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Effect of Dietary Protein Quality on the Biochemical Adaptation to High Altitude

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Abstract: The usefulness of a particular type of dietary protein for a quicker acclimatization to simulated high altitude stress was investigated in albino rats, by studying the changes occurring in the levels of urea cycle enzymes in liver. A good quality protein in (egg) and a commonly used dal protein (arhar dal, *Cajanus cajan*) were studied.

Liver arginase levels increased on starvation in the two groups of rats fed with egg and dal diets, the increase being less and gradual in the dal diet fed group. Exposure to the simulated altitude stress for various periods further increased the enzyme levels, greater changes being seen in egg diet fed group only. Similar observations were made with liver ornithine transcarbamylase which is a mitochondrial enzyme. Urea cycle enzymes were affected more in the exposed animals maintained on the egg diet than those on the dal diet. Moreover, the enzymic parameters of the latter group tended to return to the normal level much earlier than the former. It is, therefore, suggested that not-so-well-balanced dietary proteins may be well suited to overcome the initial problem of metabolic adaptation faced by subjects exposed to high altitude stress.

1. Introduction

Exposure to high altitude stress leads to appetite suppression resulting in reduced food consumption, anorexia and loss of body weight. One of the biochemical reasonings for these changes was disturbance in protein metabolism[1]. Surk[2] and Consolazio[3] had reported that some impairment in protein synthesis during the first week of altitude exposure could have occurred. Hannon *et al*[4] had demonstrated a marked reduction in ammonia excretion of rats exposed to high altitude.

High protein diets are poorly tolerated and rats actually lost weight[5,6]. It is also known[7,8,9] that during the early period of altitude stress, there is an increased adrenocortical activity which is known to increase protein degradation. Anorexia

due to loss of appetite may result in the supply of energy from body proteins. This is expected to reflect on the urea cycle enzymes and transaminases because the amino nitrogen resulting from the utilisation of amino acids for energy requirements is excreted largely as urea[10].

It was of our interest and immediate relevance to our goal to investigate whether previous dietary preparation has any effect on the experimental models, namely albino rats when exposed to simulated altitude stress particularly after the earlier observation from our laboratory that dietary protein quality has a definite role to play in the liver amino nitrogen metabolism[11]. We wanted to investigate whether the differential effect of dietary protein quality could be utilized to quickly acclimatize albino rats to altitude stress.

In the present investigation, two dietary protein sources namely, a good quality protein, egg (balanced amino acid composition) and a commonly used leguminous protein source in the Indian dietary, arhar dal (*Cajanus cajan*), were fed to experimental rates which were then exposed to 0.5 atmospheric pressure corresponding to 18,000' altitude. Two of the five enzymes of liver urea cycle were investigated and the results are presented.

2. Materials and Methods

2.1. *Materials*

All the chemicals used were of analytical grade. The biochemicals were procured from Sigma Chemical Co., USA.

White albino male rats of wistar strain weighing around 140-160 g were procured from stock colony of the Laboratory's Central Animal House Facility.

2.2. *Methods*

2.21. *Diets and Their Preparation*

Experimental diets chosen to feed the rats were at 15% protein level. One of the diets was prepared using whole egg as a standard reference diet, which satisfied the requirements for all the essential amino acids. The other diet contained arhar dal (*Cajanus cajan*). Protein content in both the diets were determined by micro Kjeldahl method. Eggs (hen) and dals were procured from the local market. Egg solids were incorporated into the diet after hard boiling and shelling. The dal was cooked in boiling water and was added to the rest of the dietary mixture.

2.22. *Exposure to Hypobaric Hypoxia of* 0.5 *Atmospheric Pressure*

Animals were exposed to hypobaric hypoxia in a decompression chamber. Low pressure was obtained in the chamber by means of a suction pump on one side and an adjustable inlet valve on the other. The chamber pressure, monitored by

a mercury barometer was adjusted to the desired level by means of the inlet valve. The open system of air flow ensured sufficient oxygen supply and prevented the accumulation of the respiratory carbondioxide. Hypobaric hypoxia of 0.5 bar could be generated within the chamber in less than 3 min. At the end of the experimental period, the animals were withdrawn after restoring the pressure to the ambient level.

Immediately after exposure, the abdominal cavity was opened under mild ether anaesthesia or sodium pentobarbitone (5 mg/100 g body wt.), Blood was collected directly from the heart, and the animal was sacrificed during the process. After coagulation of the blood, in dry centrifuge tubes, the tubes were centrifuged for 20 min. at 2000 rpm and serum was collected and stored at $-10°C$ in deep freeze till further analysis. Liver was excised and washed with ice cold saline, blotted dry and transferred to precooled glass weighing bottles and stored in deep freeze until tissue samples were drawn for homogenization.

Protein was estimated by the method of Lowry *et al*[12]. Liver arginase (EC 3.5, 3.1) was estimated by the method of Geyer and Dabich[13]. The enzyme activity was expressed as μ moles urea/min/g tissue at 37°C. Liver ornithine transcarbamylase (OTC) (EC 2.1, 3.3) was estimated by the method developed by Nuzum and Snodgrass[14]. The enzyme activity was expressed as μ moles cirtrulline formed/min/g tissue at 37°C.

3. Results

In Table 1 are summarized the arginase levels in rats maintained on the two different diets and exposed to 0.5 atmospheric pressure for various periods. The control animals were starved for the corresponding period of exposure because no food was given to experimental animals during the period of simulated high altitude exposure. Upto 40 h of starvation, the arginase levels did not change significantly from the normal level of 445.0 ± 11.0 units in the egg diet. However, at 65 h the level increased significantly by 70%. On exposure to simulated altitude, the arginase levels started increasing gradually from 6 h onwards. At 6h the increase was about 7%, at 24 h it was significant at 14%, and at 40 h the increase over the corresponding control was maximum 82%. At 65 h the level started to fall and it was only 58%.

In the group maintained on dal diet, in contrast to the egg diet group, the arginase levels of the control starved animals increased significantly by 24% at 24 h itself. At 40 h, the control values increased by 34% which remained at the same level even at 65 h. It is interesting to note that at 65 h of starvation the arginase levels were the same whether they were maintained on egg diet or dal diet. On exposure to simulated altitude for 6 h, there was no significant change. But at 24 h, the change was quite sharp at 38%. At 40 h, though the increase was about 44%, the level of arginase was 1055 ± 75.0 units and was not significantly different from the level of 937 ± 58.5 units at 24 h, neither was it significantly different from the value of 967 ± 38.7 at 65 h. However, the increase of only about 31% at 65 h as compared to about 44% at 40 h, showed that after 40 h of exposure, there was a

Table 1. Effect of 0.5 Atmospheric Pressure on Liver Arginases of Rats Maintained on egg and dal diet

Duration of exposure (h)	Egg Diet Control*	Egg Diet Exposed*	change† (%)	Level of significance (P)	Dal Diet Control*	Dal Diet Exposed*	change† (%)	Level of significance (P)
0	445.0±11.0	—	—	—	547.8±51.7	—	—	—
6	441.0±30.6	471.2±39.6	6.8	N.S	519.4±42.9	512.5±13.2	1.33	N.S
24	437.5±33.0	497.2±30.2	13.7	<0.05	679.8±56.9	937.0±58.5	37.97	<0.01
40	476.2±32.8	868.0±38.2	82.3	<0.01	735.4±64.0	1055.0±75.0	43.53	<0.01
65	755.2±32.8	1192.7±104.1	57.9	<0.01	739.6±95.7	967.0±38.7	30.7	<0.01

* μ moles urea/min/g liver tissue

† Taking the value for control as 100

* Each value represents the mean ± S. D. of six animals *ad libitum* fed at 15% protein level for a period of ten days prior to exposure. Both control and exposed animals were starved Water was allowed. *ad libitum.*

N. S. Not Significant.

Table 2. Effect of 0.5 Atmospheric pressure on liver ornithine transcarbamylase (OTC)* of rats maintained on egg and dal diets

Duration of exposure (h)	Egg Diet Control*	Egg Diet Exposed*	Change† (%)	Level of significance (P)	Dal Diet Control*	Dal Diet Exposed*	change† (%)	Level of significance (P)
0.	143.85±18.83	—	—	—	184.69±28.53	—	—	—
6	146.82±33.10	138.96±17.4	—5.35	N. S.	237.81±31.20	300.17±63.2	26.2	N. S.
24	162.27±22.20	285.31±35.7	75.82	<0.01	238.94±15.95	317.42±36.5	32.84	<0.01

*μ moles citrulline/min/g tissue

† Taking the value for control as 100

* Each value represents the mean± S. D. of six animals *ad libitum* fed at 15% protein level for a period of ten days prior to exposure. Both control and exposed animals were starved. Water was allowed *ad libitum.*

N. S. Not Significant.

tendency to come back to control levels. The changes (increase) at 40 h in dal diet group (44%) was only about half of what was seen in the egg diet group (82%).

The changes in the liver OTC levels accompanying starvation and simulated altitude exposure of rats maintained on dal as well as egg diets are presented in Table 2. On 24 h starvation, the increase was only 13% in the egg diet fed group which was not significantly different from the control level. However, on exposure for 24 h, there was a sharp and significant increase of 76% in the level of OTC activity.

In the dal diet fed group of rats, the OTC levels on 6 h starvation increased by 29% and remained at the same level even on 24 h starvation. The OTC level rose by 26% from 237.81 ± 31.2 to 300.17 ± 63.2 units on 6 h exposure itself. Even on 24 h exposure, the increase did not change significantly from the 6 h level; it was 33% as compared to the control group. The egg diet group showed a higher (76%) change in the enzyme level during the 6 h period of exposure.

4. Discussion

It was observed that loss in body weight in humans and the decrease in the growth rate in animals on exposure to actual or simulated altitudes greater than 11,000 feet have been variously attributed to dehydration[15], alteration in nutrient absorption[16] and digestibility[17], disturbances in protein metabolism[18] and anorexia[16,19,20,21]. However, reduced calorie consumption due to reduced appetite during the initial period of exposure was the frequent observation in both men and animals. Therefore, many workers in this field found it difficult to distinguish between the direct effect of hypobaric hypoxia and the effect of caloric restriction on the protein metabolism of animals at high altitude.

Schnakenberg *et al*[22] and Whitten *et al*[23] had adapted the paired-feeding technique to circumvent the problem of anorexia during simulated and actual exposure to high altitude stress. Schnakenberg *et al*[22] however concluded that since the nitrogen intake and retention at high altitude and pair-fed controls did not differ significantly during the initial period of exposure, high altitude did not elicit an increase in the net rate of protein metabolism. On the other hand Whitten *et al*[23] studying the liver transaminases and urea cycle enzymes in rats exposed to simulated altitude concluded that most of the effects on protein catabolism were due to calorie restriction, although all their data did not support this contention *in toto*.

In the present investigation we investigated effect of simulated high altitude exposure, on the role of previous dietary preparation of the subject before exposure. In fact Schnakenberg *et al*[22] suggested study of the digestibility of protein of lower biological value than casein by rats under hypoxic conditions. In other words, the quality of protein fed before the exposure may have a significant contribution on the net protein catabolism of the animal. However, to circumvent the problem of restricted intake of food on the exposed ones compared to the unexposed controls, both the groups were fasted during the period of exposure and the various enzymatic parameters studied.

When the urea cycle enzymes were investigated an interesting feature came to light. On starvation the egg diet group showed a sudden increase of 70% in arginase only at 65 h. But on the other hand the dal diet group started showing a gradual increase right from 24 h itself and steadied at 40 h with only 36% increase. Even on exposure, the egg diet group showed a greater change of 82% at 40 h than the dal diet group which showed only a 44% change. Yet another point is that the change in dal diet group was much earlier than that in the egg diet group. With respect to the terminal enzyme of urea cycle, the subjects maintained on dal diet were able to acclimatize faster, on exposure, than the ones maintained on a well balanced protein of egg. It could be due to the fact that the dal diet group had already attained an increased level of arginase. Liver OTC values also behaved in the same way on exposure. At 24 h exposure, the increase in egg diet group was twice as that in the dal diet group. OTC is a mitochondrial enzyme unlike arginase which is a cytosolic enzyme. This again proves that the previous dietary feeding on a protein of particular quality can affect the enzymic changes on subsequent exposure to hypobaric hypoxic stress.

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