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Exploiting the physiology of growth

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ABSTRACT

Muscle fibres in the adult are giant multi-nucleated cells which originate in the foetus from myoblasts and later fuse to form myotubes. Protein deposition is the balance between synthesis and degradation. The proliferation of cells and protein metabolism is controlled by a variety of growth factors and hormones which in the whole animal influence lean deposition. This endocrinological control of growth can be exploited to enhance lean deposition in animals and some of the currently available and potential methods are reviewed.

In most cases data would indicate that the animal must be fed a diet that will allow the increased potential for lean deposition to be expressed. Unfortunately many of the methods which have been shown to be safe for the consumer of the meat and the animal are not permitted for a variety of reasons by many government authorities. Future exploitation of the physiology of growth to enhance lean deposition is likely to be increasingly constrained by political decision; a luxury available to the affluent developed world.

Keywords Muscle, hormones, growth factors, lean, ruminants, pigs.

INTRODUCTION

Increasingly, the importance of balancing the dietary preferences of people with what the clinicians are recommending as an ideal diet is having a major influence on the animal production industry of the developed world. It is clear that for many animal products to remain a major component of the diet their fat content must be reduced. While recent advances in knowledge associated with the endocrinological control of fat and lean deposition in animals have suggested efficient ways of enhancing lean deposition in animals at the expense of fat deposition, many of these techniques do not currently meet with approval in many of the developed countries of the world. Despite the rejection of what are often shown to be efficacious and safe techniques there remains the continual desire to reduce the cost of food. In addition, while many parts of the world have the luxury of a more than adequate supply of food this certainly is not true of the greater proportion of the world. While arguments can be made that animal production is an inefficient utilization of resources for food production it must be acknowledged that in most areas of the world the only suitable way of utilizing the land to produce food is via the ruminant animal.

This review discusses some of the currently available methods of enhancing the growth and especially the lean depositions of animals. It deals mainly but not exclusively with the ruminant although the majority of the comments are applicable to all species, at least all domesticated food producing mammals.

GROWTH OF MUSCLE CELLS

The individual fibres of vertebrate muscle are giant cells, almost unique in higher animals in being syncytial ie possessing multiple nuclei within a continuous cytoplasm. The growth of these fibres is a complex process; since their nuclei do not divide (Allbrook et al., 1971).

In the foetus, a population of mesodermal precursor cells called myoblasts proliferate then fuse to form myotubes (Figure 1). These myotubes become contractile, synthesising actin, myosin, creatine kinase and many other proteins characteristic of muscle development. As they become innervated, deposition of myofibrillar proteins increases and their nuclei become marginated. The process of fibre formation is usually completed at, or around the time of birth in mammals,
and from this stage onwards the number is fixed (Burleigh, 1974, Kozeka and Ontell, 1982). An animal's growth potential in later life may be affected by factors which limit myoblast proliferation in the foetus. Rats runted by nutritional deprivation of the pregnant mother were shown to have decreased fibre number (Beerman et al., 1983). In a comparative study of two strains of mice selected for small and large body size, it was found that the bigger animals had more fibres per muscle; as no differences were detected between the two strains in the number of nuclei per fibre, or the time of myoblast fusion, it was concluded that myoblast proliferation in utero must have been more rapid (Penney et al., 1983).

In post-natal life the size of a muscle increases as the result of two distinct but interdependent processes. One is the proliferation of satellite cells and the other the increase in the protein content of their fibres. Satellite cells (for an early description see Mauro, 1961) are a population of mononucleate cells found between muscle fibres and their basement membranes. In vitro they share most of the properties of foetal myoblasts, though whether they are of an identical lineage is unclear. In vivo they are capable of fusing with muscle fibres, thus donating their nuclei (Moss and LeBlond, 1971). In vitro studies have shown that insulin-like growth factors (IGFs) and fibroblast growth factors (FGFs) are potent mitogens for myoblasts and satellite cells from a range of species (Gospodarowicz et al. 1976; Florini and Ewton, 1981; Kardami et al. 1985; Dodson et al. 1985; Allen et al. 1986). Surprisingly, pharmacological concentations of insulin are usually needed for mitogenesis in mammalian muscle cultures. Insulin under these conditions is thought to be acting via the IGF-1 receptor (Florini and Ewton, 1981). In contrast chick myoblasts do however divide in response to near physiological concentrations (Ridpath et al., 1984). Glucocorticoids also appear to be necessary for long-term growth of muscle cells in culture, at least for sheep and rat muscle cells (Florini and Roberts, 1979; Dodson et al., 1988); their role may be to act as antagonists to the very high concentations of insulin commonly used in defined culture media.

Myoblasts and satellite cells differentiate into myotubes (Figure 1) in the absence of a continued stimulus to divide; the time spent in G1 of the cell cycle appears to be the critical factor (Clegg et al., 1987). The greater the proliferation of the cells in the early stages of an animal's development the greater the potential to produce muscle in the adult, hence in vivo growth potential may be lost if mitogens are in short supply. Transforming growth factor beta (TGF-β) appears to have a potential regulatory role in muscle development, since in vitro it can inhibit progression to the differentiated state even in the absence of cell division (Massague et al., 1986; Florini et al., 1986).

Quite clearly, the proliferation of satellite cells is a critical component of postnatal growth, since a muscle's weight and DNA content will increase several fold between birth and maturity (see review by Young, 1985). However, the link between the two processes is not an absolute one; studies in mice treated with cytosine arabinoside to inhibit DNA synthesis showed that muscle length can increase to a limited extent in the absence of new nuclei being added to the fibres (Cardasis and Cooper, 1975). During normal maturation in a mammal a phase is reached in which DNA accumulation within a muscle drastically slows down or stops; net protein deposition does occur for some time after. It is noteworthy that this is the stage at which fat deposition begins in the muscle of a steer (see for example Trenkle et al., 1978).

Muscle contains a number of cell types in addition to satellite cells and fibres. These include endothelial cells, which may control the flow of nutrients from the blood capillaries (Vilaro et al., 1989) and fibroblasts. Fibroblasts have, along with muscle cells, an important role in laying down the connective tissue sheaths which
bind the fibres to each other and to the bones (Kuehl et al., 1982). The potential role of these populations in altering muscle growth should not be forgotten. However, the vast majority of protein within a muscle is myofibrillar, and is synthesised within the fibre.

Protein accretion is the sum of the balance between protein synthesised and that degraded. These processes are potentially influenced by a large number of hormones and growth factors. We have become interested over recent years in this aspect of muscle growth and have carried out a series of studies in vitro using differentiated muscle cultures from both foetal (7-12 weeks' gestation) and juvenile (3-6 month) sheep (Harper et al., 1987; Roe et al., 1989; J.A. Roe, J.M.M. Harper and P.J. Buttery, unpublished observations).

One of our major areas of investigation has been the influence of growth factors on both protein synthesis and degradation (Figure 2). Once again IGF-I appears to be a key factor in anabolism; it increases protein synthesis in both foetal and postnatal muscle cultures, and in the latter we have also shown increases in the activity of amino acid and glucose transporters and decreases in protein breakdown; half-maximal effects were seen at concentrations around 1 nM. Others have reported that IGFs promote differentiation in cultured chick and rat myoblasts (Schmid et al., 1983; Florini et al., 1986).

There appear to be quantitative differences in the response to insulin in the two ovine culture systems, but even in the cells from the older animals, the expected increases in nutrient transport and protein synthesis are only seen in vitro at concentrations 100-1000 fold higher than those normally found in sheep in vivo (10-100pM, Bass et al., 1987) suggesting once again that insulin's effects may be mediated by Type I IGF receptors. There is in fact little firm evidence from whole animal studies that insulin has a major role in muscle protein metabolism in ruminants (see for example reviews by Prior and Smith, 1982; Weekes, 1986). There is some evidence that nutrient transport in primary rat cultures is more sensitive to the hormone (Spencer et al., 1987).

Growth hormone (GH) has been shown to act directly on some mesodermal tissues, including rat epiphyseal chondrocytes and fibroblasts, eliciting local IGF-I production (Isaksson et al., 1982; D'Ercole et al., 1984; Cleemons et al., 1981). However, we have not found any evidence of a direct role for it in ovine muscle metabolism; protein synthesis, protein breakdown and nutrient transport were unchanged by bovine GH treatment (at concentrations up to 0.1 μM) in the satellite cell derived system. Had GH been able to induce IGF production, we might have expected to see some changes, especially over the 18 h period used in these studies for protein breakdown measurements.

Epidermal growth factor (EGF) has potent anabolic effects at low concentrations (0.1 - 10 μM) on both foetal and postnatal cultures, increasing protein synthesis, decreasing breakdown, and inducing small changes in the activity of glucose and amino acid transporters. It also increases the creatine kinase activity of muscle cultures, confirming its direct action on the muscle cells. Its effects are additive with those of IGF-I. We have identified a specific receptor on the cells with a Kd of 0.3nM. This is present in all the cultures studied, including those from clonally purified foetal myoblasts (Heywood et al, 1989) and binding is present in membranes prepared from adult sheep muscle (Hawkey, Wastie and Buttery, unpublished observations).

We have carried out some preliminary studies on the in vitro effects of steroids using differentiated myoblasts and have found no effects of near-physiological concentrations (10 μM) of oestradiol or testosterone on protein synthesis. IGF-I responsiveness

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**FIG 2** Protein synthesis in adult ovine primary muscle cell cultures. IGF-I 10nmol/l; insulin 1μmol/l; epidermal growth factor 1nmol/l; GH 100μmol/l. Bar is the SED of nine samples (from Roe et al., 1989).
was also unaffected. Roeder et al., (1986) have reported decreased protein synthesis with oestradiol benzoate in cultured muscle cells but they used pharmacological concentrations (1 μM). Whilst the synthetic glucocorticoid dexamethasone had no effects on protein synthesis, even after a 24-hour pre-incubation period, in common with other workers (Ballard and Francis, 1983), we have found marked increases in protein breakdown. Using the rat L6 continuous myoblast cell line as a model, we have been able to show an interaction between dexamethasone and insulin; 10 nM insulin desensitized the cells to the catabolic effects of dexamethasone. This parallels some observations in vivo in rats and sheep where the insulin glucocorticoid ratio was found to be correlated with growth rate (Sharpe et al., 1986). Myofibrillar proteins have been shown to be especially sensitive to glucocorticoid induced catabolism in vivo (Kayali et al., 1987; Scene et al., 1988).

How the effects of these and other individual factors are integrated to produce the responses seen in vivo remains a matter of speculation. In most cases in vivo increased anabolism for example by moderate dietary manipulation is associated with an increase in protein synthesis and a smaller increase in degradation rate (see Waterlow et al., 1978). Also there are cases where degradation rate is reduced with a smaller decrease in synthetic rate, for example in muscle following trenbolone acetate treatment (Vernon and Buttery, 1976). This contrasts with the responses in simplified culture systems, where increases in the synthesis of protein often accompany decreases in degradation. It should be noted that other factors not easily measured by biochemical techniques such as the activity of the animal are important influences on muscle protein deposition; this is well known in athletics but sometimes overlooked in studies of animal production! Studies in cultured muscle cells have shown increases in protein deposition after electrically stimulated contraction (Brevet et al., 1976). Rat limb muscles either grow or atrophy depending on whether they are immobilised in a stretched or contracted state (Goldspink et al., 1983). Chick myotubes stretched in culture increase their protein deposition (Vandenburgh and Kaufman, 1979).

Despite our lack of knowledge of the control of muscle cell proliferation, several successful methods have been developed to enhance lean deposition in animals.

**ANABOLIC STEROIDS**

Many studies have reported the efficacy of anabolic agents since Dinusson et al., (1950) first demonstrated that the oestrogenic substance dimethyl stilbesterol when implanted into heifers increased growth rate. Since that time a series of implants based on the natural sex steroids have been developed to enhance the growth of animals, especially ruminants. In the European Economic Community (EEC) an expert committee concluded that, at least for the natural hormones and compounds which are rapidly metabolised to yield natural hormones, provided they were used according to the officially approved manufacturers instructions, they presented no hazard especially to the eventual consumer of the meat (Lamming et al., 1987). Despite the scientific evidence the use of all hormone implants to promote growth of animals within the EEC is now banned (directive 85/649, EEC). However, use of anabolic implants remains a permitted practice in animal production in many other countries of the world, eg the United States of America.

Studies with anabolic agents have highlighted the importance of adequate feeding of an animal to ensure that the animal is able to express any increase in growth potential. For example, studies with silage-fed steers treated with oestradiol demonstrate that a significant growth response is only seen when animals are given supplementary protein (Gilletul. et al., 1987; Newbold et al., 1989). GH has been implicated in the mode of action of oestradiol and the interaction between the GH axis and diet offers a possible explanation for the lack of response of oestradiol treated animals when protein supply is limited (Breier et al., 1988a, see also below). A similar interaction with diet although judged not to be statistically significant has been reported for the combined implant of trenbolone acetate plus oestradiol (Galbraith et al., 1983). The data available would suggest that similar dietary-endocrinological interactions are important in other methods of enhancing lean deposition.
MANIPULATION OF THE GROWTH HORMONE AXIS

The apparent importance of the growth hormone axis in influencing the rate of lean deposition in animals has prompted many studies attempting to enhance lean deposition in animals either by exogenous administration of GH or by various novel techniques for manipulating the somatotropic axis to increase endogenous secretion. Recent developments in recombinant DNA technology have facilitated production of GH and GH-related stimulatory peptides (e.g. growth hormone releasing factor, GRF) in sufficient quantities for commercial use in animal production systems and numerous reports have now been published on their relative effects on growth and carcass composition.

In general, administration of GH to cattle, sheep and pigs increases growth rate (Peters, 1986; Pell et al., 1990; Etherton et al., 1987) but the magnitude of the response appears to be variable (8-38%; Sandles and Peel, 1987; Pell and Bates, 1987) and differences between experiments can not always be explained by differences in animal age, dose-level, duration of treatment, level of nutrition etc (see for example Johnsson et al., 1985, 1987). Most studies have been conducted over relatively short periods but Pell et al. (1990) have shown that the sensitivity to GH is maintained with long-term administration. Some workers have reported significant dose-related effects of GH (Ivy et al., 1986; Etherton et al., 1987; Kirchgessner et al., 1987) on rate of liveweight gain. In almost all cases where animals have been treated with exogenous GH, carcass protein content is significantly increased and carcass lipid reduced. The differential effects of GH on these 2 tissues will however affect overall liveweight gain changes. There are no reported deleterious effects of administered GH on meat quality (see Allen and Enright, 1989).

The response to GH is not only dose-dependent but also varies with plane of nutrition (Breier et al., 1988a, b). Many studies which have shown large responses to GH administration have been in animals which have been fed diets containing a high level of protein in an attempt to meet the increased nutrient demands of the animal. Peters (1986) showed in growing steers fed either a restricted (3.5kg DM/d) or ad libitum diet that GH can promote protein accretion at both levels of feeding (albeit to a limited extent in restricted animals), but the net reduction of adipose accretion in response to GH only occurred when there was an adequate nutrient supply (i.e. in ad lib fed animals). Although feed intake was reduced in ad lib fed steers treated with GH, the accretion of gross tissue mass was not affected indicating that GH increased overall efficiency (gain feed). Similar results were obtained by Pell et al. (1989) in a study of the effect of GH administration on young lambs fed diets containing one of 3 levels of protein (12, 16 or 20% crude protein) either ad libitum or at 3% of body weight. GH increased liveweight gains in restricted but not ad libitum-fed lambs, at all dietary protein levels. Muscle weights were increased by GH in all groups, but visceral fat was only reduced in the ad lib-fed animals. Thus, although it would appear that some anabolic effects of GH on muscle can occur irrespective of dietary intake, but that its effects in reducing fat accretion are dependent on energy intake, it should be remembered that the fat content of the animals in these restricted intake groups was already minimal. The depression in feed intake in GH treated animals fed ad lib often confounds the carcass effects seen at high doses of GH but at moderate doses it results in an increase in efficiency (gain feed). N digestibility is not altered by GH but N retention is increased (Eisemann et al., 1986; Pell et al., 1990).

Level of feeding is known to significantly affect endogenous plasma GH concentrations, levels tending to be increased at low planes of nutrition. This has been suggested to be an adaptation in an attempt to mobilise energy from adipose tissue to maintain basal metabolism and could occur either through alteration in feedback mechanisms on GH secretion, or to reduced metabolic clearance of GH. Breier et al. (1986) have stated that unless the plane of nutrition is optimised, the relationship of administered GH to its effects on growth rate may not be optimal. The mechanism of action of GH and the reasons for variability of response observed are still unclear, however, the work of Breier et al. (1988a) and of Elsasser et al. (1989) gives some insight into the mechanism of the interaction between plane of nutrition and response to GH. These workers showed that plasma IGF-I concentrations increased more in response to growth hormone when animals were on a high plane of nutrition than when poorly fed. A low plane of nutrition results in a reduction in hepatic growth hormone receptors thus reducing the extent of IGF-1
release from the liver in response to circulating growth hormone. This dietary influence on the hepatic growth hormone receptor could also explain in part the failure of oestradiol to promote growth in poorly-fed animals (see above).

The anabolic effects of GH on muscle are believed to be mediated by increases in the rate of protein synthesis (Pell and Bates, 1987; Eisemann et al., 1989) but evidence for the predominant mechanism in reducing carcass fat is equivocal. Many of the somatogenic effects of GH are believed to be mediated by IGF-I while the lipolytic and anti-lipogenic effects of GH on adipose tissue growth and development are thought to be direct (Walton and Etherton, 1986). Administration of exogenous IGF-I may therefore be an alternative means of enhancing growth and protein deposition. This possibility has not yet been extensively investigated in meat animals, probably because of the difficulty of obtaining sufficient quantities of recombinant IGF-I. However, studies with both normal growing and hypophysectomised rats have shown significant increases in body weight gain and tibial epiphyseal width after 7 days continual infusion of IGF-1 (Hizuka et al., 1987). Problems with dose and route of administration may affect the success of this technique because free IGF-I may act as an insulin mimic and cause hypoglycaemia.

GH secretion in vivo is under the dual control of 2 hypothalamic peptides, GRF and growth hormone release-inhibitory factor (somatostatin). As its name suggests, GRF is a potent specific stimulant of GH release (see Millard, 1989). There are relatively few reports of the effects of long-term administration but Etherton et al. (1986) have shown a persistent positive effect on GH secretion, growth and carcass composition in pigs although the magnitude was less than that observed for GH treated pigs. In lambs treated with 10 μg GRF/kg body weight (BW) four times daily for 42 or 56 days, effects on carcass composition were similar to those observed with 40 μg ovine GH/kg BW, although plasma GH levels were lower (Beermann et al., 1988). These and other results (Barenton et al., 1987) suggest that chronic GRF treatment does not result in pituitary refractoriness to GRF.

Hart et al. (1985) have shown that exogenously administered GRF can stimulate GH secretion in sheep maintained in both positive and negative energy balance. Although no differences in the magnitude of the response to GRF or the speed of the response were observed between the animals on the 2 planes of nutrition, the response was more persistent in the animals on restricted feed intake. Other studies have also shown that factors associated with the ingestion of feed can modulate the release of GH from the pituitary (Moseley et al., 1987; Trenkle, 1989).

Other methods of attempting to manipulate the growth hormone axis in cattle and sheep have been reported including the most notable being autoimmunisation of animals against somatostatin, to immunoneutralise the negative regulatory factor of GH secretion.

Somatostatin is a peptide of 14 amino acids and its structure is conserved across species. It is synthesised as a 92 amino acid prosomatostatin molecule from which six peptides including somatostatin itself and a 28 amino acid form of somatostatin are known to be derived (Patel, 1987). Somatostatin inhibits growth hormone secretion but in addition it also inhibits the secretion of thyrotropin from the pituitary and glucagon and insulin release from the pancreas (Patel, 1987). The concept of autoimmunising farm animals against somatostatin to enhance growth hormone release was initially explored in sheep by Spencer et al. (1983). These initial studies reported enhanced growth in the autoimmunised animals but subsequent studies by these and other workers have often yielded equivocal results. Several studies which have obtained increased growth rates have observed no increase in plasma GH levels; others have observed increased GH levels but no effect on growth (Varner et al., 1980; Lawrence et al., 1986; Laarvald et al., 1986). Bass et al. (1987) more recently obtained data which again highlighted the importance of diet in the response to a growth promoting regimen. Lambs were fed either a good quality pellet diet or cut pasture and some animals from each group were autoimmunised against somatostatin. Although plasma growth hormone concentrations were higher in both groups of immunised animals, only the animals fed the pellets showed an increased in weight gain. Presumably the forage fed animals which were on a low plane of nutrition had livers which were partially refractory to growth hormone and consequently IGF-I release (Breier et al., 1988a). It is interesting to note that the data of Bass et al. (1987) indicates that in animals showing an enhanced growth that there was some evidence, albeit...
not judged to be significant, for an enhanced deposition of fat; enhanced growth hormone activity would normally be expected to be associated with an increase in lean content (see above). Data similar to this has been obtained from studies with cattle in our laboratory (Table 1). The possibility that the changes in digesta flow seen on immunisation against somatostatin also play a major role in the response of animals needs to be considered further (see Fadalla et al., 1985). Recent evidence has indicated that digestibility of dry matter and nitrogen is increased in immunized animals (Sun et al., 1990).

**TABLE 1** Fat content of sheep and cattle immunized against somatostatin

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Immunised</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lambs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cut pasture</td>
<td>36.0</td>
<td>33.1</td>
<td>(a) Bass et al. 1987</td>
</tr>
<tr>
<td>Pellets</td>
<td>37.8</td>
<td>40.4</td>
<td></td>
</tr>
<tr>
<td><strong>Lambs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate-intake</td>
<td>35.9</td>
<td>38.7</td>
<td>(b) Sun et al. 1990</td>
</tr>
<tr>
<td>High intake</td>
<td>42.0</td>
<td>44.7</td>
<td></td>
</tr>
<tr>
<td><strong>Steers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forage-concentrate</td>
<td>25.2</td>
<td>27.0</td>
<td>(c) Dawson et al. (unpublished)</td>
</tr>
<tr>
<td>(70:30)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) fat determined by chemical analysis and expressed as percentage carcass composition;
(b) fat content calculated from regression equations between water space and empty liveweight determined in live animal. Values expressed as g body fat/100 g empty liveweight;
(c) fat determined by chemical analysis and expressed as g lipid/100 g carcass dry matter. Preliminary results.

**B-AGONISTS**

In recent years, B-agonists have been widely investigated as a potential means of manipulating growth performance and carcass composition in farm livestock. These agents, many of which are substituted phenylethanolamines, are structural analogues of the naturally occurring catecholamines, adrenaline and noradrenaline and as such, are believed to act by activation of specific membrane-bound beta-adrenoreceptors. However whether all their effects are mediated directly and whether all B-agonists have a similar mode of action is still uncertain.

The most frequently used B-agonists are clenbuterol and cimaterol but isoproterenol, ractopamine, L-640,033, L-644,969 and several others have also been used. Numerous studies have demonstrated the profound effects that administration of these compounds have on carcass composition, increasing protein accretion and reducing fat deposition which has resulted in their general description as repartitioning agents. They appear to be effective in a variety of species including rats (Reeds et al., 1986), sheep (Baker et al., 1984), cattle (Miller et al., 1985) and poultry (Dalrymple et al., 1984) but in general the magnitude of the response seems to be greater in ruminants than non-ruminants with increases of 10-15% in carcass protein and reductions of 20-30% in carcass fat consistently being observed in these species. Their effects on growth rate and feed consumption are less consistent than their effects on carcass composition, but overall efficiency of gain (feed gain) is usually improved. This is effected either by increases in liveweight gain (Quirke et al., 1988) or reductions in feed intake (Jones et al., 1985) or a combination of the two parameters (Bolhorov et al., 1987). B-agonists appear to be effective in both sexes and in castrates and at almost all ages. Mersmann et al. (1987) reported no effect of cimaterol fed to very young pigs between 10 and 60 kg weight but Williams et al. (1987) obtained significant effects in veal calves fed milk replacer containing clenbuterol.

Some studies have shown some form of a dose-response relationship with B-agonists, particularly in terms of liveweight gain and feed conversion efficiency but several have also shown that high doses become less effective (Reeds et al., 1986; Hanrahan et al., 1986). The greatest responses seen in ruminants are usually obtained with dietary inclusion levels of 1-10 ppm but this is also dependent on the B-agonist employed.

As most of the widely used B-agonists are orally active this offers a considerable advantage over growth hormone in terms of application. Some concern has been expressed over the effects of B-agonists on meat quality (Warriss et al., 1990; Dawson et al., 1990) but whether the deleterious effects can be overcome by
reducing dose rate and/or modifying slaughter and pre-slaughter handling conditions remains to be seen.

Few reports have considered the effect of nutritional status on the effectiveness of β-agonists, or whether the nutrient requirements of treated animals are increased. Kim et al. (1989) reported that cimaterol stimulated protein accretion and reduced fat deposition under both maintenance and ad libitum feeding conditions. However, Anderson et al. (1987) suggested that the growth performance and tissue partitioning effects of ractopamine in finishing pigs were improved as the dietary protein level increased. Kim et al. (1989) reported no effect of cimaterol on DM digestibility.

The mode of action of β-agonists in enhancing protein accretion and reducing fat deposition is uncertain. It has generally been believed that β-agonists act directly on adipose tissue via the β-receptor to stimulate lipolysis. This has been supported by the consistent observation of elevated plasma free fatty acids in β-agonist treated animals (Beermann et al., 1987; Eisemann et al., 1988). Although there is clear evidence that some β-agonists do act in this way, there is also increasing evidence that all species and indeed, all β-agonists do not respond identically. For example, in in vitro studies, clenbuterol has been shown to stimulate lipolysis in adipose tissue from rats (Duquette and Muir, 1985), chicken (Campbell and Scanes, 1985), sheep (Thornton et al., 1985), but not pigs (Rule et al., 1987) or cattle (Miller et al., 1988). However, isoproterenol, cimaterol and ratopamine do all stimulate lipolysis in pig adipose tissue in vitro (Peterla and Scanes, 1990). In our laboratory, we have consistently observed a significant reduction in free fatty acid and glycerol release from adipose tissue pieces obtained from young cattle treated with cimaterol (Dawson et al., 1989 and unpublished observations). Thus the in vivo effects of β-agonists on carcass fat can not always be explained by direct simulation of lipolysis. Several reports have shown that β-agonists also affect the in vitro rate of lipogenesis (Mersmann, 1989; Peterla and Scanes, 1990). In the absence of a significant effect on lipolysis, some reports have concluded that this is the predominant mechanism by which fat accretion is reduced (Miller et al., 1988). It is likely that considerable variation exists between different β-agonists in their relative potencies for inhibiting lipogenesis and stimulating lipolysis. Several reports suggest that for many of the β-agonists the potency for inhibiting lipogenesis is considerably greater than the potency for stimulating lipolysis. One major problem encountered in investigating the mechanism of action of β-agonists on adipose tissue is that these studies are conducted in vitro because of the difficulties of measuring lipid metabolism in vivo. Evidence is accumulating to suggest that the incubation conditions used for these in vitro studies are critical (Liu et al., 1989; Mersmann, 1989) as many of the responses to β-agonists are thought to be mediated by increased endogenous cAMP. It is suggested that the limited response to clenbuterol in swine adipocytes is due to a reduced capacity for adenylate cyclase activation (Mills and Liu, 1990). These differences between agonists may reflect stringent specificity by the adipose tissue receptors.

Muscle hypertrophy is believed to be due to increased fibre diameter rather than increased cell number, but whether all fibre types are affected equally appears to be equivocal (Beermann et al., 1987; Kim et al., 1987). Most evidence would suggest that type II fibres consistently respond to β-agonists. The evidence that type I fibres respond is however more open to debate (see Yang and McElligott, 1989). Several reports have concluded that the predominant mechanism by which β-agonists enhance muscle accretion is through a reduction in protein degradation rate (Reeds et al., 1986; Bohorov et al., 1987) but there is also evidence from in vivo studies for increased rates of protein synthesis (Emery et al., 1984; Claeys et al., 1989; Dawson et al., unpublished data). To these pieces of conflicting evidence has to be added that from muscle cells in culture, where little evidence for a reduction in protein degradation rate has been obtained.

Studies in cultured muscle cells have demonstrated the presence of beta receptors on ovine muscle cultures of both foetal and postnatal origin, and also in two continuous muscle lines mouse G8-1 cells and rat L6 cells. Increases in protein synthesis were observed on short term (6h) treatment with cimaterol in both rodent lines and in cultures derived from sheep satellite cells. However no changes in protein breakdown were observed (Harper et al., 1990; Symonds et al., 1990).

The cause of this reduced protein catabolism can at present only be a matter of conjecture. One possible explanation lies in the changes in the activity of the calcium activated neutral protease (calpain) system in
TABLE 2 Effect of dietary restriction or clenbuterol treatment (2 mg/kg diet) on calpains I and II (EC 3.4.22.17) and calpastatin activity in sheep *Longissimus dorsi* muscle*

(Mean values with no. of animals in parentheses)

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Control (4)</th>
<th>Restricted (6)</th>
<th>Clenbuterol Pooled SED (df 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily wt gain (kg)</td>
<td>0.347</td>
<td>0.048</td>
<td>0.360</td>
</tr>
<tr>
<td><em>Longissimus dorsi</em> wet wt (g)</td>
<td>635</td>
<td>377</td>
<td>788</td>
</tr>
<tr>
<td>Calpastatin (units/kg muscle)</td>
<td>2740</td>
<td>2340</td>
<td>5440</td>
</tr>
<tr>
<td>Calpain I (units/kg muscle)</td>
<td>570</td>
<td>430</td>
<td>490</td>
</tr>
<tr>
<td>Calpain II (units/kg muscle)</td>
<td>1250</td>
<td>1500</td>
<td>2880</td>
</tr>
</tbody>
</table>

SED, standard error of difference  
* For details, see Higgins *et al.* (1988)

the muscle of treated sheep (Higgins *et al.*, 1988). These results have subsequently been confirmed by others (Kretchmar *et al.*, 1990). As illustrated in Table 2 there is a marked increase in the activity of the natural inhibitor of the calpains, calpastatin. However, caution should be exercised in relying too strongly on this explanation since there is evidence that the main role of the calpain system in the cell is associated with degradation of hormone receptors rather than structural muscle proteins (Higgins *et al.*, 1988).

Whether all of these effects of β-agonists are exerted directly via β-receptors on muscle cells and adipocytes, or whether some of these effects are mediated through changes in blood flow or plasma hormone concentration is unknown. For example, it is possible that the change in muscle blood flow seen on administration of β-agonists (Eisemann *et al.*, 1988) contributes to the preferential growth of this tissue, while blood flow to adipose tissue is reduced.

There are several reports that plasma insulin levels are significantly reduced by β-agonists (Beermann *et al.*, 1987; Dawson *et al.*, 1989). It is possible that this contributes to the reduction in fat accretion seen in these animals by reducing the rate of lipogenesis and increasing lipolysis. Growth hormone (GH) levels were reported to be increased in sheep treated with cimaterol for 6 weeks but IGF-1 levels were 34% lower (Beermann *et al.*, 1987). Other studies however have reported no significant change in plasma GH levels (Emery *et al.*, 1984; Quirke *et al.*, 1988). James and Barker (1987) showed that β-agonists are effective in hypophysectomised rats suggesting that GH is not essential for their effect. In a recent study with veal calves given growth hormone and clenbuterol, the effects of the two treatments were reported to be additive resulting in heavier carcasses and higher feed conversion ratios than were obtained with either treatment alone (Maltin *et al.*, 1990). Although there was some evidence for an interaction between the two treatments in terms of muscle hypertrophy, it was concluded that clenbuterol does not mediate its effects via the somatotrophic axis. However, in this particular study, no effect of GH alone was noted on growth rate or muscle hypertrophy which is in contrast with much other published work.

While β-agonists have highlighted the importance of the β receptor in the control of fat and lean deposition in animals their use in animal production has not at the time of writing been approved in the developed world. They are likely to receive the same public antagonism shown towards anabolic steroids and growth hormone. The β receptor including its sub-types have now been sequenced (see for example Dixon *et al.*, 1986) and this offers the potential to manipulate the system by
means other than application of hormones and hormone analogues (eg by transgenesis or via the immune reaction).

TRANSGENESIS

Since the development of the ability to selectively modify the mammalian genome the production of transgenic animals has been reviewed many times (Clark, 1990). Reported attempts to enhance lean deposition in animals by manipulating growth hormone expression have yielded disappointing results. While for example extra copies of growth hormone gene have been inserted into livestock the animals produced often have skeletal abnormalities or difficulties in reproduction. One of the main problems however is a lack of fundamental knowledge on the control of these aspects of metabolism associated with the growth process. Even when the crucial genes have been identified then it will be necessary to understand in detail how these genes are regulated. The importance in understanding the regulatory process can be illustrated by the observation that wool production by sheep is often stimulated by an increase in the dietary supply of methionine. In principle, it should be possible to clone the genes that are responsible for producing methionine in bacteria and to insert them into sheep. However, methionine is one of the more toxic amino acids when present in excess and thus it will be essential to regulate the production of methionine such that it is only produced in quantities which slightly exceed the requirement of the animal.

CONCLUSIONS

Knowledge of the control of the deposition of fat and lean in animals has yielded a variety of techniques which safely and effectively enhance the rate of lean deposition in farm animals. Much of the conflicting evidence in the literature can be traced to the failure to recognise the interaction between nutritional status and the response of the animal. It is essential if enhanced lean deposition is to be achieved that there is an adequate supply of both energy and amino acids available to the tissues.

While the scientific community can effectively design techniques to produce meat of decreased fat content and at a reduced cost they are not always acceptable to a very vocal section of the consuming public. There is considerable suspicion by many of the public of the benefits of modern biotechnology. The only way that these advances stand any chance of being accepted by public is continued attempts by the scientific community to explain their science to the public and for the scientists only to promote techniques and products which are conceptually sound.

The luxury of rejecting the advances in science is one that is only applicable to the overfed affluent western world.

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