Excess energy intake promotes the development of hypoalbuminaemia in rats fed on low-protein diets

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1. A group of rats were given *ad lib.* a diet with a protein-energy: total energy (P:E) value of 0·03. Other animals received the same protein intake (g/kg body-weight per d) as this group, but had their energy consumption reduced to either 90, 80, 70, 60 or 50% of the *ad lib.* value.

2. The restricted growth rate of rats fed on the P:E −0·03 diet *ad lib.* has been shown to be due entirely to their insufficient protein consumption. In contrast, energy intake was far in excess of that required for maintenance and the limited amount of growth.

3. Carcass analysis demonstrated that some of the excess energy intake was stored as fat, but a greater part had been dissipated, presumably by diet-induced thermogenesis.

4. The plasma concentration of triiodothyronine (T_3) was elevated in all animals consuming excess energy and was significantly related to both the total surplus and the amount of energy dissipated.

5. In the group of animals restricted to 50% of the *ad lib.* intake, energy rather than protein appeared to be the factor limiting growth. Energy intake was below estimated requirements for maintenance and was associated with values for plasma T_3 that were lower than those found in well-fed control rats.

6. Although all the animals had similar protein intakes, plasma albumin concentration differed between the groups and was found to be inversely proportional to the energy intake. Thus it was lowest in animals receiving food *ad lib.* and rose to near normal values in the most-severely-restricted rats.

7. It is suggested that hypoalbuminaemia, and perhaps other features of protein deficiency, seen in animals fed on low-P:E diets may occur as an undesirable consequence of the metabolic response required to deal with excess energy consumption.

It has become generally accepted that rats offered diets of low-protein content voluntarily reduce their food intake and thus suffer from a shortage of energy as well as protein. It has been argued, therefore, that it is difficult to know whether the metabolic abnormalities seen in such animals are a result of the low protein-energy: total energy (P:E) value of the diet, or occur as a response to the voluntary food restriction. However, the findings of Coward *et al.* (1977) showed that food consumption, when expressed as a function of body-weight, was not reduced in rats maintained on diets of low P:E despite severely-restricted growth rates. Moreover, a theoretical estimation of energy requirements for maintenance and growth (using the values of Evans & Miller, 1968) suggests that far from being short of energy, the animals may be facing the problem of how to deal with a surplus.

The present paper describes a practical investigation into the relative deficiencies and excesses of protein and energy consumed by rats offered *ad lib.* a low-P:E diet. The relationship between these dietary constituents and plasma albumin concentration, an accepted index of protein deficiency, is also examined.

EXPERIMENTAL

Males from a specific-pathogen-free colony of hooded rats kept at the Dunn Nutrition Laboratory were used in the experiment. Fifty animals were weaned at 3 weeks on to a synthetic diet of P:E 0·20, on which they were maintained for a further 2 weeks. After this time, they were randomly assigned to six experimental groups of five and two control groups of ten. Rats in one of the control groups were immediately killed to provide baseline values for a carcass composition study.

Animals in experimental group 1 received a diet of P:E 0·03 *ad lib*. Those in groups 2–6 were given the same amount of protein as that consumed by group 1 rats (on a body-weight basis), but with progressively greater restrictions of energy. This was achieved by making up a series of isoenergetic diets of P:E 0·0333, 0·0375, 0·0429, 0·0500 and 0·0600 and feeding these at 90, 80, 70, 60 and 50 % of the intake of the group 1 animals respectively (see Table 1). The remaining ten control animals continued to be fed on the P:E −0·20 weaning diet. A general description of the basic dietary composition is given by Lunn *et al.* (1976).

After 14 d of these dietary treatments, the animals were killed by exsanguination under diethylether anaesthesia. The blood was weighed and heparinized plasma prepared and stored at −20° until analysed.

Carcass fat and fat-free dry weight (FFDW) were calculated from estimates of total body water measured directly by desiccation (for a review, see Sheng & Huggins, 1979). A correction was made for the weight of blood taken for analysis, which was found on average to contain 190 g solid material/kg. It was assumed that water constituted 732 g/kg fat-free wet weight of the animals, and that protein constituted 800 g/kg FFDW (Pace & Rathbun, 1945).

Triiodothyronine (T_3) was measured by radioimmunoassay using a kit obtained from The Radiochemical Centre, Amersham, Bucks. Plasma albumin was determined with bromo-cresol green (Northam & Widdowson, 1967).

RESULTS

Rats given the P:E 0·03 diet *ad lib*. ate, on average, 135·1 g food/kg body-weight per d. This contained 4·1 g protein, and the mean (±SE) weight gain over the 14 d experimental period was 0·74±0·13 g/d, a value much lower than that of control animals, 5·43±0·09 g/d. Table 1 shows, however, that so long as the protein intake was maintained at 4·1 g/kg body-weight per d, energy consumption could be reduced to 60 % of the *ad lib*. intake with only a small and non-significant further reduction in growth rates. A more pronounced and significant fall in growth was observed between groups given 60 and 50 % of the *ad lib*. energy intake.

Carcass analysis showed, however, that some changes in body composition had occurred (Table 2). FFDW gain was not reduced by lowering energy to 60 % of *ad lib*. intake; in fact animals in groups 3, 4 and 5 showed slightly better gains than those seen in group 1. Only in the most-severely-restricted animals (group 6), was a mean reduction in FFDW observed. In contrast, there were marked differences in carcass fat content. In addition to the gain which could be attributed to growth, rats in groups 1–4 showed an increased proportion of body fat. Expressed as a percentage of body-weight, fat was found to have increased by (mean±SE) 4·9±0·9, 4·3±0·8, 1·4±0·8 and 0·1±0·6 in groups 1–4 respectively. In groups 5 and 6, some reduction in body fat stores was evident.

Balance studies

In order to obtain a clearer picture of the relative deficiencies and excesses of the various diets, an examination of the protein and energy balance of the animals was carried out. The results are presented in Table 3 but, in order to calculate the results, a number of assumptions were necessary and were as follows. To obtain the carcass energy content, fat was assumed to represent 39·3 kJ/g and FFDW 18·8 kJ/g (Canolty & Koong, 1976). Maintenance requirements for nitrogen and energy for the strain of rats used, and under similar experimental conditions, have been found to be $243 \times$ body-weight $(W)^{0.75}$ mg N/d and $586 \times W^{0.75}$ kJ/d respectively, and the cost of energy deposition to be 10·8 kJ/kJ deposited. Protein was calculated as $N \times 6.25$.

The protein balance results confirm that the low consumption of this nutrient was

Table 1. *The effect of energy restriction on weight gain of rats receiving a constant level of protein*

Group no.	Protein intake (g/kg rat per d)	Energy intake (kJ/kg rat per d)	Protein-energy: total energy	Wt gain (g/d) Mean	SE
1	4·1	2108	0·0300	0·74	0·13
2	4·1	1897	0·0333	0·73	0·22
3	4·1	1686	0·0375	0·65	0·09
4	4·1	1475	0·0429	0·62	0·04
5	4·1	1264	0·0500	0·46	0·07
6	4·1	1054	0·0600	−0·19	0·31*
Control	23·4	1953	0·2000	5·43	0·9

* Value significantly different from group 1 value: $P < 0.05$ (Student's t test).

Table 2. *Changes in carcass composition in response to dietary treatments*

Group no.†	Body-wt (g) Mean	SE	Body water (%) Mean	SE	Carcass FFDW (g) Initial‡	Final	Change Mean	SE	Carcass fat (g) Initial‡	Final	Change Mean	SE
1	109·2	1·4	65·3	0·7	25·0	26·1	1·1	0·3	5·85	10·87	5·02	0·63
2	111·4	3·0	65·4	0·4	26·2	27·1	0·9	0·1	6·03	11·45	5·42	0·85
3	108·2	3·2	67·9	0·5*	25·0	26·9	1·9	0·3	5·90	9·04	3·14	0·23*
4	112·8	2·4	68·9	0·5***	26·3	28·5	2·2	0·1*	6·10	6·74	0·64	0·72***
5	108·2	2·8	70·2	0·6***	25·7	27·8	2·1	0·1*	5·95	4·54	−1·41	0·89***
6	113·2	1·9	70·0	0·5***	28·3	28·3	−0·2	1·0	6·61	4·84	−2·03	0·75***

FFDW, fat-free dry weight.
Values significantly different from group 1 value; * $P < 0.05$; *** $P < 0.001$ (Student's t test).
† For details of groups, see Table 1.
‡ Initial values for FFDW and fat were calculated from the weight of individual animals at the start of the experiment and the carcass composition values from control animals killed at this time. In these control animals mean ($\pm$ SE) values obtained were (/kg body-weight) body water 69.07 ± 0.15, FFDW 25.22 ± 0.06, fat 5.85 ± 0.22.

responsible for the limited growth of most of the rats. Protein excess, defined as dietary protein not used for either maintenance or growth, was close to zero in animals of groups 1–5, but in group 6 rats, 1·8 g of protein could not be accounted for in this way. Rats in groups 3, 4 and 5 seemed to be more efficient in protein utilization than those fed *ad lib*. On the other hand, the results show clear evidence of excessive energy intakes in all but the most-severely-restricted group. As the percentage of fat in the carcass of rats in groups 1–4 had increased above the baseline value of 5.85 ± 0.22, the energy represented by this rise has been added to that dissipated to give the total excess energy eaten. For example, it can be seen that animals fed on the P:E 0·03 diet *ad lib*. consumed on average 2955 kJ energy, of which 452 kJ was deposited as fat and 879 kJ dissipated. As the energy intake was reduced, this surplus decreased until eventually, in group 6, consumption was in fact less than the estimated maintenance requirement.

A further indication that the high energy consumption of these animals was an excess, and not an extra requirement for maintenance at times of low protein intake was provided by the thyroid-hormone T_3 results, which are shown in Fig. 1. Plasma concentrations of

Table 3. *Protein and energy balance study†*

Group no.‡	Total food (g) Mean	SE	Protein (g) Food	Maintenance	Growth	Protein excess (g) Mean	SE	Energy (kJ) Food	Maintenance	Growth	Fat stores Mean	SE	Energy excess (kJ) Dissipated Mean	SE	Total Mean	SE
1	190	12	5·96	3·90	0·89	0·90	0·11	2955	1502	121	456	50	875	180	1332	188
2	159	3	5·31	3·98	0·72	0·60	0·07	2481	1536	96	502	71	351	54*	849	21*
3	140	4	5·27	3·88	1·52	0·13	0·21**	2188	1498	130	197	67*	364	67*	560	21**
4	131	3	5·60	4·01	1·76	−0·17	0·14***	2034	1532	121	50	54***	331	84*	381	46***
5	110	2	5·51	3·99	1·68	−0·16	0·13***	1716	1486	59	—	—	159	33**	171	33***
6	98	3	5·91	4·11	—	1·80	0·71	1532	1586	—	—	—	—	—	—	—

Values significantly different from group 1 value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's *t* test).

† See under *Balance studies* for assumptions made in order to construct this table.
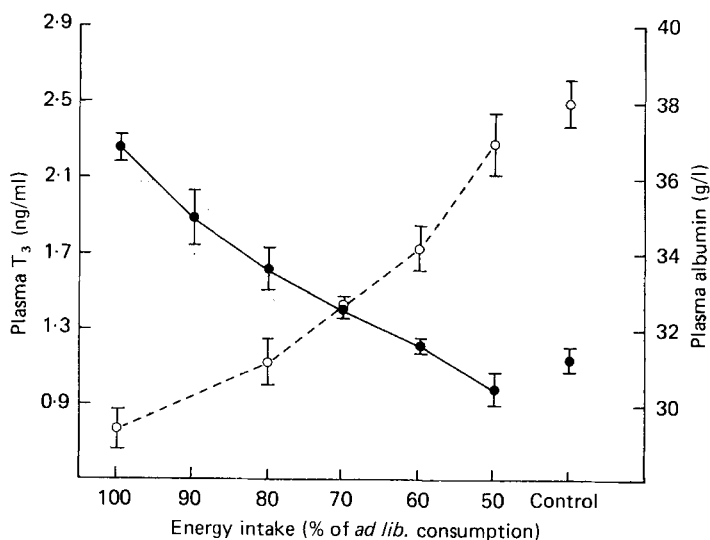
‡ For details of groups, see Table 1.

Fig. 1. Relationship between plasma T_3 and albumin concentrations with energy restriction at constant protein intake. Values are means and their standard errors for groups of five rats. ●——●, T_3; ○---○, albumin.

this hormone in rats of groups 1–5 were elevated above those seen in normal well-fed animals of similar age. The highest values, associated with both the greatest dietary energy excess and the highest rate of energy dissipation was seen in group 1, and concentrations fell as the energy surplus was decreased by dietary restriction. In group 6 rats, the plasma T_3 value was below normal and was thus in keeping with the primary energy deficiency indicated by the balance study.

A highly-significant correlation was found between plasma T_3 concentration and the amount of energy dissipated (r 0·78, $P < 0·001$), but the relationship between the hormone level and total energy excess was even stronger (r 0·91, $P < 0·001$).

Fig. 1 also demonstrates an important relationship between plasma albumin concentration and the level of energy restriction. Although there were no differences in over-all growth rates or gain in FFDW of rats in groups 1–5, plasma albumin concentration increased as energy consumption decreased, despite the constant protein intake. The highest albumin concentration was seen in rats restricted to 50% of the *ad lib.* energy intake, and this was not significantly different from the mean ($\pm$ SE) value of 38·0$\pm$0·6 g/l observed in well-fed control animals.

DISCUSSION

The results demonstrate that the restricted growth of rats given *ad lib* a diet of P:E 0·03 was due entirely to an insufficient protein intake. Furthermore, the results show that, on the level of protein eaten by these animals, i.e. 0·41 g/d per kg body-weight, energy consumption could be restricted to at least 60% of the *ad lib.* intake without reducing growth rates, or the gain in FFDW.

The explanation for the absence of an effect of energy reduction can be seen in the balance study where it is clear that rats in groups 1–5 were all consuming energy in excess of their requirements despite the 'restricted' intakes of groups 2–5. Group 1 animals, for example, ate 2955 kJ food during the 14 d experimental period, but only 1623 kJ, i.e. approximately 55% of this was actually needed for maintenance and the limited amount of growth allowed

by the low protein intake. Thus energy consumption in these animals was approximately 82% in excess of requirements so, clearly, even a 60% reduction of this level of intake (group 5 animals) would still provide more than adequate amounts of energy. Such high intakes could be expected to result in raised body fat stores and indeed an increase in the proportion of body fat was observed in the rats of groups 1–4. These rises, however, could only account for part of the excess energy consumption. Again using group 1 as an example, 34% of the surplus energy had been utilized in this way, and the remainder dissipated presumably by the mechanism of diet-induced thermogenesis (Miller & Payne, 1962; Stirling & Stock, 1968).

The fact that rats fed on protein-deficient diets do have energy surplus to their requirements makes the elevated plasma T_3 concentration, which we and others (Edozien *et al.* 1978; Tulp *et al.* 1979) have observed in such animals, more understandable. Plasma levels of this hormone invariably rise during hyperphagia, and there is evidence to indicate that it plays some part in stimulating thermogenesis (Danforth *et al.* 1979). Our results suggest that the plasma T_3 concentration does in fact give a good indication of the excess energy eaten and the amount eventually dissipated. Only in the most-severely-malnourished animals was the plasma T_3 value below the level seen in well-fed controls. Rats in this group differed from the others as they lost both body-weight and FFDW during the experimental period. Moreover, the balance study indicated that energy, rather than protein, had become the primary deficiency limiting growth. This result is therefore in keeping with previous reports that reduced plasma T_3 levels occur under conditions of dietary energy restriction (Jung *et al.* 1980).

It can be seen in Fig. 1 that plasma T_3 concentration would reach the level found in control animals at a level of energy restriction between 50 and 60%. This would clearly be very close to the point at which there would be no dietary energy excess, and where the relative extents of protein and energy restriction were equal.

A correct knowledge of the energy status of animals fed on diets of low P:E is important as it makes a number of observations more easily understood, e.g. the reduction in gluconeogenesis in protein-deficient rats reported by Heard *et al.* (1977) is to be expected as the pathway is clearly not needed. However, the results are of greater importance in defining the impact of energy intake on the appearance of classical symptoms of kwashiorkor. Although dietary protein consumption remained constant, plasma albumin concentration varied with energy intake. It was lowest when the food contained the greatest excess of energy, and increased towards normal as this surplus was reduced by dietary restriction. Similar changes in albumin levels have been reported previously in rats receiving 50% of *ad lib.* intakes of low-protein diets (Coward *et al.* 1977), but those studies showed that other metabolic features characteristic of protein deficiency were also improved by this treatment. Plasma amino acids returned to a more normal pattern, the non-essential:essential amino acid value decreased and evidence of inappropriate body protein distribution, i.e. 'dysadaptation' (Gopalan, 1968), disappeared. More recent results show that the rate of albumin synthesis was higher in hepatocytes isolated from rats fed on low-protein diets in restricted amounts than from those fed on the same diet *ad lib.* (J. E. Smith & P. G. Lunn, unpublished results). The suggestion made by Coward *et al.* (1977) that the reason for these changes was a switch from primary protein deficiency in the *ad lib.* animals to primary energy deficiency in the 50%-restricted group has been confirmed by the present results. It is also known that rats fed on reduced amounts of adequate-protein diets do not show metabolic features of protein deficiency (Lunn & Austin, 1982).

The slight but significant improvement in protein utilization when energy intake was reduced to 80, 70, or 60% of the *ad lib.* consumption was an unexpected finding. It is possible

that this effect occurred because of an increased requirement for protein to deal with the excessive energy intake of the *ad lib.*-fed animals, or by an unavoidable increase in amino acid oxidation as a result of the high rate of thermogenesis. It is, nevertheless, also feasible that this small difference is an artefact resulting from one of the assumptions made in estimating the carcass composition. There is good evidence that the calculation of body fat and FFDW from measurements of body water remains valid over a wide range of conditions, e.g. in normal rats of differing weight (Rothwell & Stock, 1979), in lean and obese mice (Jagot *et al.* 1980) and in protein-deficient and starved rats (Mendez & Kollias, 1977). However, the assumption that the proportion of protein in FFDW also stays constant under various conditions is probably not entirely correct and could thus be a source of error. Consequently, it would be unwise to draw any firm conclusions from this particular result without confirmation of the findings by direct body protein measurements.

Although the plasma concentrations of T_3 and albumin both change in response to the energy intake, the results do not indicate any cause and affect relationship, but such a possibility cannot be ruled out at present. Thyroid hormones do stimulate muscle protein synthesis (Brown *et al.* 1981) and in this respect have an effect similar to insulin, but the effects of the two hormones on protein degradation are probably different (Jefferson *et al.* 1977; Flaim *et al.* 1978). Moreover, low plasma albumin concentrations are usually associated with low, not high, thyroid hormone values (Peavy *et al.* 1981 *a, b*). Thus further work is clearly required to show whether the changes in T_3 concentration can be implicated in the same way as insulin and the corticosteroids in the development of hypoalbuminaemia. Nevertheless, whatever the mechanism proves to be, it seems that a major part of the reduction in plasma albumin and, perhaps, the appearance of other abnormalities seen in animals fed on the low-protein diets develop as a consequence of the metabolic response to excess dietary energy.

This rat model clearly provides considerable support for the traditional explanation of the aetiology of kwashiorkor (Whitehead & Alleyne, 1972; Coward & Lunn, 1981). The results are in keeping with the more frequent occurrence of this form of protein-energy malnutrition in those areas of the world where energy foods are in plentiful supply relative to protein food availability.

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