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Adaptation to Restricted Intake of Protein and Energy

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Abstract. Adult rats that maintained nitrogen balance on a diet containing 5% casein fed *ad libitum* were restricted to 70% of their normal food intake for 31 days. This resulted in a negative N balance, loss of body weight and increased activities of hepatic glutamic pyruvic transaminase and arginase — all of which persisted for 10 days. After this time there was no further weight loss, the negative N balance returned to equilibrium and the activities of the two enzymes returned to normal indicating adaptation to the dietary restriction. Radio-isotope studies with labelled methionine showed that dietary restriction led to an increase in the radioactivity of the liver and a decrease of that of the muscles indicating maintenance of liver protein at the expense of muscle protein. Resting oxygen consumption decreased by 34% after 20 days of dietary restriction and this would account for the adaptation observed.

Introduction

With an inadequate intake of energy the efficiency of utilisation of dietary protein is reduced as evidenced by a negative nitrogen balance (4). However, some degree of adaptation can follow through a reduction in energy output (5).

The extent of such adaptation to restriction of both dietary energy and protein was investigated in adult rats.

Materials and Methods

Animals and Diets

Adult male rats, 6–7 months old of the Sprague-Dawley strain were housed in individual metabolic cages at 21–22 °C. They were fed *ad libitum* on a diet found just adequate to maintain body weight and N equilibrium (8), namely 5% casein, 5% corn oil, 2% vitamin mixture, 4% mineral mixture and 84% corn starch.

After 7 days the average food intake was determined and when fed the restricted diet 70% of this amount was offered in two portions morning and evening as a wet paste to reduce spillage.

Urine, faeces and shed hair were collected in 1 *N* H₂SO₄ from each individual rat and 4 or 5 pooled daily collections from each were homogenised for 2–3 min in a large blender and diluted to volume with distilled water for N determination by the Kjeldahl method.

Oxygen Consumption

Rats were fasted for 16 h and their oxygen consumption was measured for 10-min periods in a metabolic chamber consisting of a large glass desiccator fitted with a soda-lime container to absorb carbon dioxide. Oxygen consumption was calculated from the change in pressure in the chamber as shown by a calibrated water-filled manometer.

Analytical Methods

Liver. Dry weight was determined by drying to constant weight at 105 °C. Crude protein (N × 6.25) was determined on 1-gram samples by the Kjeldahl method.

Liver glutamic pyruvic transaminase (EC 2.6.1.2) was measured by the method of Rosen *et al.* (16) and arginase (EC 3.5.3.1) estimated by the method of Archibald (1).

Diet. Nitrogen content was determined by the Kjeldahl method and the energy value was estimated in the ballistic bomb calorimeter (12).

Statistical Methods. The Mann-Whitney U test was used to determine the statistical significance of the results (11).

Procedure

Experiment I

42 rats were fed on the experimental diet *ad libitum* for 3 weeks and then divided randomly into 7 groups of 6 animals. All were injected with *L*-methionine-S³⁵ (2 μCi/100 g body weight) intraperitoneally 1 day before the start of restrictive feeding. One group of animals was killed a day later (day 0) three groups selected at random received food *ad libitum* and three groups were fed the restricted diet – 70% of their normal intake – for 10, 20 and 31 days.

Each group of rats was killed at the end of its respective feeding period by a blow on the head, the liver was rapidly removed, cooled and weighed. A sample of the liver and the gastrocnemius muscle were prepared for radioactive counting (7).

Arginase was measured at the same times. Glutamic-pyruvic transaminase was estimated after 10 and 31 days.

Experiment II

6 adult rats were fed *ad libitum* for 3 weeks on the experimental diet and their oxygen consumption measured for 10-min periods.

They were fed on restricted diets – 70% of their normal intake – and their oxygen consumption measured after 10, 20 and 31 days.

Results

Experiment I

Body weights, food intake and N balances of rats fed *ad libitum* and 70% of their normal intake are shown in table I and figure 1.

Table 1. Average body weight, food intake and nitrogen balance of rats fed an *ad libitum* (A) or restricted diet (B)

	Days	Body weight, g	Food intake		N balance mg N/kg ^{3/4} /day
			kcal/kg ^{3/4} /day	mg N/kg ^{3/4} /day	
<i>For 10 days</i>					
(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
FR	6-10	566	103	221	-64.4
	11-15	554	105	224	-51.7
<i>For 20 days</i>					
(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
FR	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
<i>For 31 days</i>					
(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
FR	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

FR = Food restriction.

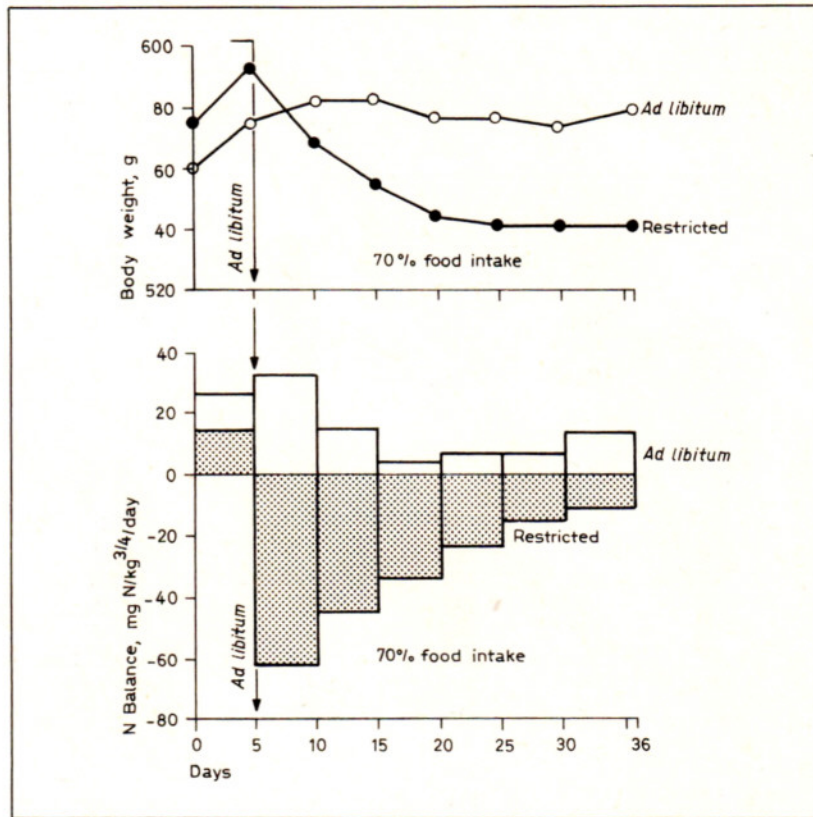


Fig. 1. N balance and body weight change on 5% protein diet.

All maintained weight or gained very slightly when fed *ad libitum*. On the restricted diets they lost 40 g (7%) in 10 days, 66 g (11%) in 20 days and 51 g (9%) in 31 days. Figure 1 shows that the body weights reached a plateau after 20–25 days on restricted diets and that there was no further weight loss in group 3 during the last 15 days on experiment.

N equilibrium was maintained during *ad libitum* feeding (fig. 1) when energy intake averaged 150 kcal and N intake 321 mg/kg^{3/4}/day. On the restricted diet of 105 kcal and 226 mg N/kg^{3/4}/day there was an immediate increase in urinary N excretion and N balance became negative. The animals then adapted and N loss gradually decreased. In the three periods 6–10 days, 21–25 days and 31–36 days N balances averaged –61.3, –24.5 and –12.0 mg N/kg^{3/4}/day.

Table II. Liver composition of rats fed *ad libitum* or restricted diet (mean values)

Treatment	Days on diet	Liver weight		Liver composition		
		total g	g/100 g body weight	total protein g	protein %	dry matter %
<i>Ad libitum</i>	10	17.7	2.95	2.94	16.6	32.5
Restricted		12.3	2.2	2.38	19.3	33.2
<i>Ad libitum</i>	20	18.1	2.98	2.94	16.2	32.9
Restricted		12.6	2.3	2.50	19.8	29.6
<i>Ad libitum</i>	31	17.0	2.94	3.06	18.0	34.4
Restricted		13.9	2.56	2.94	21.2	31.2

Table III. Radioactivity in liver and muscle of rats after an injection of *L*-methionine-S³⁵ (mean values)

Dietary treatment	Days after injection	Liver dpm		Muscle dpm	
		per mg tissue	per mg N	per mg tissue	per mg N
<i>Ad libitum</i>	1	91.6	2,700.0	17.5	500.0
<i>Ad libitum</i>	10	33.4	1,289.0	18.4	508.0
Restricted		44.7	1,358.0	16.5	435.0
<i>Ad libitum</i>	20	20.0	740.0	20.5	501.0
Restricted		27.6	763.0	18.5	469.0
<i>Ad libitum</i>	31	14.4	481.0	22.7	535.0
Restricted		18.7	534.0	20.3	520.0

Table IV. Effect of food restriction on the activities of hepatic glutamic-pyruvic transaminase (mmol pyruvate produced/h) and arginase (mg urea N produced/30 min)

Treatment	Days on diet	G-p T activity		Arginase activity	
		specific U/g protein	total U/liver	specific U/g protein	total U/liver
<i>Ad libitum</i>	0	12.0	37.0	92.0	272.0
<i>Ad libitum</i>		12.5	36.7	91.2	268.0
Restricted	10	20.2	48.8	134.2	317.5
<i>Ad libitum</i>		—	—	94.7	280.0
Restricted	20	—	—	115.4	295.0
<i>Ad libitum</i>		11.7	35.5	90.4	270.0
Restricted	31	8.9	33.1	82.0	263.0

Liver weights were reduced both in absolute terms and when calculated per 100 g body weight.

The proportion of protein was increased but because of the smaller size, total liver protein was less than on *ad libitum* diets. Dry matter was reduced after 20 and 31 days on restricted diets.

Radioactive Count. There was a rapid uptake of labelled methionine by the liver with a marked fall after 10, 20 and 31 days in both groups (table III). The fall was less marked, however, in the restricted group when calculated per milligram tissue.

The difference was significant at each period of experiment; after 10 days $p < 0.048$, after 20 days $p < 0.032$ and after 31 days $p < 0.008$.

When radioactivity was calculated per milligram liver N there was still a difference between the restricted and fully fed groups but not significantly so. Thus, the difference was more likely to be due to chemical composition than to the extent of labelling of the protein.

Changes in the radioactivity of the muscle tissue were the opposite of those taking place in the liver, increasing continuously after 10, 20 and 31 days. The increases were less in the restricted group at each period but the differences were not significant.

Liver Enzymes. Glutamic-pyruvic transaminase increased after 10 days both in specific activity ($p < 0.001$) and total liver activity ($p < 0.021$).

After 31 days some degree of adaptation was observed since the activities of the enzyme, although slightly less in the restricted group, were no longer significantly different.

Arginase was elevated in the restricted group after 10 days, specific activity by 50% in the restricted group and total activity by 20%. Some adaptation occurred after 20 days when the increases in specific and total activity were only 20 and 5%, respectively, non-significant with a slight non-significant fall after 31 days. The difference between total activity of this enzyme in the livers of the restricted groups at 31 and 10 days were significant ($p < 0.05$).

Experiment II

Oxygen consumption is shown in table V for the experimental group while being fed *ad libitum* ('0 days') and after the three periods on the restricted diet, together with the heat production calculated at 4.8 kcal/l oxygen consumed (9).

There was no change after 10 days on restricted diet, when the body weight had fallen by 6%. After 20 days there was a marked fall to 34% of initial oxygen consumption ($p < 0.001$) when body weight had fallen by 8.6%. In the final 11 days there was no further change in body weight and oxygen consumption persisted at the same reduced level.

Table V. Oxygen consumption and heat production of rats fed *ad libitum* or restricted diet (mean values)

	<i>Ad libitum</i>	70% food intake		
	0 days	10 days	20 days	31 days
Oxygen consumption, cm ³ O ₂ /min/kg ^{3/4}	7.4	7.0	4.9	4.8
Heat production, kcal/day	28.5	26.1	18.0	17.5
Energy intake, kcal-metabolizable/day	84.0	59.0	59.0	59.0

Discussion

There are many reports of adaptation to dietary restriction. For example *Infantellina and Ribino* (6) showed this in rats and pigeons, and *Rosenthal and Allison* (18) showed it in rats and dogs. *Waterlow* (20) reported that organs can be maintained at the expense of the muscles.

Measurement of N balance and body weight changes in the present experiments show that adult rats adapt to restriction of both dietary protein and of energy. The early loss of nitrogen indicates protein catabolism followed by a decrease in the extent of loss following adaptation, similar to that reported by *Rosenthal and Allison* (17, 18).

Administration of labelled methionine resulted in greater radioactivity in livers of rats fed restricted diets than those fed *ad libitum* and the reverse was found in the muscles. This was explained by *Waterlow* (20) who suggested that with inadequate dietary supplies of protein there is a concentration of protein synthesis in the internal organs at the expense of muscle and skin.

Such an effect could be mediated through cortisol and insulin which, in some respects, have reciprocal actions on protein metabolism. Cortisone appears to promote protein synthesis in the liver at the expense of peripheral tissues (13) while insulin promotes the uptake of amino acids by muscle (2, 10) but not by liver (19).

The maintenance of more essential tissues at the expense of muscle is, however, only part of the adaptive mechanism which would not account for the observed change from severe negative N balance to N equilibrium. This appears to be partly due to enzymic adaptation and partly due to a fall in overall metabolic rate. *Das and Waterlow* (3) showed amino acid conservation through a rapid adaptation of glutamic-pyruvic transaminase and arginase within 30 h of changing from high protein (135 g casein/kg diet) to low protein diet (45 g casein/kg) and the reverse.

In the present work glutamic-pyruvic transaminase increased after 10 days of dietary restriction but returned to normal after 31 days. Adaptation of arginase occurred after 20 days.

The loss in body weight took place during the first 10 days of dietary restriction; after 20 days the metabolic rate fell by 34%, a similar figure to that found by *Infantellina and Ribino* (6).

These various types of adaptation may help to explain how adult human beings appear to maintain health on dietary intakes well below the established physiological requirements. For example, *Nicol and Phillips* (14) showed that Nigerian adult males require about 30% less protein for maintenance than young North Americans. *Payne* (15) suggested that this probably reflects adaptation to a low habitual intake.

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