Iron and Zinc Status of Protein-Energy Malnourished Rats

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SUMMARY

Rats were divided into three groups and the following two types of diets were given. Group 1, 15% protein diet (control), group 2, 5% protein diet (low-protein group). These two groups, were on a diet ad libitum. Group 3 was fed a 60% amount of 5% protein diet taken by group 2 (low protein-low energy group). Iron concentrations were significantly low in blood and high in almost all other tissues of malnourished rats (group 2 & 3). The same phenomenon was observed in zinc concentrations of group 3 rats, but to a lesser extent. The results indicate that, there was accumulation of iron in solid tissues of malnurished rats in spite of negative balance in the body.

INTRODUCTION

Disturbances in mineral metabolism is frequently associated with protein -energy malnurition (PEM). PEM is still now a major public health probelm in many areas of the world, especially in the developing countries, which cause high mortality and morbidity. Seventy five percent of world children live in underdeveloped nations, 60% of whom are malnourished and nearly 90% have no access to basic health service. 80,000 children die from malnutrition every day[1].

Iron is absolutely essential for transport of oxygen to the tissues and maintenance of oxidative systems within the cells. It is important for formation of hemoglobin, myoglobin, cytochromes, peroxidase and catalase[2]. Zinc is an essential element for a large -number of enzymes, such as carbonic anhydrase, carboxypeptidase, alkaline phosphatase and alcohol dehydrogenase[3]. Although the essential role of trace elements is recognised, data still insufficient concerning their concentrations in different tissues, especially in relation to malnutrition. This experiment was carried out to clarify the effects of low -protein and low-energy diets on iron and zinc status in the body.

MATERIALS AND METHODS

Animals and Diet

Fifteen albino weanling male Wistar rats weighing 40–45 gram of three weeks old, were distributed randomly into three groups of 5 animals each. Compositions of different diets are shown in Table 1 which differed only in the proportion of protein and carbohydrate, where the carbohydrate was inversely proportional to the protein. They were kept in stainless steel cages with a raised wire bottom. Temperature was between 20–25°C and a 24 hour light and dark cycle was maintained. Body weight and diet intake were measured daily. All the rats were given free diet except group 3, which was given 60% of the diet taken by group 2.

Table 1. Composition of experimental diets

Group No.	1	2 & 3
Casein	15.00	5.00 (%)
Sucrose	38.30	48.30
Starch	30.00	30.00
Olive oil	10.00	10.00
Cellulose	2.00	2.00
Salt mixture*	4.00	4.00
Vitamin mixture†	0.50	0.50
Choline chloride	0.20	0.20

*Composition:–

	Per 100g diet
NaCl	0.43g
$K_3C_6H_5O_7.H_2O$	0.95g
K_2HPO_4	0.31g
$CaHPO_4.2H_2O$	1.42g
$CaCO_3$	0.65g
$MgCO_3$	0.16g
$FeC_6H_5O_7.3H_2O$	64.00mg
$MnSO_4$	5.00mg
$CuSO_4.5H_2O$	0.70mg
$K_2Sl_2(SO_4)_3.24H_2O$	0.36mg
$CoCl_2.6H_2O$	0.36mg
KI	0.18mg
$ZnCO_3$	0.18mg
NaF	3.50μg

†Composition:–

	Per 100g diet
Vitamin A palmitate	1250 I.U.
Calciferol	100 I.U.
Thiamin nitrate	500 μg
Riboflavin	750 μg
Niacin	5000 μg
Pyridoxine hydrochloride	500 μg
Folic acid	250 μg
Cyanocobalamine	0.5 μg
Pantothenic acid	2500 μg
Ascorbic acid	18750 μg
dl-α-tocopherol	500 μg

Before killing, the rats were fasted for 12 hours to reduce metabolic rate to basal levels and then anesthetised with pentobarbital sodium. Five ml blood was collected from the aorta into syringes and the organs were collected.

Analytical Methods

The tissues were ashed by wet method with nitric acid and perchloric acid in the ratio of 4:1, which was completed by the appearence of white fumes. Iron and zinc were measured by using atomic absorption flame emission spectrophotometer (Shimadzu Model AA 670). All reagents were of the best commercially available grade. Student's t-test was used for data analysis.

RESULTS

There was (Fig. 1.) little body weight gain in low-protein group (group 2) and slight loss in low -protein low energy group (group 3). Appetite was decreased (Fig. 2.) in low -protein rats (group 2) compared to control rats (group 1). The malnourished groups (groups 2 & 3) showed fall of body hair which was noticed by about 20th day of experiment. All organs (Fig. 3.) of both groups of malnourished rats were significantly low in weight.

In group 3, iron concentration was (Fig. 4.) decreased significantly in blood and were increased significantly in all other tissues except in muscle. In group 2, the concentrations were decreased significantly in blood and muscle while significantly increased in brain, heart, kidney and spleen. Zinc concentrations (Fig. 5.) were significantly decreased in blood and bone, and increased in liver, heart, kidney and testis of group 3 rats. In group 2 rats, the concentrations were significantly decreased in blood, muscle and bone, while increased in kidney only.

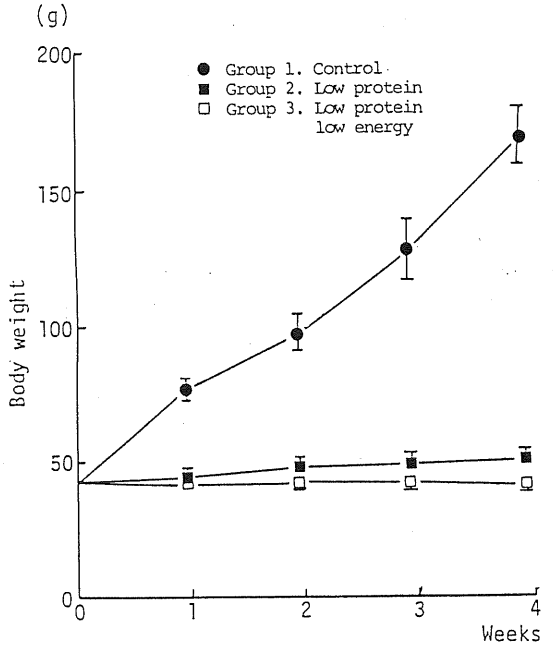

Fig. 1. Body weight curves.

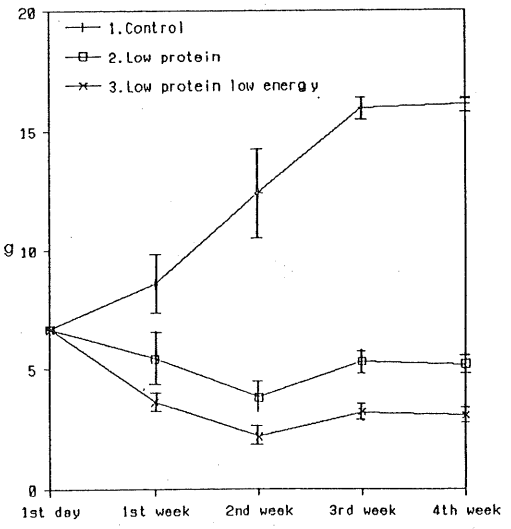

Fig. 2. Food intake curves (g/day).

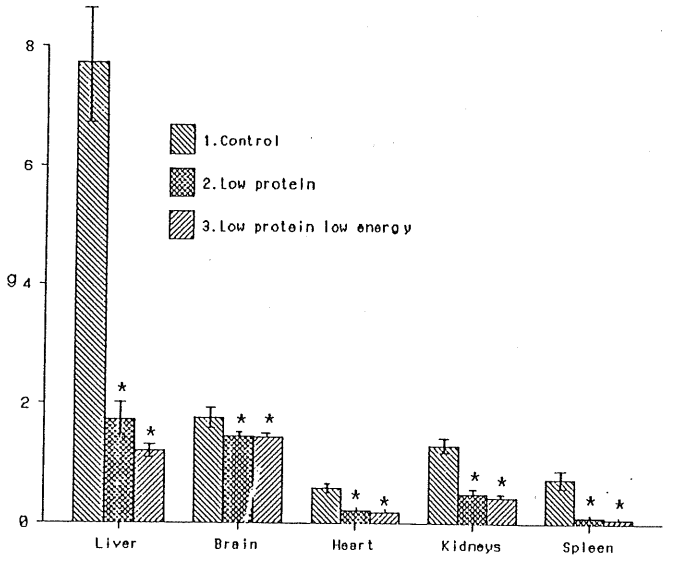

Fig. 3. Weights of various organs (g).

The values represent means ± S.E.M. of 5 rats. *Significant difference at $p<0.001$ as compared to group 1.

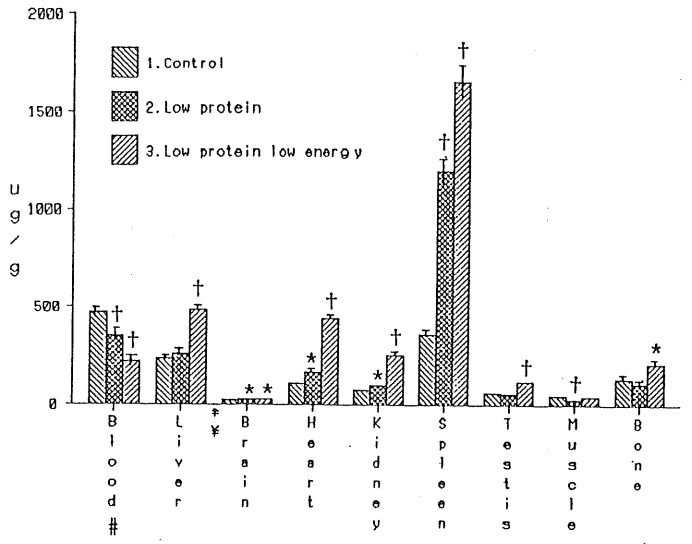

Fig .4. Iron concentrations in various tissues (μg/g).

The values represent means ± S.E.M. of 5 rats. #(μg/ml) *Significant difference at $p<0.05$ as compared to group 1. †Significant difference at $p<0.005$ as compared to group 1.

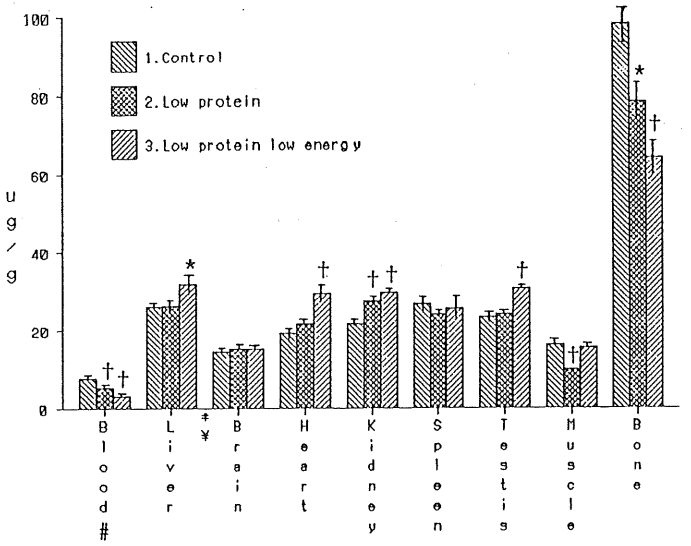

Fig .5. Zinc concentrations in various tissues (μg/g).

The values represent means ± S.E.M. of 5 rats. #(μg/ml) *Significant difference at $p<0.05$ as compared to group 1.†Significant difference at $p<0.005$ as compared to group 1.

DISCUSSION

Regarding iron distribution, our results reenforces the fact that, malnutrition causes anemia following iron deficiency. Because about 65% of total body iron is present in blood in the form of hemoglobin[2] and our malnourished rats had significantly low level of blood iron, they should be considered iron deficient, despite of high level of tissues iron. The deficiency was due to more carbohydrate in the diet[4] and decreased absorption of iron. Klavins *et al.* had reported that, 15—18% protein was necessary to meet the normal needs as far as iron absorption was concerned[5]. The mechanism involved in the higher concentrations of iron in tissues cannot be stated clearly without further investigation. A probable explanation might be as follows. Iron is stored in tissues (mainly in liver) in two forms[2]. Ferritin, the usual and greater storage pool, which is readily available for blood i.e. regulate blood iron concentration. The second storage form is hemosiderin which occures in case of the absence of free apoferritin and which is not easily available for blood. It is possible that, malnutrition caused greater storage pool of iron in hemosiderin form rather than ferritin. As a result accumulation of iron occured in tissues which could not go to blood stream, despite of low blood iron concentration. We have previously reported[6] that protein energy malnutrition also causes accumulation of calcium and magnesium in solid tissues despite of overall negative balance in rat. Wallwork et al[7] found that femur iron was increased in low -protein rather than high -protein diet. Klavins *et al*[5]. has reported less total liver iron in 5 and 10 percent protein diets, compared to 18 percent protein diet. This discrepancy might be due to the difference in age and strains of the

rats. We got significantly lower levels of zinc in blood and bone of the malnourished rats and higher levels in some solid tissues. At present there is no single laboratory parameter that indicates the body status of zinc[8]. Becasue of the lack of data concerning tissues zinc concentrations in animal, it is not easy to say whether malnutrition causes negative zinc balance in the body or not. Low animal protein and high wheat flour intake has been reported by Prased *et al.*[9-11] to cause zinc deficiency in Iran and Egypt. Moreover, several characteristic features of malnutrition are also found in zinc deficiency indicating a possible positive correlation between them. The features include growth retardation, poor appetite, mental lethargy, delayed wound healing and impaired bone formation.[11]. In this light malnutrition may be considered to cause zinc deficiency.

From the above result and discussion we can conclude that, malnutrition causes iron accumulation in solid tissues in addition of iron deficiency. It also changes the zinc concentrations in different tissues.

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REFERENCES

1. Kappenberger, M. G. (1982): The benefits to mankind from Albino laboratories' research. in Chelated Mineral Nutrition in Plants, Animals and Man. ed. by Ashmead, D. and Thomas, C. C., Springfiels, pp.5–9.
2. Guyton, A. C. (1981): Red blood cells, anemia and polycythemia, in Text book of medical physiology. ed. by Guyton, A. C., Saunders, W. B. Company, Philadelphia, pp.56–64.
3. Durnin, J. V. G. A. (1980): Food, energy and nutrition, in Text bood of physiology. ed. by Bell, G. H., Emslie-Smith, D. and Peterson, C. R., Churchill Livingstone, New York, pp.8–35.
4. Truswell, A. S. (1977): Nutritional factors in disease. in Adavidson's Principles and Practice of Medicine. ed. by John Macleod, Pitman Press, Great Britain, pp.99–151.
5. Klavins, J. V., Kinney, T. D. and Kaufman, N. (1962): The influence of dietary protein on iron absorption. Br. J. Exp. Path., 43, 172–180.
6. Ahmed, S. M., Kimura, M. and Itokawa, Y. (1987): Calcium and magnesium distribution in protein -energy malnourished rats. J. Nutr., in press.
7. Wallwork, J. C., Johnson, L. K., Milne, D. V. and Sandstead, H. H. (1983): The effect of interactions between dietary egg white protein and zinc on body weight, bone growth and tissue trace metals in the 30-day old rat. J. Nutr., 113, 1307–1320.
8. Abdulla, M. (1983): How adequate is plasma zinc as an indicator of zinc status? in Zinc deficiency in human subjects. ed. by Prased, A. S. *et al.*, Alan R. Liss Inc., New York, pp.171–183.
9. Prased, A. S., Halsted, J. A. and Nadimi, M. (1961): Syndrom of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am. J. Med., 31, 532–544.
10. Prased, A. S., Miale, A., Farid, Z., Sandstead, H. H. and Darby, W. J. (1963): Biochemical studies on dwarfism, hypogonadism and anemia. Arch. Int. Med., 111, 407–423.
11. Prased, A. S. (1983): Zinc deficiency in human subjects. in Zinc deficiency in Human Subjects. ed. by Prasad, A. S. *et al.*, Alan R. Liss Inc., New York, pp.1–33.