Defence Science Journal, Vol. 54, No. 2, April 2004, pp. 169-178

SHORT COMMUNICATION

Biochemical Changes in Lowlanders on Descent to Plains after Prolonged Stay at High Altitude–A Cross-sectional Study

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ABSTRACT

The biochemical changes taking place in human subjects following de-induction to plains from high altitude (5700-6100 m) after a prolonged stay of 9-13 months were investigated in 120 soldiers in four groups of 30 each on day 5, 30, 60 and 90 after their descent. One group of soldiers, who were never posted at high altitude served as control. Haemoglobin levels, which were initially high after 5 days of de-induction dropped to significantly lower levels in the group studied on day 60 after de-induction. No clinically abnormal changes were noted in activities of serum enzymes, ie, aspartate aminotransferase, alanine aminotransferase and γ-glutamyl transpeptidase. Alanine aminotransferase activity was found significantly elevated in 5-day group (53.1 ± 0.24 IU/*l*). Cholesterol and triglyceride levels were higher in 30-day group in comparison to groups studied on day 5, 60 and 90 after de-induction from high altitude. Activity of glutathione *S*-transferase was more in groups studied on day 30 and 60 and may be in response to increased demand for detoxification of certain metabolites. Endogenous creatinine clearance was impaired with concomitant increase in serum creatinine levels. The creatinine clearance returned to normal only in group studied on day 90 after de-induction. Testosterone and estradiol levels were within normal physiological limits. However, there was increase in testosterone levels following de-induction. Results of this cross-sectional study indicate that it takes 1-3 months after de-induction from high altitude for biochemical parameters to reach normal levels.

Keywords: De-induction, high altitude, biochemical changes, creatinine clearance, liver function, analysis of variance (ANOVA)

1. INTRODUCTION

Exposure to high altitude is known to cause alterations in physiological variables, hormonal status, and body biochemistry as an adaptive response to hypobaric hypoxia. Above 5,500 m, which is a critical altitude, successful acclimatisation does not take place and leads to ill effects, such as weight loss (a consequence of anorexia) and a decline in mental and physical work capacity. There are very few studies on biochemical changes following prolonged stay at high altitude and results have often been conflicting. Do these changes persist, both immediately and later on, after return to sea

Revised 23 January 2003

Table 1. Physical characteristics, life style, and food habits of subjects in different groups

Study group	Age (Y)	Height (cm)	Weight (kg)	Vegetarian / non-vegetarian	Alcoholic / non-alcoholic	Smoker / non-smoker
HA de-inducted						
5-day group	27.2 – 0.9	168.3 – 0.8	60.8 – 1.4	4 / 26	4 / 26	1 / 29
30-day group	25.1 – 0.8	169.5 – 0.9	61.9 – 1.4	3 / 27	2 / 28	2 / 28
60-day group	26.7 – 0.8	173.8 – 0.8	62.1 – 0.9	5 / 25	3 / 27	3 / 27
90-day group	26.7 – 0.8	172.6 – 0.7	62.2 – 1.0	7 / 23	3 / 27	9 / 21
SL control group	23.1 – 0.4	168.0 – 1.0	58.2 – 1.0	5 / 25	0 / 30	0 / 30

Values are mean ± SEM, n = 30.

level and later? Little is known of this de-adaptation process. Few studies pertaining to body composition changes, haematological response, hormonal changes, plasma proteins, blood glucose, and lipid profile are available[1-9]. In the present paper, findings of a cross-sectional study on serum enzymes related to liver function, ie, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT); kidney function assessed by endogenous creatinine clearance; activities of erythrocyte enzymes, ie, transketolase (TK) and glutathione *S*-transferase (GST), and serum lipid profile along with hormones testosterone and estradiol of Indian soldiers who were de-inducted to plains after 9-13 months of stay at heights 5,700-6,100 m in Eastern Himalayas have been reported.

2. MATERIALS & METHODS

2.1 Study Subjects

A study was conducted on Indian soldiers who were de-inducted to plains (200-250 m) from the Eastern Himalayas (altitude 5700-6100 m) after a continuous stay for the past 9-13 months (mean ± SEM, 10.7± 0.4; median, 11) of their two-year tenure at high altitude. A total of 120 subjects in four batches of 30 each were selected randomly from among the candidates willing to participate in the study from four Army units on their arrival to plains. The study subjects were asked not to go on long leave during the study period. They were taking food from the common mess of their respective units with authorised ration scale for sea level. These were designated as 5-day, 30-day, 60-day and 90-day groups on the basis of period at plains after their de-induction from high altitude*. One group of soldiers who were never posted to high altitude and stayed at sea level served as control (SL control). The subjects were explained about the nature and purpose of the study and their written consent was obtained. The physical variables (mean age, weight, and height), life style, and dietary habits of these groups have been compared (Table 1). Majority of them were non-vegetarian, non-smoker, and few of them were consuming alcohol occasionally.

2.2 Sample Collection

The venous blood samples were drawn after overnight fast and divided into two tubes, one heparinised for plasma separation and the other without anticoagulant for serum. Plasma and serum samples were separated by centrifugation at 1,000 x g for 15 min at 4 °C. Erythrocytes were separated and washed thrice with chilled saline and lysates were prepared. Samples were divided into aliquots, transported to the laboratory in frozen condition and were stored at –70 °C until assayed for various biochemical variables. Twenty-four hour urine samples were also collected for estimation of urinary creatinine and creatinine clearance.

* The Ethics Committee of the Defence Institute of Physiology & Allied Sciences (DIPAS), Delhi, approved the study.

2.3 Biochemical Estimation

Blood haemoglobin and total serum proteins were analysed colorimetrically[10,11]. Enzymic activities of aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) *in serum* were estimated by a method of King[12]. γ-Glutamyl transpeptidase (EC 2.3.2.2) activity was determined using substrate γ-glutamyl *p*-nitroanilide[13]. Enzymic activities of transketolase (EC 2.2.1.1) and glutathione *S*-transferase (EC 2.5.1.18) were estimated in erythrocyte lysates respectively by methods described by Bamji[14] and Habig and Jakoby[15]. Urinary and serum creatinine were estimated using alkaline picrate method[16]. Endogenous creatinine clearance was expressed as UV/S where U and S are the concentrations of creatinine in urine and serum respectively and V is the rate of urine flow in ml/min. Serum total cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride (TG) were estimated using commercially available kits (Quali TEST, Rashmi Diagnostics Pvt Ltd, Bangalore). Low density lipoprotein (LDL)-cholesterol was calculated using Friedwald Formula [LDL = total cholesterol–(HDL cholesterol + TG/5)]

2.4 Statistical Analysis

A comparison between different groups was made using one-way analysis of variance (ANOVA) and post-hoc testing was carried out using Bonferroni *t*-test and p value < 0.05 was considered significant.

3. RESULTS

Haemoglobin levels of group studied after 5 days of de-induction were significantly higher in comparison with SL control and remained in higher range even in the subjects who were studied on day 30 of de-induction. However, in the group studied on day 60, haemoglobin levels were in lower range and of 90-day group, haemoglobin levels were again found elevated (Table 2). Total plasma protein levels were significantly more in 5-day group and less in 30-day group in comparison to SL control group though the values were within normal range (Table 2).

Serum asparate aminotransferase (AST) activity was found elevated in 60-day group though it was well within its normal range whereas alanine aminotransferase (ALT) activity was significantly high in 5-day group and remained higher in groups studied on day 30 and day 60 after de-induction. γ-Glutamyl transpeptidase (γ-GT) activity was more in 5-day group though it was not significant. However, a significant decline on comparison with this group was noted in 30-day and 90-day groups (Table 3).

Total cholesterol levels were significantly high in 30-day and 60-day groups studied in comparison to SL control group as well as the 5-day group.

Table 2. Haemoglobin and plasma protein levels of human subjects de-inducted from high altitude and of SL control group

Study group	Haemoglobin (g/dl)	Total plasma proteins (g/dl)
HA de-inducted		
5-day group	19.9 ± 0.4^{+}	8.27 ± 0.08^{+}
30-day group	17.3 ± 0.2^{+*}	6.70 ± 0.14^{+*}
60-day group	13.3 ± 0.1^{+*}	7.74 ± 0.09^{+*}
90-day group	16.6 ± 0.2^{+*}	6.79 ± 0.10*
SL control group	14.7 ± 0.2	7.09 ± 0.09

Values are mean ± SEM (n = 30), + and * significantly different in comparison with SL control group and 5-day group, respectively.

Table 3. Serum enzyme activities of human subjects de-inducted from high altitude and of SL control group

Study group	AST (IU/*l*)	ALT (IU/*l*)	γ-GT (IU/*l*)
HA de-inducted			
5-day group	3.89 ± 0.23	53.10 ± 0.24^{+}	7.11 ± 0.70
30-day group	2.78 ± 0.25	11.52 ± 1.12*	5.24 ± 0.34*
60-day group	17.02 ± 1.64^{+*}	15.50 ± 2.33^{+*}	5.45 ± 0.51
90-day group	3.27 ± 0.37	3.27 ± 0.37*	4.65 ± 0.37*
SL control group	5.31 ± 0.34	7.79 ± 1.08	5.99 ± 0.25

Values are mean ± SEM (n = 30), + and * significantly different in comparison with SL control group and 5-day group, respectively.

Table 4. Serum lipid profile of human subjects de-inducted from high altitude and of SL control group

Study group	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Triglycerides (mg/dl)
HA de-inducted				
5-day group	153 ± 5	38 ± 2	99 + 5	70 ± 5
30-day group	218 ± 14^{+*}	46 ± 10^{+*}	152 ± 12^{+*}	102 ± 9^{+*}
60-day group	192 ± 10*	42 ± 2	109 ± 8	200 ± 12^{+*}
90-day group	188 ± 13	43 ± 2	121 ± 13	113 ± 7^{+*}
SL control group	163 ± 6	37 ± 3	113 ± 6	66 ± 5

Values are mean ± SEM (n = 30), + and * significantly different in comparison with SL control group and 5-day group, respectively.

Both the HDL cholesterol and the LDL cholesterol were more in 30-day group. Triglyceride (TG) levels were more in de-inducted groups except that of 5-day group. In this case, triglyceride levels were similar to SL control group (Table 4).

Serum creatinine levels were higher in group studied on day 60 (176.7 ± 2.8 μ mol/l, normal range 70–133 μ mol/l). Creatinine clearance values of de-inducted groups were significantly low except for 90-day group (normal clearance for men 120 ml/min, range 95-140) (Table 5).

Activity levels of glutathione S-transferase were similar to SL control group in case of 5-day and 90-day groups, however a rise was noted in 30-day and 60-day groups. Transketolase activity was also found elevated in 30-day, 60-day, and 90-day groups, whereas in 5-day group, it was near to SL control group value (Table 6).

Levels of testosterone and estradiol were within the normal limits (normal values for men, testosterone 8.3–41.6 n mol/l and estradiol 55–220 p mol/l) (Table 7).

Table 5. Serum and urinary creatinine levels and endogenous creatinine clearance of human subjects de-inducted from high altitude and of SL control group

Study group	Serum creatinine (mol/l)	Urinary creatinine		Creatinine clearance (ml/min)
		m mol/l	m mol/ 24 h	
HA de-inducted				
5-day group	117.5 – 2.8^{+}	8.68 – 0.8	7.0 – 0.4^{+}	42.2 – 2.5^{+}
30-day group	119.2 – 3.7^{+}	4.9 – 0.5^{+*}	9.0 – 0.4^{+*}	53.6 – 2.9^{+*}
60-day group	176.7 – 2.8^{+*}	11.6 – 1.2	8.9 – 0.6^{+*}	35.2 – 2.1^{+*}
90-day group	114.0 – 2.2^{+}	16.0 – 1.7^{+*}	19.9 – 1.7^{+*}	119.9 – 9.8*
SL control group	89.3 – 7.8	10.2 – 0.7	12.8 – 0.6	110.3 – 7.0

Values are mean ± SEM (n = 30), + and * significantly different in comparison with SL control group and 5-day group, respectively

Table 6. Transketolase and glutathione *S*-transferase activities in erythrocytes of human subjects de-inducted from high altitude and of SL control group

Study group	Transketolase (mol/ml RBC)	Glutathione *S*-transferase (*n* mol/min/mg protein
HA de-inducted		
5-day group	6.84 ± 0.55	35.40 ± 1.25
30-day group	15.16 ± 1.41^{+*}	59.76 ± 2.25^{+*}
60-day group	15.07 ± 0.99^{+*}	63.69 ± 2.55^{+*}
90-day group	21.62 ± 0.59^{+*}	33.70 ± 1.29
SL control group	5.99 ± 0.62	33.45 ± 0.78

Values are mean ± SEM (*n* = 30), + and * significantly different in comparison with SL control group and 5-day group, respectively.

Table 7. Plasma testosterone and estradiol levels of human subjects de-inducted from high altitude and of SL control group

Study group	Testosterone (*n* mol/*l*)	Estradiol (*p* mol/*l*)
HA de-inducted		
5-day group	20.9 ± 0.8	138.6 ± 8.4
30-day group	27.4 ± 1.3^{+*}	158.6 ± 14.2
60-day group	28.2 ± 1.4^{+}	153.6 ± 9.1
90-day group	28.4 ± 1.0^{+}	132.1 ± 6.0
SL control group	19.3 ± 0.6	158.6 ± 15.0

Values are mean ± SEM (*n* = 30), + and * significantly different in comparison with SL control group and 5-day group, respectively

4. DISCUSSION

Increase in haemoglobin levels is well characterised at high altitude in response to hypoxia and the haemoglobin levels remain high throughout the stay[17-19]. Heath and Williams[18] have reported that haemoglobin levels decline after descent from high altitude and reach to normal sea level value after 6 weeks. Savourey[5], *et al.* have also reported the persistence of increased red cells mass and packed cell volume for 30 days. It is interesting to note that destruction of newly formed erythrocytes takes place (neocytolysis) and the situation resembles to that of return from a space flight[9, 20]. Wolski[21], *et al.* have reported immediate and progressive decrease in red cells production and inhibition of erythropoietin activity at descent to sea level and minimum levels are seen after two to three weeks. Berglund[22] has mentioned decreased athletic performance few months after descent to sea level due to decrease in haemoglobin levels and occurrence of relative anaemia (lower haemoglobin levels in comparison to basal). Similar changes are reported in high altitude natives on descent to sea level[23]. Low haemoglobin level has been observed in the group studied on day 60 after de-induction and this appears to be a transient state as again higher haemoglobin levels (16.6 ± 0.2 g/dl) were seen in the group studied on day 90 after de-induction and these values are even more in comparison to SL control group.

Serum total protein levels were found increased in the group studied after 5 days of return from high altitude. In earlier studies on prolonged stay at high altitude, only initial increase in protein levels during the first two weeks was recorded and on return, no significant changes were seen. This initial increase in the protein levels is believed to be the result of haemo-concentration[4,24]. But in the present study, it appears that high protein levels were maintained throughout the stay. Since no initial data of these human subjects before their ascent to high altitude or during their stay at high altitude is available, it is difficult to come to some conclusion.

Estimation of levels of enzymes, viz., asparate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase are clinically important and used as markers of injuries to organs, such as liver, heart, and muscles. During the early period of natural or stimulated high altitude exposure, blood levels of asparate aminotransferase and alanine aminotransferase have been shown to be increased both in the experimental animals and in the human beings[25-28]. In the present study also, levels of alanine aminotransferase in group just de-inducted from high altitude (5-day group) were found to be significantly high. However, the increase is only marginal over the normal range. Liver is highly resistant to hypoxic stress[29,30], and the results indicate no clinical abnormality in liver function. The data is consistent with the earlier studies on prolonged stay at high altitude[28].

Values of total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride were within the clinical normal limits and changes observed during the present study may be due to individual variations and may not be due to altitude effect alone. At this point, changes due to difference in dietary intake are expected but this possibility is not there since all the groups were having similar lifestyle and dietary habits and were on the common ration scale of Indian Army for sea level. There was not much difference in 5-day group and SL control group, however, an increase in total cholesterol and triglyceride in 30-day and 60-day groups was noted. Both the HDL cholesterol and the LDL cholesterol were increased in 30-day group.

During Operation Everest II, Young[31], *et al.* have shown that in the fasting subjects, total cholesterol and the HDL cholesterol concentration decreased, whereas concentration of triglyceride increased about two-folds but free fatty acid levels were unchanged. However, Férézou[34], *et al.* have reported a decrease in the total cholesterol concentration without change in HDL cholestrol, at high altitude. Savourey[6], *et al.* in a longitudinal study (longitudinal study means same human subjects studied at different locations and cross-sectional study means different human subjects studied and different locations and times) on 9 subjects have reported increased total cholesterol and triglyceride levels in human subjects on expedition to the Himalayas, which returned to normal levels after 30 days of de-induction. Results of the present study in 5-day group are indicative of normal lipid profile after 9-13 months stay at high altitude. However, de-induction appears to have increased cholesterol synthesis. A longitudinal study on this aspect needs to be conducted to get a clear picture of the lipid profile for Indian subjects at high altitude, and after their de-induction to plains over a period of time.

Urinary creatinine excretion was significantly low in comparison to SL control group in all de-inducted groups except that of 90-day group. It is reported that lowlanders exposed to an altitude beyond 3500 m and natives of high altitude areas excrete larger amount of urinary protein and also have reduced creatinine clearance[33]. Studies by Rennie[34], *et al.* on both acclimatised and unacclimatised lowlanders at 5,400 m on Mount Logan after 6 weeks of stay also indicated decreased creatinine clearance with increase in plasma creatinine levels. These changes appear to be independent of acclimatisation and polycythemia.

Glutathione *S*-transferase is a drug-metabolising enzyme[35]. Significant rise was noted in 30-day and 60-day groups whereas it was in normal range for 90-day group. This increase may be due to stress response as this enzyme protects from oxidative stress and is inducible in response to exposure of toxic products of lipid peroxidation[36]. Increased levels of this enzyme are suggestive of more oxidative stress during de-induction from high altitude, which may be due to changes in environmental conditions.

Transketolase, an enzyme of pentose phosphate pathway (which provides NADPH and pentose sugars for nucleotide synthesis) was found increased in groups de-inducted from high altitude except in 5-day group and may be in response to an increase in the demand of products of this pathway. Activity of this enzyme is dependent on thiamine intake. In earlier studies on the human subjects at high altitude, low levels of erythrocyte transketolase were observed in subjects stationed for about 6 months at moderate altitude[19].

Various studies indicate initial decline in plasma testosterone levels at high altitude but on return to sea level, levels of this hormone get normalised within seven days[37,38]. In an earlier study by Basu[39], *et al.*, testosterone levels of subjects who were at high altitude for 6 months, were found similar to SL control group. Decreased urinary levels of testosterone (~ 40 per cent) after two-year stay at 3505 m have been reported by Brahmachari[40], *et al.* Since in the present study, the human subjects were at high altitude for a longer period (for 9-12 months), the concentration of testosterone may have reached normal range as an effect of acclimatisation

6. CONCLUSION

Results of the present study indicate no clinical abnormality upon descent from high altitude after prolonged and continuous stay of 9-12 months, and all the studied variables returned to their normal levels within 1-3 months. There is need for a detailed and longitudinal study to evaluate kidney functions since impaired creatinine clearance was observed up to 60 days of return from high altitude.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the Director Dr P.K. Banerjee, and Dr W. Selvamurthy, CC R&D for their constant interest and encouragement throughout the study. Thanks are also due to soldiers of the Indian Army who participated as subjects for this study.

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