Vol. XII (3-4), 1975

THE EFFECT OF FOOD RESTRICTION ON THE ACTIVITY OF HEPATIC ARGINASE OF RAT

M. Akmal Khan*

The effect of a diet low in both protein and calorie on the activity of hepatic arginase was studied in adult mature rats. The activity of the enzyme increased and the liver nitrogen decreased after 10 and 20 days, indicating that protein was used as a source of energy. The subsequent decrease in the liver enzyme activity and liver nitrogen after 31 days indicate conservation of protein in the liver. The suppression of this pathway means that not only the liver but also the body as a whole benefits from the more economical use of amino acids when restricted amount of food is consumed.

INTRODUCTION

Many investigators have studied the effects of diets deficient in protein or energy on the enzyme concentration in the tissues, particularly liver tissue. Generally the results of such studies indicate a depressing effect of such deficiencies on the concentration of many enzymes. The level of protein in the diet has direct effect on the activity of urea cycle enzymes. High protein diets raise the arginase level (Miller, 1950; Mandelstam and Yudkin, 1952; Ashida and Harper, 1961) and low protein diet or adrenalectomy, which decrease protein catabolism, causes a decrease in the activities of most of the urea cycle enzymes (Mclean, 1961 and Freedland, 1964). On the other hand these enzymes were more active in rats which were starved (Schimke, 1962).

Since the formation of urea is a vital segment of protein catabolism in the mammals, this stage provides a natural place to look for metabolic

*Department of Nutrition University of Agriculture, Lyallpur

controls. A study was thus undertaken to see the effect of a diet low in both protein and calorie on the activity of hepatic arginase of rats fed for different length of times.

MATERIALS AND METHODS

Thirty six adult male rats, 6–7 months old of Sprague-Dawley strain were randomly divided into six groups of six rats each and were kept in individual metabolic cages. All the rats were fed ad–libitum, an experimental diet (Khan, 1972) containing 5 per cent protein for 3 weeks before the start of the experiment. The animals on restricted diet received 70 per cent of their normal food intake (Khan, 1971). *Ad-libitum* and restricted diets were randomly assigned to six groups in such a way that three groups received food ad-libitum and the other three groups were fed a restricted diet for 10, 20 and 31 days.

The animals were killed by a blow on the head, the liver was rapidly removed, cooled and weighed. A small sample was homogenized with ice-cold distilled water 1:200 and the enzyme activity was determined. The assay was based on the liberation of urea from arginine, followed by the colour determination of urea with 1- phenyl-1, 2- propanedione -2- oxime by the method of Archibald (1945). One gm sample of liver was analysed for nitrogen by Kjeldahl method and protein was calculated by multiplying the values for nitrogen by 6.25. All the assays were performed in duplicate. The data were subjected to statistical analysis by using Mann-Whitney U-Test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The average food intake, liver nitrogen and activity of arginase of rats fed *ad-libitum* or restricted diet for 10, 20 and 31 days are presented in Table 1.

TABLE 1. *Average food intake, liver nitrogen and activity of arginase of rats fed ad-libitum or restricted diets.*

Days on diet	Treatment	Food intake per Kg 3/4 per day		Liver total nitrogen (gm)	Arginase Activity*	
		Kcal	Nitrogen		Specific**	Total***
10	Ad-lib.	152	326	0.47	91.2	268.0
	Restricted	106	224	0.38	134.2	317.5
20	Ad-lib.	153	326	0.47	94.7	280.0
	Restricted	106	225	0.40	115.4	295.0
31	Ad-lib.	156	344	0.49	90.4	270.0
	Restricted	106	226	0.47	82.0	263.0

* = Mg urea N produced per 30 minutes..
** = Units per gm of protein
*** Units per liver.

Liver Nitrogen :

Average total nitrogen in restricted and *ad-libitum* groups fed for 10 days was 0.38 gm and 0.47 gm and the difference was statistically significant ($P<0.032$). After 20 days the nitrogen was significantly ($P<0.021$) lower in the restricted groups, the average values were 0.40 gm and 0.49 gm in restricted and *ad-libitum* groups respectively. No significant difference was observed between restricted and *ad-libitum* groups fed for 31 days, indicating conservation of nitrogen in the liver. Khan (1974) found that the activity of L-Methionine-S35 in the liver was greater in the restricted rats than the rats fed *ad-libitum* conversely the activity in the muscle was less in the restricted than in *ad-libitum* groups. Waterlow (1959) has suggested that in protein depleted animal there is a concentration of protein synthesis in the internal organ at the expense of muscle and skin.

Arginase Activity :

The specific activity of arginase in *ad-libitum* and restricted groups killed after 10, 20 and 31 days was (91.2 and 134.2)* (94.7 and 115.4) and (90.4 and 82.0) respectively. Significant differences were noticed only after 10 and 20 days. The specific activity reduced after 31 days but the difference was non-significant, however the specific activity in the restricted group at 31 days was significantly ($P<0.001$) lower than the restricted group at 10 days.

The total activity in the restricted animals increased significantly ($P<0.047$) compared with the rats fed *ad-libitum* for 10 days. No significant difference was observed between restricted and *ad-libitum* groups after 20 and 31 days. The total activity in the livers of rats fed restricted diet for 31 days reduced significantly ($P<0.047$) as compared with the restricted group at 10 days.

It is apparent from the results that rats on restricted diet showed an increase in the activity of the enzyme with a decrease in liver nitrogen after 10 and 20 days, indicating that protein was used to provide energy. When the food restriction was prolonged to 31 days the activity of the enzyme did not reduced significantly which could be due to high variation in the results but the liver nitrogen indicates conservation of nitrogen in the liver as there was no significant differenee in nitrogen contents of livers of the rats fed *ad-libitum* or restricted diet. The animals adapt to reduced food intake and need less energy to maintain their body weights so do not exidise protein for energy (Khan and Bender, 1974).

There are some observation on the effect of protein content of the diet on the activity of certain liver enzymes. Schimke (1962) has shown that on protein free diet the activity of the most of the urea cycle enzymes is reduced. Mariani *et al.* (1963) observed that a low protein diet causes an increase in the activity of amino acid activating enzymes in the liver. It was postulated that amino acid activating enzymes in the liver are regulated by the size of free amino acid pool. When the pool decreases the enzymes increase in activity and so does the fraction of free amino acid directed towards protein synthesis.

The adaptive changes in the enzyme activity must presumably be that a free amino acid molecule, entering liver pool, whatever its origin, has a larger chance of being incorporated in protein and a smaller chance of being degraded to urea. The end result would be an increased economy or re-utilization of amino acids in the liver. Since urea is formed only in the liver, partial suppression of this pathway means that not only the liver, but the body as a whole benefits from this more economical use of amino acids.

LITERATURE CITED

Archibald; R.M. 1945. Colorimetric Determination of Urea. J. Biol. 157, 507-517.

Ashida, K. and A.E. Harper, 1961. Liver arginase activity during adaptation to high protein diet. Proc. Soc. Exp. Biol. Med. 120. 352-356.

Freedland, R.A. 1964. Urea cycle adaptations in intact and adrenalectomized rats. Proc. Soc. Exp. Biol. Med. 116, 692-696.

Khan, M.A. 1971. Ph.D. Thesis, University of London.

Khan, M.A. 1972. Minimum dietary protein required to maintain nitrogen equilibrium in adult rats. Pak. J. Biochem. 5, 19-21.

Khan, M.A. 1974. Effect of food restriction on the *turnover* rate of liver and muscle proteins in rats. Pak. J. Agri. Sci. 11, 21-24.

Khan, M.A. and A.E. Bender. 1974. Ths effect nf food restriction on body weight, nitrogen balance and liver composition of adult rats. Pak. J. Sci. Ind. Res. 17, 10-20.

Mandelstam, J. and J. Yudkin. 1952. The effect of variation in dietary protein upon the hepatic arginase of the rat. Biochem. J. 51, 681-686.

Mariani, A., M.A. Spadoni, and G. Tomassi, 1963. Effect of protein depletion on amino acid activating enzymes of rat liver. Nature, 199, 378-379.

Mclean, P. 1961. Effect of adrenolactomy on the activity of enzymes of urea cycle in rat liver. Nature, 191, 1302-1303.

Miller, L.L. 1950. The loss and regeneration of rat liver enzymes related to diet protein. J. Biol. Chem. 186, 253-260.

Schimke, R.T. 1962. Adaptive characteristics of urea cycle enzymes in the rat. J. Biol. Chem. 237, 1921-1924.

Schimke. R.T. 1962. Adapative characteristics of urea cycle enzymes in the rat. J. Biol. Chem. 237, 459-468.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. The Iowa State University. Ames, Iowa, U.S.A.

Waterlow, J.C. 1959. Effect of protein depletion on the distribution of protein synthesis, Nature. 184, 1875-1876.