Letters to the Editors

Thyroid status and metabolic rate in protein-deficient rats

Lunn & Austin (1983) proposed that the hypoalbuminaemia of protein deficiency results from 'dysadaptation' which in turn reflects the hyperthyroid state due to the excess dietary energy which follows the consumption of protein-deficient diets. In the protein-deficient rat there can be inverse changes between free and total triiodothyronine (T_3) due to an increased binding capacity for T_3, and plasma free and not total T_3 reflects the metabolic rate (Cox *et al.* 1981, 1984; Smallridge *et al.* 1982; Young *et al.* 1982). Sawaya & Lunn (1985) argue that an increased total T_3 in protein-deficient rats does indicate a hyperthyroid state and an increased metabolic rate, since it is accompanied by increased free T_3 as well as other hyperthyroid and thermogenic indices. We have compared the kit used by Sawaya & Lunn (1985) (Diagnostic Products) with a second kit (Lepetit) to measure free T_3 in rats (Table 1). As shown, the two kits gave quite different results. Only the Lepetit kit gave values which reflected the changes in the metabolic rate.

Table 1. *Free T_3 in plasma from protein-deficient rats as measured by two different assays*

Treatment (g protein/kg diet)	Wt (g)	Oxygen consumption* (litres/d per kg body-wt)	Free T_3 (pg/ml)	
			Lepetit†	Diagnostic‡
200	81	67·8	5·9	1·29
50	87	46	4·0	2·27
50+T_3	96	73	10·5	2·3

* Measured as described in Cox *et al.* (1984).
† Lepetit, distributed by Metachem Diagnostics, 29 Forest Road, Piddington, Northampton NN7 2DA.
‡ Diagnostic Products (UK) Ltd, Wallingford, Oxon OX10 0EL.

The Diagnostic kit uses a labelled T_3-analogue in the radioimmunoassay which does not bind to human thyroid-binding globulins. If this T_3-analogue binds to the rat T_3-binding globulins, then any increase in T_3-binding proteins should result in an erroneous apparent increase in measured free T_3, as found. However, this would not explain why all results are much lower than those found with the Lepetit kit which separates free T_3 on a column before the radioimmunoassay and is insensitive to the binding proteins present in the serum. Furthermore, it indicates similar values to those obtained by equilibrium dialysis of rat plasma from protein-deficient rats (Smallridge *et al.* 1982).

Given the variation in the response of rats to protein-deficient diets between laboratories, our finding of a decreased metabolic rate in rats on such a diet should not detract from the findings of Sawaya & Lunn (1985) that there was an increased oxidative capacity of brown adipose tissue in the protein-deficient rats suggesting an increased metabolic rate although they did not measure it on this occasion. However, the increased activities of hepatic thyroid-related enzymes cannot be used as an indicator of overall thyroid status since (*a*) increased total T_3 could induce increased hepatic intracellular free T_3 because of uptake of the bound hormone into the liver by endocytosis (Pardridge & Mietus, 1980) and (*b*) since the deiodination of thyroxine occurs at several sites, 'thyroid status' varies between tissues (Silva *et al.* 1978). In any case the resolution of the theories of Lunn and co-workers relating hypoalbuminaemia to thyroid status and increased thermogenesis requires the adoption of a better assay for free T_3 as well as, in the case of this recent study, an actual measurement of the metabolic rate.

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Reply to letter by Cox & Millward

We do not accept the criticisms of Cox & Millward concerning our paper (Sawaya & Lunn, 1985) for the following reasons.

The question of which assay of free triiodothyronine (T_3) gives the correct value cannot be decided by discussion and until tests can be made must remain a matter of opinion. We have, however, no reason to doubt our results as they are in keeping with other indices of thyroid status in our animals. We would distrust any assay which depended on a physical separation of one component of a system in equilibrium as such measurements will inherently give values which are higher than the true levels. Moreover, in the Lepetit assay, a reduction in the rate of dissociation of protein-bound T_3 due to the increase in binding affinity which seems to occur in protein-deficient animals could result in the apparent fall in free-T_3 concentrations quoted in Cox & Millward's letter.

It was precisely because free T_3 measurements must be treated as potentially unreliable that physiological evidence of increased thyroid activity was sought and found in the protein-deficient rat. Despite the findings of Pardridge & Mietus (1980) suggesting that protein-bound T_3 can enter the liver, the hepatic enzymes and shuttle systems which we investigated are still recognized as being indicative of increased thyroid activity (Muller & Seitz, 1984; Nauman *et al.* 1984) and to our knowledge the criticism that this might not be the case has never previously been made. In any case, changes consistent with a metabolic hyperthyroidism are not restricted to the liver. T_3 undoubtedly plays a role in brown adipose tissue thermogenesis (Himms-Hagen, 1983; Sundin *et al.* 1984) and the increasd thermogenic activity which occurs in this tissue when rats are given a diet with a low protein energy: total energy (P: E) value is delayed by thyroid blockade (A. L. Sawaya and P. A. Lunn, unpublished results) and abolished by thyroidectomy (Tulp & Krupp, 1984). Elsewhere, increases in Na^+K^+-ATPase, another thyroid-sensitive enzyme, have been observed in various tissues (Pimplikar & Kaplay, 1981) and Tulp *et al.* (1979) have found evidence of raised thyroid hormone synthesis. All reports agree that total plasma T_3 values are increased.

The picture appears to be closely analogous to that seen during energy, particularly carbohydrate, hyperphagia and is thus in keeping with the observation that rats fed on diets of low P: E value, perhaps in an attempt to satisfy their protein requirements, eat excess amounts of food and have to cope with a considerable energy surfeit (Lunn & Austin, 1983). Under such conditions a hyperthyroid state is to be expected.

In contrast to the results of Cox & Millward, most workers in fact report an increase in resting metabolic rate in rats fed on protein-deficient diets and an associated increase in food consumption (Tulp *et al.* 1979; Balmagiya & Rozovski, 1983; Swick & Gribskov, 1983). However, we suggest that oxygen consumption measurements can at best give only a superficial assessment of energy status as the method is unable to differentiate between different components of energy utilization, i.e. maintenance, growth and thermogenesis. In the present context this failing makes the results quite misleading. For example, the energy cost of 7·6 g/d growth in body-weight (value from Cox *et al.* 1984) is about 80 kJ/d. For a rat of 81 g, this represents about half of its total energy expenditure and an even greater proportion of its resting metabolic rate. In the non-growing protein-deficient animal, the energy cost of growth is of course zero, so before oxygen consumption figures can be meaningfully compared between the groups a correction must be made for this difference in growth. Such a correction would at least halve the value quoted by Cox & Millward for well-fed animals and in consequence would show the protein-deficient animals to have the higher metabolic rate. Because of the high proportion of energy used in growth it is quite possible for a non-growing protein-deficient rat to have an energy surfeit and a high rate of diet-induced thermogenesis but show a resting metabolic rate below that of a well-fed, growing counterpart. Because of these difficulties in interpretation of resting metabolic rates we have always preferred to use comparative carcass analysis and measurement of food consumption to assess energy status in our animals.

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Letter from Schweizer & Würsch

In a recent article in your journal, Millard & Chesson (1984) criticize several methods for the measurement of dietary fibre. In particular, these authors find the methods inadequate for measuring microbially degraded fibre material collected at the terminal ileum of pigs. Millard & Chesson seem to overlook the fact that dietary fibre has invariably been defined to include food constituents and that methods such as ours (Schweizer & Würsch, 1979) have therefore been designed to measure dietary fibre in food, hence they may not be adequate for measuring microbially degraded dietary fibre. Their comparison of the analytical methods is misleading, even assuming that their reference values are the best ones and that the methods considered are applicable. Initially, the authors should base their comparisons on the sum of soluble and insoluble constituents since the separation is done in different conditions in each method considered.

As far as our own method is concerned (Schweizer & Würsch, 1979), it reflected uronides in digesta samples quite adequately, namely 92% of Millard & Chesson's value (Table 6). In contrast to what the authors state on p. 590, the overall recovery of 417 g/kg digesta obtained by our method would be more satisfactory than the one obtained by the extraction methods. However, as we suspect that the extremely high values for phenolics found by the authors may be an artefact, we prefer to base the comparison on carbohydrate constituents only. In this case, the recovery is 409 g/kg digesta which is 89% of the authors' 'reference value'.

In their discussion of the comparative feed fibre analysis in Table 5, the authors claim that our method 'overestimated all the main fibre components' although it resulted in 281 g carbohydrate constituents/kg feed compared with the 305 g/kg given as reference value. Obviously, both these values will be higher than the 187 g recovered from digesta in view of the fibre degradation occurring anterior to the terminal ileum. Similarly, they are inconsistent when they say that our method recovered more cellulose from digesta than was present in the feed. Seemingly, the 126·1 g in Table 5 are not very different from the 121·5 g in Table 1. Why does this latter value decrease to 105·9 g in Table 5? In addition, the 200 g in Table 6 seem to be close to the value of 202 g preferred by the authors.

In conclusion, we feel that the authors are not justified in attempting to disqualify analytical methods either in a context for which they have not been designed (digesta samples) or based on the questionable assumption that their preferred method gives the true dietary fibre contents (feed samples). In addition, some examples of the authors' comments which are not consistent with the corresponding figures are shown above. We certainly welcome any contribution towards a better understanding of the physiological action of dietary fibre, and we realize the need for measuring microbially degraded fibre in addition to food fibre. However, we think that a study on two single pigs and one single fibre source is an insufficient basis to justify the various statements of Millard & Chesson concerning dietary fibre analysis.

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Reply to letter by Schweizer & Würsch

We accept that the methods of dietary fibre analysis, including that of Schweizer & Würsch (1979), cited in our article (Millard & Chesson, 1984) were designed solely to measure the dietary fibre content of food. We are not attempting to disqualify these methods on the grounds of their methodology, nor do we seek to supersede them with our own analytical methods, which were simply an attempt to measure all components of food and digesta without prejudging what constitutes dietary fibre in these samples. What we are seeking to stress is that analysis of fibre in food is not capable of providing a sufficiently adequate description of fibre or fibre components in the gut which can be related to their physiological effects.

Soluble and insoluble fractions of dietary fibre differ in the extent to which they are modified in the digestive tract. This makes it difficult to accept the suggestion of Schweizer & Würsch (1979) that our comparisons should have been based on the sum of the soluble and insoluble components. The fact that the fractionation methods used in the various methods we examined differed, and produced different results, was the point of the comparison. It is true that the method of Schweizer & Würsch (1979) adequately reflected the total uronide content of the digesta sample, but it also produced a very different distribution of uronide between the soluble and insoluble fractions compared to our own 'reference method' (Table 6).

It also seems to us arbitrary to choose to discount the phenolic content of food. This represents a potentially highly reactive dietary component which might be expected to contribute directly or indirectly to the physiological effects of fibre in the gut. Similar levels of total phenolics have been found for a large number of swede samples (Millard *et al.* 1984) and others have shown that swede roots contain a variety of phenolic compounds (Rhodes & Wooltorton, 1973). There seems no reason to suppose that our values are an artefact.

Schweizer & Würsch (1979) compare results presented in Tables 1 and 5, pointing to a number of apparent inconsistencies. The values presented in Table 1 are the means of nine experiments, while, as is clearly stated in the text (p. 588), those shown in Table 5 derive from a single sample of feed and its corresponding digesta. The results in these tables though similar, are not directly comparable.

In their letter Schweizer & Würsch claim that we are not justified in attempting to disqualify analytical methods in a context for which they were not designed. In this we concur; we do not question the methods of fibre analysis applied to foods. We do, however, question the context in which they are considered and, more importantly, the biological significance ascribed to the results they produce.

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