various high altitude studies have one common observation, i.e, decrease in appetite even when best possible food is available[1-7]. This appetite loss leads to decrease in body weight and when physical activity is combined, this can lead to serious consequences. Tschöp and co-workers were the first investigators, who have established possible neuroendocrinological factors implicated in pathogenesis of high altitude anorexia[8]. They identified a significant increase in serum leptin levels in male mountaineers during two ascents to 4559 m. Subjects with acute mountain sickness have higher circulating leptin levels. Leptin is

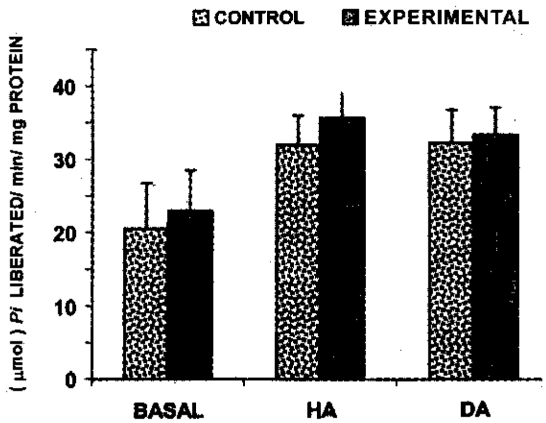

Figure 3. Changes in 5' nucleotidase activity in women mountaineers with and without zinc suplementation. HA= High altitude, DA= De-inducted from high altitude.

a 16 kDa protein encoded by ob gene, secreted from adipocytes and acts as signalling molecule for satiety and energy stores to hypothalamus where receptors for this are located[9,19]. In hypothalamus, action is mediated through neuropeptide Y[20]. It appears that increased leptin at high altitude acts as false signal to brain that energy stores are more than adequate, so reduce feeding which, in turn, is met by decreased neuropeptide Y production. In our earlier study, decreased hypothalamic neuropeptide Y and galanin in rats exposed to simulated altitude (7620 m) were observed[21].

In the present study, we have observed very low basal leptin levels (3.12 ± 0.57) in women mountaineers in comparison with that of normal lean nonathletic females (8.35 ± 0.35) studied separately[22]. Although, body mass index is marginally low in present study group in comparison to earlier study where it was (22 ± 1.6), exercise training is reported to decrease leptin levels, and this may be the reason of low leptin levels observed[14]. The leptin levels increased in control group at high altitude even though subjects were engaged in excessive physical work of mountaineering. In case of zinc-supplemented group increase in leptin level was not observed and reason of this is not clear and unexplainable at present. In both the groups, levels of zinc as well as leptin were different at basal levels and this became a limitation of the study. It is reported that circulating leptin levels are reduced in zinc deficiency and there is increase in hypothalamic neuropeptide Y[23-25]. Zinc deficiency causes anorexia whereas low leptin levels increase food intake. To explain this, a resistance to neuropeptide Y action is speculated in a review on this aspect[12].

Basal zinc levels are also marginally low in these subjects when one considers acceptable/desirable levels of zinc (0.85-1.25 µg/ml) in nutritional status studies[26]. No change in plasma zinc levels in non-supplemented group was observed during this study and it may be due to mobilisation of muscle zinc in response to hypoxia and exercise[15]. Dietary intake during study is close to recommended dietary allowances of zinc for Indians, i.e., 15.5 mg/day. In earlier studies on male subjects at 3500 m, zinc levels were found reduced significantly in control group[27] along with increase in alkaline phosphatase activity for 10 days. Decrease in plasma zinc levels of rats exposed to hypobaric hypoxia was also observed and was associated with decreased food intake[28].

During the present study, increase in alkaline phosphatase activity was observed at de-induction. 5'nucleotidase activity was more at high altitude as well as de-induction from high altitude. In zinc-supplemented group, activities of these enzymes were marginally higher. Activity of zinc-dependent enzyme 5'nucleotidase appears to be responsive to acute change in zinc intake and is being considered better marker in comparison to alkaline phosphatase[26]. Increased urinary losses of zinc are reported at high altitude as well as during exercise[13, 15]. In the present study, estimation of urinary zinc was not possible due to limitation of getting 24 hr urine sample.

5. CONCLUSIONS

Increased leptin levels at high altitude can be considered as one of the neuroendocrine factors for appetite loss though several other factors may be involved, as human eating behaviour is complex in nature. The observation of no increase in leptin level of zinc-supplemented women mountaineers at high altitude needs to be studied further. The zinc supplementation at high altitude may be useful in the prevention of anorexia as observed in the present study. Zinc also acts as antioxidant and may help in overcoming oxidative stress which is reported to be more at high altitude[29,30]. Low plasma zinc concentration and leptin levels in female mountaineers need to be evaluated for their clinical importance as these are involved in regulation of many other metabolic activities, i.e., immunity and reproduction, etc.

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