Biological Sciences Section

Pakistan J. Sci. Ind. Res., Vol. 17, No. 1, February 1974

THE EFFECT OF FOOD RESTRICTION ON BODY WEIGHT, NITROGEN BALANCE AND LIVER COMPOSITION OF ADULT RATS

M. AKMAL KHAN

Department of Nutrition, Agricultural University, Lyallpur

A.E. BENDER

Department of Nutrition, Queen Elizabeth College, University of London, London

(Received October 31, 1973; revised December 6, 1973)

Abstract. Adult male rats were fed a 5% protein diet, restricted to 70% of their normal food intake for 31 days. Dietary restriction resulted in increased catabolism of protein and large negative nitrogen balance, decreased body weight persisting for 10 days. After 20 days the loss of body weight ceased and negative nitrogen balance returned towards equilibrium. Although continued food restriction reduced the liver size but conservation of nitrogen was observed at the end of the experiment. It was suggested that adaptation to food restriction could be due to reduced nitrogen turn over or reduced caloric requirements.

It has been recognized that continued dietary restriction ultimately results in adaptation to a lowered intake of protein[1] or of calories,[2] Bosshardt and his coworkers[3] have shown that an animal responds in two ways to a systematic reduction of the caloric value of the diet. A limited reduction causes the animal to draw on existing reserves to obtain the energy its food is not supplying; then more drastic deprivation forces it to use both food and body protein for energy purposes.

A restriction in caloric intake results in certain metabolic adjustments that are directed towards correction of the deficit in energy intake. At the same time essential anabolic functions are continued. Allison and Anderson[4] and Allison *et al.*[5] found that a marked restriction in caloric intake in dogs could prevent attainment of nitrogen equilibrium with retention of dietary nitrogen at low nitrogen intakes.

The effects of inadequate diets on infants and growing animals have been thoroughly investigated and it was considered worth extending similar studies to mature adult rats fed dietary regimen similar to those consumed by adults in developing countries. Such diets are low in protein and inadequate in calories.

Materials and Methods

Thirty six mature adult male rats, 6–7 months old, of Sprague–Dawley strain were divided randomly into six groups of six rats each and were housed in individual metabolic cages in a room maintained at 70–72°F. All the rats were fed *ad libitum* an experiment diet[6] containing 5% protein for 3 weeks before the start of the experiment to acclimatise the animals. Food was offered in a small pot fitted into a large outer pot, covered with a wire mesh to collect spilled food.

The animals on restricted diet received 70% of their normal food intake[7] and the quantity of restricted diet was calculated from the average, normal 7 days intake. Since rats are likely to consume the limited amount of food as soon as provided and, therefore, go short of food for the rest of day, the restricted diet was offered in two portions morning and evening as a wet paste to reduce spillage.

Ad libitum and restricted diets were randomly assigned to these group in such a way that three groups received food *ad libitum* and three groups were fed a restricted diet for 10, 20 and 31 days. Urine, faeces and shed hair were collected in 1N H_2SO_4 from the individual animal and four or five days pooled collections from each rat were homogenized for 2–3 mm in a large blender and diluted to volume with distilled water. A 5-ml homogenized sample in duplicate was used to determine nitrogen by Kjeldahl method.[8]

At the end of the experimental period the rats were killed and livers were excised, weighed and stored in deep freeze. The moisture content was determined by drying a portion to constant weight at 105°C. About 1 g samples of liver in duplicate were used for nitrogen estimation by Kjeldahl method. Protein was calculated by multiplying the values for nitrogen by 6.25.

The caloric value of the diet was determined in the ballistic bomb calorimeter[9] and the nitrogen content was estimated in duplicate samples by Kjeldahl method.

The data were subjected to statistical analysis by using Mann–Whitney U-test.[10]

Results and Discussion

The average body weights, food intake and nitrogen balance of rats fed 5% protein diet, *ad libitum* or

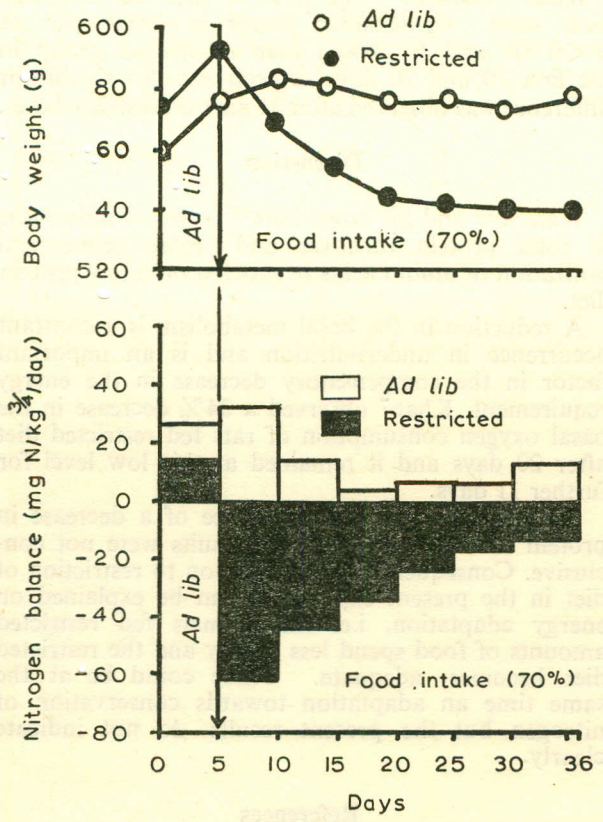

Fig. 1. Nitrogen balance and body weight changes of rats fed *ad libitum* or restricted diet.

restricted to 70% of their normal food intake, for 10, 20 and 31 days are presented in Table 1.

Body Weights. All the animals on restricted diets lost weight, the degree of loss depended on the length of time on the diet. Table 1 shows the rats just maintained their body weights when fed *ad libitum* and the weight loss in the restricted group was 6.7% in 10 days. Rats fed *ad libitum* for 20 days gained an average 14 g and restricted group lost 66 g body weight (10.9%).

The animals lost 50 g in the first 20 days (8.5%), Table 1. No change in the body weight was observed in the last 11 days of dietary restriction. This reflected adaptation of the body to the reduced intake. *Ad libitum* fed group was maintaining their body weight during this period (Fig. 1).

Nitrogen Balance. Table 1 (3B) shows the changes in average nitrogen balance which is associated with reduced food intake for 31 days. During *ad libitum* period the animals ate on average 150 kcal and 321 mgN/kg¾/day which was sufficient to maintain nitrogen equilibrium. The restricted diet provided 105 kcal/kg¾/day and 226 mg N/kg¾/day. The excretion of urinary nitrogen increased and nitrogen balance became negative immediately the diet was restricted. However, the animal began to adapt since the large initial N loss gradually decreased. The average values of nitrogen balance (mg N/kg¾/day) were —61.3, — 24.5 and — 12.0 for 6–10, 21–25 and 31–36 days respectively (Fig. 1).

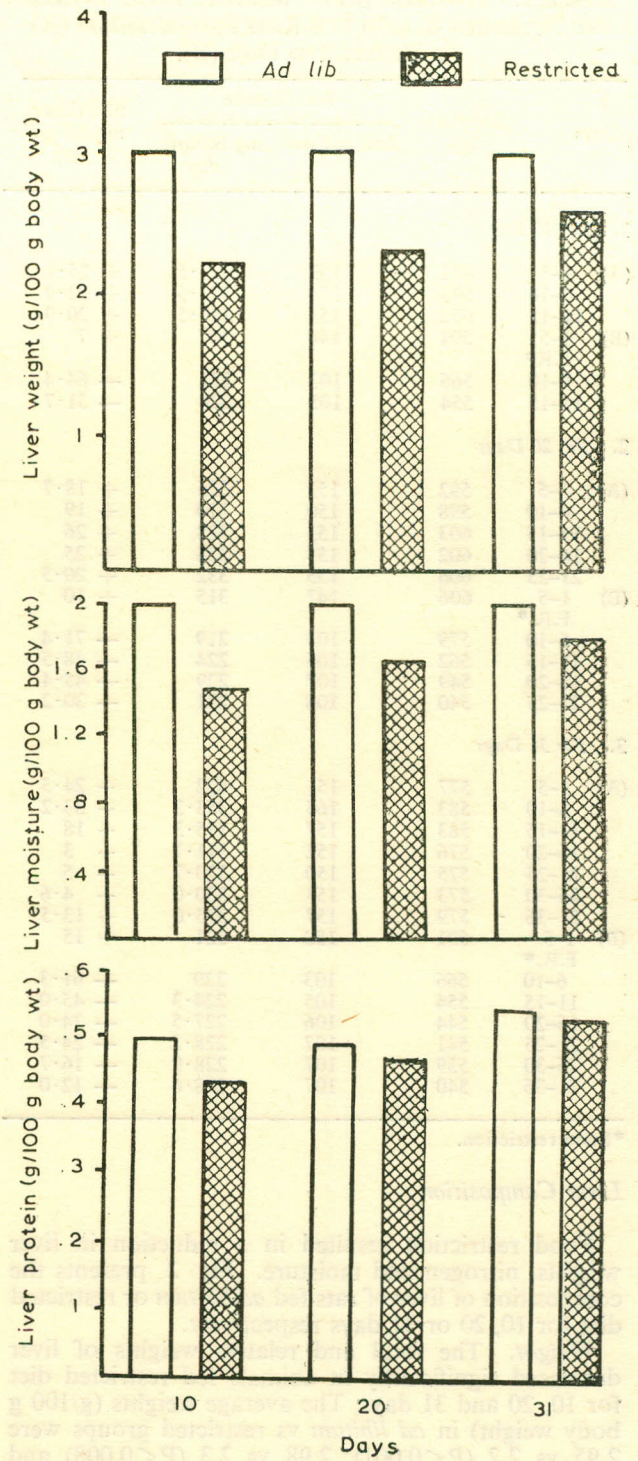

Fig. 2. Composition of liver of rats fed *ad libitum* or restricted diet.

The loss of nitrogen during the early periods of restriction is an indication of catabolism of protein for gluconeogenesis and energy, and the subsequent decrease of nitrogen catabolism suggests an adaptive mechanism for conserving protein.

TABLE 1. AVERAGE BODY WEIGHT, FOOD INTAKE AND NITROGEN BALANCE OF RATS FED *ad libitum* (A) OR RESTRICTED DIET (B).

Days		Body wt (g)	Food intake		N balance mg N/kg$^{\frac{3}{4}}$/day
			kcal/kg$^{\frac{3}{4}}$/day	mg N/kg$^{\frac{3}{4}}$/day	
1. *For 10 Days*					
(A)	1–5	602	153	327·5	+ 25·1
	6–10	598	151	323·5	+ 22·9
	11–15	602	153	327·5	+ 20·9
(B)	1–5	594	144	307	+ 7
	F.R.*				
	6–10	566	103	221	— 64·4
	11–15	554	105	224	— 51·7
2. *For 20 Days*					
(A)	1–5	592	153	326	+ 18·7
	6–10	598	150	320	+ 19
	11–15	603	153	328	+ 26
	16–20	602	153	328	+ 25
	21–25	606	155	332	+ 20·5
(B)	1–5	606	147	315	+ 10
	F.R.*				
	6–10	579	103	219	— 71·4
	11–15	562	105	224	— 48·5
	16–20	549	107	229	— 45·4
	21–25	540	108	231	— 30·2
3. *For 31 Days*					
(A)	1–5	577	151	422	+ 24·5
	6–10	583	165	353·3	+ 33·2
	11–15	583	157	335·3	+ 18
	16–20	576	152	311·7	+ 3
	21–25	575	150	320·7	+ 5
	26–30	573	153	330·0	+ 4·6
	31–36	579	157	335·0	+ 13·5
(B)	1–5	591	150	321	+ 15
	F.R.*				
	6–10	566	103	220	— 61·3
	11–15	554	105	224·3	— 45·0
	16–20	544	106	227·5	— 34·0
	21–25	541	107	228·2	— 24·5
	26–30	539	107	228·9	— 16·7
	31–36	540	107	228·6	— 12·0

*Food restriction.

Liver Composition

Food restriction resulted in a reduction in liver weights, nitrogen and moisture. Fig. 2 presents the composition of liver of rats fed *ad libitum* or restricted diet for 10, 20 or 31 days respectively.

Weight. The total and relative weights of liver decreased significantly in animals fed restricted diet for 10, 20 and 31 days. The average weights (g/100 g body weight) in *ad libitum* vs restricted groups were 2.95 vs 2.2 ($P<0.001$), 2.98 vs 2.3 ($P<0.008$) and 2.94 vs 2.56 ($P<0.013$) respectively.

Water Content. The liver of rats fed restricted diets were significantly lower in water content ($P<0.001$ and $P<0.008$) than *ad libitum* group in the first 10 and 20 days respectively. No significant difference was observed after 31 days on restricted diet.

Discussion

Waterlow and his coworkers[11] reported a decrease in total protein turnover and more economical utilization of amino acids in rats fed on a low protein diet.

A reduction in the basal metabolism is a constant occurrence in undernutrition and is an important factor in the compensatory decrease in the energy requirement. Khan[7] observed a 34% decrease in the basal oxygen consumption of rats fed restricted diet after 20 days and it remained at this low level for further 11 days.

He failed to find clear evidence of a decrease in protein turnover although the results were not conclusive. Consequently the adaptation to restriction of diet in the present experiment can be explained on energy adaptation, i.e. the animals fed restricted amounts of food spend less energy and the restricted diet becomes adequate. These could be at the same time an adaptation towards conservation of nitrogen but the present results do not indicate clearly.

References

1. H.J. Deuel, K. Sandiford and W.M. Boothby, J. Biol. Chem., **76,** 391 (1928).
2. A. Keys, J. Brozek, A. Henschel, O. Mickelsen and H.L. Taylor, *The Biology of Human Starvation* (Minnesota Press, Minneapolis, 1950), vol. 1, p. 338.
3. D.K. Bosshardt, W.J. Paul, K.O'Doherty and R.H. Barnes, J. Nutr., **36,** 773 (1946).
4. J.B. Allison and J.A. Anderson, J. Nutr., **29,** 413 (1945).
5. J.B. Allison, J.A. Anderson and R.D. Seeley, Ann. N.Y. Acad. Sci., **47,** 245 (1946).
6. M.A. Khan, Pakistan J. Biochem., **1,** 19 (1972).
7. M.A. Khan, Ph.D. thesis, University of London, (1971).
8. R. Markham, Biochem. J., **236,** 795 (1942).
9. D.S. Miller and P.R. Payne, Brit. J. Nutr., **13,** 501 (1959).
10. H.B. Mann and D.R. Whitney, Ann. Math. Statist., **18,** 52 (1947).
11. J.C. Waterlow, G.A.O. Alleyne, H. Chan, J.S. Garrow, A. Hay, P. James, D. Picou and J.M.L. Stephen, Arch. Latinom., Nitr. **16,** 189 (1966).