Letters to the Editors

Plasma insulin, 3,5,3′-triiodothyronine concentrations and muscle growth in the rat

In a recent publication by Jepson *et al.* (1988) the effect of dietary protein and changes in the plasma concentrations of insulin, total and free 3,5,3′-triiodothyronine (T_3) were measured in relation to the control of muscle growth in the rat. However the fact that a decrease in muscle growth rate occurred between the baseline group (BL) of rats (killed after a 4 d period of acclimatization to animal-house conditions) and those fed on a similar diet but killed 10–16 d later (AC 180; fed on 180 g casein/kg) was completely ignored by these authors. This point is particularly important given the correlation Jepson *et al.* (1988) illustrated between plasma insulin concentration and both the rate of protein synthesis and growth of muscle. Therefore if the BL rats were included in Figs. 5, 6 and 7 in this paper, it would obscure any such relations given that the mean plasma insulin concentrations in these groups ranged from 18·6 to 41·0 ng/ml and the mean rate of muscle growth was 8·0 to 10·0 %/d.

Muscle growth was 50% lower in the AC 180 group compared with the BL rats (BL mean muscle growth 9·3 %/d *v.* AC 180 mean muscle growth 4·9 %/d). This effect was primarily due to a fall in the rate of protein synthesis and indicates that the growth of these rats was actually falling as a result of long-term adaptation to the diet. Consequently the positive relation between plasma insulin and both protein synthesis rate and muscle growth is not maintained if the BL values are included. Furthermore Jepson *et al.* (1988) state that this relation is for rats of similar ages, which is not the case given that M1 rats were studied for 16 d compared with 10 d in groups M3 and M4. A possible explanation for these effects is a change in insulin levels with age, although at no point in this paper are the ages of the animals given. This is crucial if one is to obtain a complete understanding of this paper given the physiological and metabolic adaptations occurring following weaning (see Henning, 1981).

It is also possible that a difference in the strain of the rats between groups M1, M2 (Charles River, Margate, Kent) and M3, M4 (Olac, Bicester, Oxon) may explain the occurrence of hyperphagia in response to low-protein diets in both M3 and M4 groups. The mean plasma total T_3 concentration was 41% higher in the M3, M4 groups (1·66 ng/ml) compared with M1, M2 groups (1·18 ng/ml) fed on 180, 80 or 45 g casein/kg diet. Furthermore, the reason why the M2 rats fed on 80 g casein/kg grew 260% faster than M1 rats consuming the same amount of diet may be because of their 17% heavier body-weight at the start of the experimental period. This indicates an increased age of the M2 rats and more efficient utilization of feed for growth purposes.

An alternative conclusion to the results of Jepson *et al.* is that plasma insulin concentrations are only positively correlated with protein synthesis in the rat under very limited conditions, such as during the initial response to re-feeding following a 3–4 d fast (Millward *et al.* 1988) when animals of the same body-weight and strain are studied. However, plasma free T_3 concentration appears to be positively related to both the rate of protein synthesis and degradation irrespective of age of the rat. Jepson *et al.* (1988) failed to show if free T_3 levels were actually related to muscle growth, hence the significance of this result also remains in doubt.

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Diet–hormone interactions and muscle growth

Dr Symonds expresses concern over several points raised in our recent paper on diet–hormone interactions in muscle growth, in which we analysed the statistical relations between the intakes of protein and energy, plasma insulin and 3,5,3′-triiodothyronine (T_3), and the determinants of muscle protein turnover, in rats given various low-protein diets (Jepson *et al.* 1988).

Dealing first with the minor points, Dr Symonds suggests possible explanations for the variable hyperphagia of our animals given the protein-deficient diets, raising the question of strain differences as one explanation. As we argued in the paper, strain differences did not entirely explain the response, and subsequent studies with rats from both suppliers have also involved variable responses which are independent of age and which remain inexplicable. He also pointed towards differences in plasma total T_3 levels as possible indicators of strain differences which might relate to the hyperphagia. As we argued in this and previous papers (Cox *et al.* 1984), the rat exhibits increases in thyroid-binding proteins when fed on protein-deficient diets which increase total circulating T_3 without influencing free T_3, metabolic rate or muscle protein turnover. Another minor point related to the fact that we did not report the relation between T_3 and muscle growth. As clearly stated in the paper, we deliberately chose not to do this since in the data set analysed, which only included the rats of the same age, there was a non-linear relation between muscle growth and plasma T_3 (as there was with insulin), with a plateau at high hormone levels. For this reason, the fractional growth rate was not included in the partial correlation analysis.

However, the major concern of Dr Symonds appears to relate to what he perceives as data selectivity in the correlation analysis of our results, arguing that we omitted data obtained from our baseline groups (animals examined at the start of the dietary treatments) which we ought to have included. He points out that because of the higher growth rates and lower insulin levels in these animals, their inclusion in the analysis would have negated our conclusions about the relative importance of insulin in the regulation of muscle growth and protein turnover. This is indeed the case. As we stated in the paper, plasma insulin increases with age in the rat, the change being particularly marked in the few weeks after weaning, and reflects a decrease in insulin sensitivity with age. For this reason, the analyses were limited to the animals at the end of the treatment, when age differences were a maximum of 3 d, and when we could consequently expect differences in insulin levels to have functional importance.

As for the significance of the decrease in muscle protein synthesis and consequent growth rate in the control rats which occurred during the course of the experiments, which we 'completely ignored', according to Dr Symonds, this involves reduced growth as a result of long-term adaptation to the diet. Dr Symonds is on somewhat dangerous ground here. In fact, had our control rats in group M1, which started at 74 g body-weight, continued growing at their initial growth rate of 10%/d, at the end of the 16 d of the experiment they would have attained 366 g, rising to 1·5 kg after a further 2 weeks and achieving about

10^{16} kg after a year. Fortunately rodent growth is not exponential. In fact, the *absolute* growth rate did not fall with age. It *increased* (in group M1 from 7·4 to 9·2 g/d) during the experiments. Fractional rates can be appropriate descriptors of growth especially when growth rates are related to rates of protein synthesis and degradation expressed as fractional rates in the context of discussions of regulation. However, when a fractional growth rate is used, a fall with age in the rate will be inevitable because of the fact that body-weights increase towards a plateau value and not, fortunately, exponentially. Thus the fall in the fractional growth rate is absolutely as expected and there is no reason whatsoever to suggest any adaptive growth inhibition on our control diets.

Dr Symonds' own conclusion is that plasma insulin is only correlated with protein synthesis under very limited conditions, such as demonstrated in our previous fasting–refeeding studies (Millward *et al.* 1983, 1988). We would not disagree with this, having shown that in the catabolic states induced by glucocorticoids (Odedra *et al.* 1983) or endotoxins (Jepson *et al.* 1986) plasma insulin and muscle synthesis are not correlated because of the insulin resistance of muscle protein synthesis induced by these conditions. The important point of our present studies is that in rats of similar ages, in the absence of anti-insulin factors, insulin (and T_3) can be shown to have a key role in the mediation of the anabolic influence of dietary protein on muscle growth and protein turnover.

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