High Altitude and Protein Metabolism in the Rat[1] (34931)

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Exposure to moderately high altitudes is associated with a prompt and sustained loss of body weight in humans and a suppression of growth in small laboratory animals (1). The factors responsible for these changes are not completely understood. In part, they are attributable to anorexia, since a voluntary reduction in caloric intake is a common observation in all species taken abruptly to altitude (1). It is becoming increasingly apparent, however, that other factors are involved. Thus, altitude exposure has been shown to reduce the efficiency of food utilization (2) and alter normal gastrointestinal function (3). But even more important, evidence of defects in the intermediary metabolism of various assimilated food stuffs, particularly protein, has been accumulating. High-protein diets, for example, are poorly tolerated; rats fed such diets not only fail to grow at altitude but actually lose weight (4, 5). Negative nitrogen balances in both animals (6, 7) and man (8, 9) and alterations in the excretion pattern of nitrogenous metabolites are also observed (6, 10–13). Serum concentrations of essential amino acids are reduced (14), and the turnover of serum albumin is increased (8). Finally, the rate of leucine-^{14}C into hepatic proteins was recently shown to be reduced in rats breathing hypoxic gas mixtures (15).

The investigation described here was designed to provide further information on the intermediary metabolism of tissue proteins. Accordingly, we studied the *in vivo* incorporation of alanine and glutamic acid into the proteins of various tissues of rats acutely and chronically exposed to high altitude. We also determined the effects of such exposures on hepatic arginase and arginine synthetase activities.

Methods. Male Holtzman rats ranging in weight from 150–180 g were randomly segregated into two groups and placed in individual wire cages. They were fed a synthetic diet, *ad libitum*, for 10 days prior to experimentation and throughout the entire experimental period. The diet contained the following components by weight: Crude casein 18%, sucrose 75%, corn oil 3%, USP Salt Mix No. XIV 4%, and a complete vitamin supplement. Water was also available *ad libitum*. After the 10-day dietary adjustment period, one group of animals was transported to the summit of Pikes Peak, Colorado (14,110 ft), a trip requiring 2.5 hr, and housed in a laboratory trailer for either 2 days (acute exposure) or 30 days (chronic exposure). The control group remained in the laboratory in Denver, Colorado (5280 ft). Other than altitude the environmental conditions at both locales were quite similar. Changes in body weight and food intake were measured periodically.

At the end of the indicated exposure periods groups of 5–7 animals were injected intraperitoneally with 3 μCi/100 g body weight of uniformly labeled L-alanine-^{14}C or L-glutamic-^{14}C acid (sp act 142 mCi/mmole, and 240 mCi/mmole, respectively). Immediately after injection each animal was placed in a metabolism cage for a period of 4 hr, and the expired CO_2 was collected at hourly intervals in a solution of ethanolamine in ethylene glycol monomethylether (1:2 v/v). At the end of the collection period the animals were sacrificed, and the desired tissues (liver, spleen, duodenum, and thigh muscle) were quickly removed and chilled in

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

TABLE I. Effect of High-Altitude Exposure on Average Gain in Body Weight, Average Daily Food Intake, and Hepatic Arginase and Arginine Synthetase Activities.

	Control (days)		Altitude (days)	
	2	30	2	30
Av daily gain, g	4.2 ± 0.3[a]	4.1 ± 0.4	2.0 ± 0.1*	3.1 ± 0.5*
Av daily food intake, g	14.9 ± 0.1	16.2 ± 0.2	10.2 ± 0.2*	15.9 ± 0.3
Arginase[b]	1120 ±80	1060 ±90	1990 ±130*	1140 ±90
Arginine synthetase[b]	4.6 ± 0.3	4.5 ± 0.3	8.1 ± 0.2*	4.3 ± 0.5

[a] Average 5–7 animals ± SEM.

[b] Micromoles product/min/100 g body weight.

* Different from controls $p \leq .05$, same period.

ice-cold saline. Tissue proteins were isolated according to the procedure described by Siekiewitz (16). Briefly, homogenates were prepared with 10% trichloracetic (TCA), centrifuged, and decanted. The protein precipitate was twice resuspended in 5% TCA, recentrifuged, and decanted. This process was then repeated once with hot (90°) TCA to remove nucleic acids. The protein residue was centrifuged again and washed once with warm 95% alcohol, twice with warm 2:2:1 alcohol:ether:chloroform, once with warm acetone, followed with ether, and then air-dried. A weighed amount of the protein was dissolved in 1 ml of Hyamine solution by heating at 60° for 2 hr; it was then diluted with 15 ml toluene-*p*-phenyloxazole (PPO-POPOP) and the radioactivity was assayed in a Packard scintillation spectrometer. Radioactivity in the expired $^{14}CO_2$, was collected as indicated above and according to the procedure of Jeffay and Alvarez (17).

For enzyme assays, five animals from each experimental group were decapitated and exsanguinated after which their livers were carefully and completely removed and weighed. A portion of each liver was homogenized in 9.0 vol of 0.1% solution of hexadecyltrimethyl ammonium bromide in water and assayed for arginase and arginine synthetase activities according to the method of Brown and Cohen (18).

Results. The effect of high altitude on growth, food intake, and activities of the two urea cycle enzymes are summarized in Table I. A significant growth decrement was observed after 2 days' exposure on Pikes Peak, and recovery from the loss did not occur even after 30 days. There was a concomitant decrease in food intake observed at high altitude. Activities of hepatic arginase and arginine synthetase were significantly increased after 2 days but not after 30 days on Pikes Peak.

Table II indicates the effect of high altitude on $^{14}CO_2$ production from labeled alanine and glutamic acid. With both amino acids, increased rates of respiratory $^{14}CO_2$ loss were observed after 2 days' exposure. This effect was apparent the first hour after injection and continued throughout the entire collection period. By contrast, the animals exposed to high altitude for 30 days expired approximately the same amount of radioactivity as the corresponding control groups at low altitude.

The data showing incorporation of alanine or glutamic acid into proteins of selected tissues are presented in Table III. On the second day of exposure, decreased incorporation of either amino acid into liver, spleen, duodenal, and adrenal proteins was observed. A slight decrease in the mean specific activities of muscle proteins was also obtained but this was not statistically significant. Altitude exposure for 30 days had no effect on the incorporation of these amino acids into proteins of any of the tissues studied.

Discussion. Acute altitude exposure, as seen in the present study is associated with anorexia and this, in turn, with a reduction in caloric intake. The latter would appear to be the prime factor underlying the reduced growth rates which are observed in young

mammals and the body weight loss which is observed in adult mammals, including man. It would also appear that the reduced caloric intake is responsible for most, if not all, of the changes in protein metabolism which were seen in this study. Under conditions of caloric deficiency a greater fraction of dietary protein would be required to meet the energy demands of the animal; consequently, a lesser fraction would be available to support other protein requirements including growth. Therefore, it may be anticipated that the dilution of the injected isotopic amino acid would be different between low and high altitude, which in turn would affect specific activities of the isolated tissue protein. The differences in $^{14}CO_2$ production between the controls and the animals acutely exposed to altitude could be also interpreted as dilution effects rather than metabolic effects. Further work will be required to determine amino acid pool sizes at high altitude since the recent work from this laboratory indicates that the circulating levels of serum glutamic acid is markedly elevated in human subjects acutely exposed to an altitude of 14,110 feet (34). Unfortunately, the plasmic levels of glutamic acid or alanine were not determined in the present experiment.

Increased activities of the two urea cycle enzymes plus a decrease in the incorporation of alanine or glutamic acid into tissue proteins in acutely exposed rats suggests that the catabolic pathways of amino acids and protein metabolism are enhanced during the initial stages of high altitude exposure. Elsewhere, the studies of Evans (6) and Brunquist *et al.* (7) showing negative nitrogen balances under conditions of acute hypoxia provided early evidence of elevated protein catabolism. More recently, similar observations were made by Surks (8) and Consolazio *et al.* (9). Surks also observed (8) a greater rate of serum albumin degradation and a probable reduction in albumin synthesis in acutely exposed humans. Sanders *et al.* (15) in studies more akin to those reported here, reported a decreased incorporation of ^{14}C-leucine into the liver protein of acutely hypoxic rats.

TABLE II. Effect of High-Altitude Exposure on $^{14}CO_2$ Production from Alanine-^{14}C or Glutamic-^{14}C Acid (dpm in thousands).

Altitude/days:	2				30			
Amino acid:	Alanine		Glutamic		Alanine		Glutamic	
Altitude/feet:	5,280	14,110	5,280	14,110	5,280	14,110	5,280	14,110
Hours after injection								
1	660 ± 60	990 ± 90	620 ± 50	880 ±100	750 ± 100	780 ± 80	650 ± 70	690 ± 100
2	1080 ± 70	1440 ± 70	880 ± 70	1350 ± 60	1000 ± 90	990 ± 110	790 ±120	880 ± 40
3	940 ± 40	1280 ± 40	1080 ± 90	1290 ± 80	940 ± 100	860 ± 100	900 ± 50	930 ± 110
4	600 ± 40	800 ± 80	520 ± 70	800 ± 80	900 ± 60	840 ± 120	610 ± 90	530 ± 80
Total	3280 ±100	4520 ± 80*	3090 ± 80	4310 ± 90*	3600 ± 90	3460 ± 110	2960 ± 80	3020 ± 100

* Values are significant from controls at $p < .05$, $n = 5$–7 animals.

TABLE III. Effect of High-Altitude Exposure on Amino Acid Incorporation into Tissue Proteins.

Altitude/days:	2				30			
Amino acid:	Alanine		Glutamic		Alanine		Glutamic	
Altitude/feet:	5,280	14,110	5,280	14,110	5,280	14,110	5,280	14,110
Tissue								
Liver	79 ± 8[a]	53 ± 6*	50 ± 7	28 ± 6*	85 ± 6	81 ± 12	53 ± 10	60 ± 9
Spleen	85 ± 12	43 ± 10*	155 ± 11	108 ± 7*	83 ± 10	79 ± 12	144 ± 12	134 ± 13
Duodenum	78 ± 7	49 ± 6*	118 ± 9	65 ± 7*	74 ± 9	68 ± 8	111 ± 10	118 ± 7
Muscle	23 ± 5	19 ± 8	24 ± 5	21 ± 7	24 ± 4	21 ± 6	25 ± 5	28 ± 4
Adrenals	138 ± 10	98 ± 9*	154 ± 12	108 ± 10*	123 ± 13	126 ± 15	148 ± 11	153 ± 12

[a] Disintegrations per minute per milligram protein ± SEM.
* Values are significant from controls at $p < .05$, $n = 5$–7 animals.

Increased rates of protein or amino acid oxidation and reduced rates of protein synthesis are not the only effects of acute altitude exposure. An enhancement of gluconeogenesis from amino acid precursors also appears likely. The report of Burlington and Klain (19) showing an increase in hepatic glutamic–pyruvic transminase activity during acute exposure in rats would favor such an interpretation. So would the report of Timiras *et al.* (20) showing a reduction in the blood sugar of such rats. Humans, it should be noted, rarely show this latter response although they do show other evidence of defects in protein metabolism (14).

The factors directly responsible for altered urea cycle enzyme activities and the decreased incorporation of labeled amino acids into tissue proteins in acutely exposed rats remains to be fully elucidated. However, it is well established that the enzyme activities of the urea cycle tend to parallel urinary urea production (21, 22) falling when a low protein or protein-free diet is fed and increasing during starvation and when excess protein is ingested. Elevated rates of protein catabolism during acute exposure could produce similar effects. It is also known that the activities of the urea cycle enzymes increase markedly after administration of corticosteroids (23, 24) although the effect appears to be nonspecific; that is, the enzyme levels are changed only so much as the corticosteroids increase protein breakdown and urea excretion. It is well known that increased adrenocortical activity (25–27) and increased concentration of plasma free thyroxine (28) occur during the early period of altitude exposure. There is general agreement among studies concerned with effects of glucocorticoids on protein metabolism that cortisol increases protein degradation (29, 20). Although thyroxine stimulates incorporation of amino acids into rat liver protein (31), Surks reported a possible interference with the stimulation of protein synthesis by throxine under hypoxic conditions (8). Therefore, increased activities of the urea cycle enzymes observed in acutely exposed rats may be due to a combination of decreased food intake and of hormonal imbalances. An increase in protein degradation un-

der these conditions would tend to increase the amino acid pool size, thus diluting radioactivity and decreasing specific activities of tissue proteins.

As the data herein indicate, animals exposed chronically to high altitude reach a new metabolic steady state in the process of acclimatization to hypoxia. One aspect of these metabolic alterations is an increase in food intake which equals that of the control animal. Another aspect may involve the size of the adrenal gland. For example, in contrast to the rise in the urinary excretion of steroids observed in the newcomers and temporary residents (32), no differences have been found in the excretion of 17-ketosteroids and 17-hydrocorticoids (32, 33) between subjects at the sea level and men living at high altitudes. Such metabolic alterations could, in turn, affect overall nitrogen metabolism.

Summary. Activities of hepatic arginase and arginine synthetase and incorporation of alanine or glutamic acid into tissue proteins were studied in rats exposed acutely or chronically to an altitude of 14,110 feet. Compared to the controls, activities of the two enzymes were significantly increased after 2 days but not after 30 days at altitude. By way of contrast, a decrease in amino acid incorporation into liver, spleen, duodenal, and adrenal proteins was observed in rats exposed to altitude for two days. Altitude exposure for 30 days had no effect on amino acid incorporation into tissue proteins. The data demonstrate an increase in protein catabolism during an acute exposure to hypoxia.

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