MANIPULATION OF ENERGY AND PROTEIN METABOLISM

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Summary.

The effects of variation of dietary energy and protein on normal growth rate are well established particularly for non-ruminant domestic animals. The effects of exogenously applied hormones and hormone analogues on protein deposition are also well recognised, but less attention has been given to the possibility that hormones may affect the way that available energy is directed. This review is focussed on energy partitioning and its interaction with protein metabolism. Although we have concentrated on effects of steroids and cationic amines which represent our primary research interests, this topic may be equally relevant to the actions of a number of other hormones.

I. INTRODUCTION

It is striking how attitudes to growth differ according to discipline. Thus for nutritionists the significance is primarily how availability of food dictates growth. They tend to take for granted that an animal has an adequate internal 'environment' and assume that limitation of growth will be determined by the quantity and quality of food that is eaten. For endocrinologists it is assumed that food is not limiting; they are concerned with whether the hormonal environment is optimal. For them stunting is a matter of some hormonal imbalance. For the molecular biologist, the controlling events are the expression of genes - if growth is less than optimal this can only be because of a lack of transcriptional (or perhaps translational) control. The neurologist will surely say that it is all determined by the way appropriate neuronal pathways are laid down. Perhaps if one considers also human nutrition there are additional possibilities - the psychologist may take the view that food intake is determined by whether it is an alternative to mother-love; or perhaps to sex! Although these comments may be a caricature of real life they do serve to emphasise that study of growth may at times be restricted by the 'training' blinkers with which one is equipped.

II. NUTRITIONAL LIMITATIONS TO GROWTH

These observations were prompted by considering how growth may be stimulated. In general the first limiting nutrient is energy. In studies with pigs, Dunkin et al. (1986) have shown clearly to what extent this is so; they also showed how protein and energy interact. Given these limitations on effects of increasing supply of energy and protein how do hormones stimulate growth? While they may stimulate appetite, it is clear that this is not a general phenomenon. There are very simple models that one may devise for the growth of pigs.(see e.g Whittemore 1976). One takes the energy content of nutrients absorbed, assesses the amount required for maintenance and the rest was 'available'.One then estimates the amount of the available protein that can be used, based on the biological value. The surplus is oxidised, and one can then estimate how much of the surplus energy is needed for the deposition of the protein; the remaining surplus energy is used to lay down fat. Alternatively, one may assume there is a fixed relation between protein and fat laid down. It is not hard with either assumption to show how such approaches are unduly simplistic. Yet they can actually give a fair approximation to normal growth.

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III. HORMONAL CONTROL OF GROWTH

The mechanism by which hormones act to affect growth has usually been focussed on the way they might alter protein deposition - a stimulation of protein synthesis being the most generally favoured. However, it is attractive to consider also that they might affect energy utilisation - they could reduce maintenance needs; or the efficiency of nutrient deposition. It was inevitable that we contemplated this proposition in considering how to stimulate growth of cattle in the tropics. Growth is sporadic - it can even be negative in the dry season; nutrient availability is frequently limited. Thus any kind of successful endocrine manipulation would need to improve the efficiency of utilisation of nutrient energy.

(a) Action of steroids

We examined first the possibility of altering basal metabolic rate. Hunter and Vercoe (1987) were able to show that the growth promoter trenbolone could reduce the basal metabolic rate of cattle by 12-15%. One consequence for cattle eating poor quality tropical pasture was a decrease in voluntary food intake. This is the result of a decrease in the concentration of rumen ammonia due to a decrease in blood urea recycling to the rumen. It could be eliminated by supplying urea, while the decrease in metabolic rate remained. It has also been shown that when trenbolone-treated animals are given fodder inadequate to maintain their body weight, their loss of body weight (over 7-8 weeks) is appreciably less than control animals matched for food intake.

In contrast when some naturally occurring growth promoters were examined, it was found that neither oestrogen (Hunter and Vercoe 1988) nor testosterone (Hunter 1989) were able to modify basal metabolic rate. The finding with oestrogen is supported by a study by Rumsay and Hammond (1990) in which they studied the growth stimulation of cattle by oestrogen or diethylstilboestrol at three values for energy intake. Only with a substantial energy intake was there any increased deposition of energy or nitrogen over control animals. The finding with testosterone is perhaps not surprising, since there is some indication that bulls have a significantly higher basal metabolic rate than steers (Webster et al. 1977). However, in our studies there was no appreciable difference either way in basal metabolic rate due to testosterone. This suggests that if bulls have a greater basal metabolic rate than steers it is not directly due to elevated circulating testosterone. It is perhaps also worth observing that testosterone is an extremely powerful growth promoter in cattle - when given in large dose it can double weight gain, so that cattle on a lucerne diet can gain at up to 1.5 kg/day -as good as growth in a feedlot (O'Kelly 1985; Hunter 1989)

Trenbolone was originally selected as having potential as an energy saver because there was some evidence that it was unusual in stimulating deposition of protein by reducing the rate of protein degradation, rather than stimulating protein synthesis. Thus for a given rate of gain, the energy cost of protein deposition should be less. We were able to show that there was a significantly reduced excretion of urinary 3-methyl histidine in trenbolone-treated animals, and hence probably a reduced rate of breakdown of skeletal muscle. However, careful analysis of the energy saving provided in this way, showed that this would not be sufficient to explain the attenuation of weight loss found in treated undernourished cattle.

Trenbolone is a xenobiotic and the question immediately arises as to how it acts. It is an androgen superficially structurally similar to testosterone yet in metabolic terms clearly the two differ significantly. There is evidence that the growth-promoting action of trenbolone might involve inhibiting the action of corticosteroids (see Sharpe et al. 1986a) which are known to inhibit normal growth at high concentrations. It is known that trenbolone stimulates the growth of female rats, but not of males and Sillence and Rodway (1990) have shown that it also reduces the plasma corticosterone concentration of female, but not of male rats. Moreover while testosterone also stimulates growth in female rats, it is much less effective than trenbolone - and it results in an elevation of plasma corticosterone rather than a decrease. Sharpe et al. (1986b) suggested that in lambs while trenbolone reduced total plasma cortisol concentration, the concentration of free cortisol was actually increased. However, they found that although trenbolone had only weak ability to bind to glucocorticoid receptors in lamb muscle it did reduce the receptor density as

measured by dexamethasone binding. There is thus appreciable evidence that growth stimulation by trenbolone may at least in part involve effects on the glucocorticoids. How far is this true for effects on the utilisation of energy? Observations of Coyer et al. (1985) suggest the effect is complex. In young male rats given a moderate subcutaneous dose (50 mg/kg/day) of corticosterone metabolisable energy (ME) intake fell to about 70% of untreated animals while energy deposition was only about 55%, the adverse effect being almost entirely in deposition of protein. When the dose of corticosterone was doubled, ME intake fell further although not significantly. However,energy deposition fell below zero (that is, there was mobilisation of tissue reserves) and in contrast to the lower dose, there was an increase in oxygen consumption. In contrast, Woodward and Emery (1989) observed (using a dose of corticosterone the same as the low dose of Coyer et al.) that energy expenditure per unit of fat-free mass was significantly increased by injection. The effect was seen in both males and females, although growth stunting was much more striking in males than females. To further complicate the story, Hardwick et al. (1989) have studied the action of the drug RU486, which is said to be a potent glucocorticoid antagonist. Following injection of this drug into rats, there was an immediate increase in oxygen consumption of about 15%.Since the same response could be obtained by intraventricular injection of about 1/50 dose, it seemed the response was primarily effected through the central nervous system, and since it could be largely blocked by propranolol, they considered it was probably mediated via ß-adrenergic receptors. It is striking that chronic treatment with RU486 inhibits weight gain. Thus although it is a corticoid antagonist, it has the same qualitative effect as corticosteroid treatment. However by blocking negative feedback RU486 does increase the plasma concentration of ACTH and corticosterone. Perhaps there is a population of receptors responding to glucocorticoids which is not inhibited by RU486.

In studies with cattle, we attempted to immunise animals against ACTH. In those animals which developed a significant anti-ACTH titre there was a significant decrease in plasma cortisol.(Jones et al. 1990). Nevertheless there was no effect on basal metabolic rate was weight loss attenuated in undernourished animals. In contrast Sillence and colleagues (unpublished) have been able to show in female rats, passively immunised against ACTH, that growth is enhanced. Thus while it seems probable that the growth-promoting action of trenbolone involves an effect on the glucocorticoid system, whether the effect on metabolic rate is part of the response is uncertain.

(b) Action of cationic amines

In assessing the role of RU486, as discussed above, it was suggested that the catecholamine system is involved. We have for some time been interested in the role of this system in the control of metabolic rate. The calorigenic effect of adrenaline has long been known. What is not always appreciated however is just how sensitive the response is. In studies with humans, Staten et al. (1987) showed there was a significant increase in oxygen consumption with an infusion of 0.1µg/min. Sensitivity to noradrenaline is substantially less. Nevertheless in humans there is a significant increase of about 20% in metabolic rate with an infusion of about 0.1µg/kg/min. (Jung et al. 1979). Other species seem to be less sensitive. In rats (Macdonald and Siyamak, 1990), sheep (Webster et al. 1969) and in cattle (Hunter and Magner, unpublished) the value is between 0.4-1µg/kg/min. However, this difference in sensitivity may be only apparent - it is possible that variation in metabolic rate may be more controllable in humans so that a significant increment may be more easily detected.

Some new adrenergic agonists can enhance weight loss of obese subjects on restricted diets, probably by a sustained increase in thermogenesis (Connacher et al. 1988). What is less clear however is whether inhibition of appropriate ß-adrenergic receptors will reduce metabolic rate below normal. Stock and Rothwell (1986) have shown that ß-blockers can substantially attenuate the enhancement of metabolic rate produced by several ß-agonists including the natural agonists noradrenaline and adrenaline. Ma et al. (1986) have shown that dietary -induced thermogenesis (DIT) in rats can be reduced long-term by propranolol (a non-specific ß-blocker) although Morgan et al. (1986) were unable to demonstrate a similar effect in humans; however Thorin et al. (1986) and Acheson et al. (1987) could demonstrate an effect on glucose-induced thermogenesis in humans. In addition in a study of nearly 4000 middle-aged men and women of the effect of

propranolol over several years (Rossner et al. 1990) there was a significant weight increase. It was suggested that metabolic efficiency was improved, perhaps through fall in metabolic rate. However, Christin et al. (1989) were unable to demonstrate any acute effect of propranolol on resting metabolic rate.

Manipulation of the ß-adrenergic system did not seem therefore to be a promising way to interfere with metabolic rate. However a well-defined action of α_2 agonists is inhibition of the release of noradrenaline. These agonists therefore potentially of value in reducing metabolic rate, and indeed Thompson et al. (1984) did demonstrate a modest fall in resting metabolic rate (about 7%) in humans given 7µg/kg of an α_2 agonist, clonidine. We made initial studies with a putative α_2 agonist, guanfacin. In studies with mice, we were able to show that metabolic rate was reduced in a dose-responsive manner by guanfacin - eventually by up to 50% (Sillence et al. 1990). It would therefore be expected that guanfacin would enhance weight gain. Surprisingly all doses of guanfacin (within the range studied) resulted in a significant stunting of growth. Spiers et al. (1990) using the same dose in rats showed that there was a significant inhibition of muscle growth. It was shown that at least in part this was a consequence of a substantial urinary energy loss - through a glycosuria; this appeared to be due to enhanced plasma corticosterone - approximately a doubling in concentration, a range which Sillence and Etherton (1991) have shown is quite sufficient to stunt growth. In more recent studies with rats, (Gazzola, unpublished) it has been shown that metabolic rate can be reduced with much lower concentrations of guanfacin, and in these circumstances it is possible to use doses which will reduce metabolic rate, yet produce no glycosuria. Whether growth is enhanced in these conditions is being tested.

In studies with cattle, low doses of guanfacin can reduce fasting metabolic rate by up to 20%, again with no significant glycosuria. Moreover, in cattle maintained on a sub-maintenance diet it has been shown that with prolonged infusion of guanfacin there is a significant attenuation of weight loss (Hunter 1991). However, it seems doubtful at least in cattle that the mechanism of action is as originally postulated, since guanfacin does not seem to change significantly the plasma concentration or turnover rate of noradrenaline.

While guanfacin, like trenbolone, reduces metabolic rate and attenuates weight loss in undernourished cattle, it does not reduce 3-methyl histidine excretion, and thus probably does not reduce protein degradation in skeletal muscle.

In contrast, the ß-agonist clenbuterol has been shown to increase metabolic rate in rats (Reeds et al. 1987), sheep (MacRae et al. 1988) and cattle (Huntington et al. 1990) **and** to increase the deposition of muscle protein (although the mechanism is not certain: there is evidence both of a capacity to increase rate of protein synthesis (Emery et al. 1984) and to reduce the rate of protein degradation in skeletal muscle (Reeds et al. 1986; Eadara et al. 1989). However, the increased deposition of muscle protein and other facets of metabolism such as reduced fat deposition are not inevitably interlinked. Reeds et al. (1987) showed that changes in metabolic rate and protein deposition could be dissociated and we have also been able to separate the diminished fat and enhanced protein deposition. Propranolol can attenuate effects such as enhanced metabolic rate and reduced fat deposition while not affecting muscle protein deposition.

Thus it seems that reducing metabolic rate and thereby making more energy available does not necessarily increase the rate of protein deposition in growing animals. Trenbolone reduces MR, and may enhance protein deposition; guanfacin also reduces MR but does not enhance protein deposition; while the combination of propranolol plus clenbuterol will not change metabolic rate, but does result in enhanced deposition of muscle.

IV. NUTRIENT PARTITIONING

There is some evidence that there is competition between adipose tissue and muscle for any surplus energy. Panton et al.(1990), have shown that in rats immunised against adipose tissue

membranes there is extensive loss of adipose tissue and animals do not eat less food - instead there is enhanced protein deposition.

It is striking also that if one compares growth of pigs raised at 10° and 35°, with food intake varied, one can obtain conditions where at 10°and a high energy intake, pigs grow at about the same rate as those at 35° and a low energy intake (Weaver and Ingram 1979). Nevertheless,the shape of the animals in these two groups is markedly different, and analysis of their body composition shows that the 10°/high energy intake group have much more protein, the 35°/low energy intake much more fat (Dauncey and Ingram 1983). Thus although the 'available' energy is fairly similar, it is partitioned very differently. It is also noteworthy that there is a difference in whole body protein turnover with the group at the lower temperature having about 30% greater value for similar fractional rate of protein deposition (Lindsay et al. 1988).

A different partitioning can be seen in studies of rats with ventromedial lesions in the hypothalamus (Jeszka et al. 1991). In such rats it is well known that there is a huge stimulus to food intake, so that the animals become grossly obese. What is striking in this recent study however, is that if food intake of control (normal) rats is limited to about 65% ad libitum intake, and the animals with lesions are pair-fed to this level, there are striking differences in body composition. The normal animals continue to grow (just), but do so by maintaining protein deposition, with extensive loss of body fat.The animals with lesions have a reduced metabolic rate and improved efficiency of utilisation of food; their deposition of protein is suggestively lower, (although not statistically significantly less than that of the control group). However, they succeed in depositing increasing amounts of body fat. These effects are seen even more strikingly in rats maintained at 10°. Thus the hypothalamic lesion has significantly altered the efficiency of utilisation but has not permitted any improvement in the deposition of protein. In passing, the effect of reducing environmental temperature is quite different in rats and pigs. This could well be a consequence of differences in mechanisms for producing heat, which is primarily by activation of brown adipose tissue in rats, but not in pigs which have very little brown adipose tissue.

It is tempting to consider that the partitioning of nutrients is determined primarily by the relative activity of different cationic amine receptors, with the drive in the direction of adiposity being controlled by the balance of α_2/β_{1-3}-receptors, the drive to protein by β_2-receptors. This is certainly a gross over-simplification. We know for example that purinergic receptors play some part in energy balance. It is nevertheless a useful preliminary working hypothesis.

We conclude by drawing your attention to some peculiar features on the effects of exercise, in human studies. Mulligan and Butterfield (1991) showed that while in control (non-active) women, there was good agreement between energy intake and expenditure, this was not true for the active runners, whose expenditure was some 20% greater than their intake. It was stated that the participants had been adapted to their activity for 6 months, energy intake and output was monitored for about 2 months, and in this period there was no body weight change. There was also no change in resting metabolic rate. Similar findings are reported by Bingham et al. (1989) who used a more objective assessment of energy output (double isotope technique) - again there was no appreciable change in body weight but total energy output was also about 20% greater than input, although there was no change in resting metabolic rate. However in this case body composition was monitored; there was an increase in fat-free mass, and a suggestive (in this case not statistically significant) decrease in body fat.

This situation seems to be quite analogous to that discussed above, of pigs raised at a temperature of 10°. The increased energy requirement has resulted in some repartitioning of nutrients in the direction of protein deposition. Since the subjects were all adult it clearly is not the drive for growth that is the important feature.

In a recent leader in the British Medical Journal, Garrow (1991) refers to the treatment of obesity, with a sub-heading 'the first law of thermodynamics still holds'. We would suggest rather we should define the laws of thermodynamics as related to nutrition in an Orwellism 'all forms of energy are equal; but some are more equal than others.'

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REFERENCES

ACHESON, K., JEQUIER, E. and WAHREN, J. (1987). J.Clin.Invest. 72: 981.
BINGHAM, S.A., GOLDBERG, G.R., PRENTICE, A.M. and CUMMINGS, J.H.(1989). Br.J.Nutr. 61: 155.
CHRISTIN, L., RAVUSSIN, E.,BOGARDUS, C. and HOWARD, B.V. (1989). Metabolism. 38: 439.
CONNACHER, A.A., JUNG, R.T. and MITCHELL, P.E.G. (1988). Brit.Med.J. 296: 1217.
COYER, P., COX, M., RIVERS J.P.W. and MILLWARD D.J.(1985). Br.J.Nutr. 53: 491.
DAUNCEY, M.J. and INGRAM, D.L.(1983). J.Agric.Sci.(Camb). 101: 351.
DUNKIN, A.C., BLACK, J.L. and JAMES, K.J. (1986). Br.J.Nutr. 55: 201.
EADARA, J.K., DALRYMPLE, R.H., DeLAY, R.L., RICKS, C.A. and ROMSOS, D.R. (1989). Metabolism 38: 883.
EMERY, P.W., ROTHWELL, N.J, STOCK, M.J. and WINTER, P.D. (1984). Biosci.Rep. 4: 83.
GARROW, J.S.(1991). Br.Med.J. 302: 803.
HARDWICK, A.J., LINTON, E.A. and ROTHWELL N.J. (1989). Endocrinol. 124: 1684.
HUNTER, R.A. and VERCOE, J.E. (1987). Br.J.Nutr. 58: 477.
HUNTER, R.A. and VERCOE, J.E. (1988). J.Agric.Sci.(Camb). 111: 187.
HUNTER, R.A. (1989). J.Agric.Sci.(Camb). 112: 257.
HUNTER, R.A. (1991). Br.J.Nutr. In Press.
HUNTINGTON, G.B., EISEMANN, J.H. and WHITT, J.M. (1990). J.Anim.Sci. 68: 1666.
JESZKA, J., GRAV, H.J., HOLM, H., HUSTVEDT, B., LOVO, A. and UELAND O. (1991). J.Nutr. 121: 386.
JONES, M.R., HUNTER,R.A., MAGNER,T., HOSKINSON, R.M. and WYNN, P.C. (1990). Proc.Aust.Soc.Anim.Prod. 18: 500.
JUNG, R.T., SHETTY, P.S., JAMES, W.P.T., BARRAND, M.A. and CALLINGHAM, B.A. (1979). Nature 279: 322.
LINDSAY, D.B., DAUNCEY, M.J., BARKER, P.J. and INGRAM, D.L. (1988). J.Therm.Biol. 13: 79.
MA, S.W.Y., NADEAU, B.E. and FOSTER, D.O. (1986). Can.J.Physiol.Pharmacol. 65: 1802.
MACRAE, J.C , SKENE, P.A., CONNELL, A., BUCHAN, V. and LOBLEY, G.E.(1988). Br.J.Nutr. 59: 457.
McDONALD, I.A. and SIYAMAK, A.Y. (1990). Exp.Physiol. 75: 639.
MORGAN, J.B., YORK, D.A. and WILKIN, T.J. (1986). Ann.Nutr.Metab. 30: 386.
MULLIGAN, K. and BUTTERFIELD, G.E.(1991). Br.J.Nutr. 64: 23.
O'KELLY, J.C. (1985). Nutr.Rpts.Int. 32: 935.
PANTON, D., FUTTER, C., KESTIN, S. and FLINT, D. (1990). Am.J.Physiol. 258: E985.
REEDS, P.J., HAY, S.M., DORWOOD, P.M. and PALMER, R.M. (1986). Br.J.Nutr. 56: 249.
REEDS, P.J., HAY, S.M., DORWOOD, P.M. and PALMER, R.M. (1987). Comp.Biochem.Physiol. 89C: 337.
ROSSNER, S., TAYLOR, C.L., BYINGTON, R.P. and FURBERG, C.D. (1990). Br.Med.J. 300: 902.
RUMSEY, T.S. and HAMMOND, A.C. (1990). J.Anim.Sci. 68: 4310.
SHARPE, P.M., HAYNES, N.B. and BUTTERY, P.J. (1986a). In 'Control and manipulation of animal growth', p.207, eds. P.J.Buttery, N.B.Haynes and D.B.Lindsay. (Butterworths: London.
SHARPE, P.M., BUTTERY, P.J. and HAYNES, N.B. (1986b). Br.J.Nutr. 56: 289.
SILLENCE, M.N. and ETHERTON, T.D.(1991). J.Anim.Sci. 69: In Press.

SILLENCE, M.N., MATTHEWS, M.L., SPIERS, W.G. and LINDSAY, D.B.(1990). Proc.Nutr.Soc.Aust. 15: 170.
SILLENCE, M.N and RODWAY, R.G (1990). J.Endocrinol. 126: 461.
SPIERS, W.G., SILLENCE, M.N. and LINDSAY, D.B.(1990). Proc.Nutr.Soc.Aust. 15: 172.
STATEN, M.A., MATTHEWS, D.E., CRYER, P.E. and BIER D.M. (1987). Am. J.Physiol. 253: E322.
STOCK, M.J. and ROTHWELL, N.J. (1986). In 'Control and manipulation of animal growth', p. 249, Eds P.J.Buttery, N.B.Haynes and D.B.Lindsay. (Butterworths: London)
THOMPSON, D.A., PENICAUD, L., and WELLE, S.L. (1984). Am.J.Physiol. 247: R560.
THORIN, D., GOLAY, A.,SIMONSEN, D.C., JEQUIER, E., FELBER, J.P. and DeFRONZO, R.A. (1986). Metabolism 35: 524.
WEAVER, M.E and INGRAM, D.L.(1979). Ecology 50: 710.
WEBSTER, A.J.F., HEITMAN, J.H., HAYS, F.L. and OLYNYL G.P. (1969). Can.J.Physiol.Pharmacol. 47: 719.
WEBSTER, A.J.F., SMITH ,J.S. and MOLLISON, G.S.(1977). Anim.Prod. 24: 237.
WHITTEMORE, C.T. (1976.). Proc.Nutr.Soc. 35: 383.
WOODWARD, C.J.H. and EMERY, P.W. (1989). Br.J.Nutr. 61: 445.