FACTORS CONTROLLING FAT DEPOSITION IN RUMINANTS

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INTRODUCTION

It has been shown that domesticated cattle and sheep have similar basic patterns of protein and fat deposition (see Fig.1 ; Haecker 1920; Moulton et al. 1922; Hedrick 1968; Searle et al. 1972; Searle and Graham 1975). Figure 1 indicates that protein accumulation is virtually linear, but fat deposition increases dramatically when empty bodyweights of approximately 300 kg for cattle and 30 kg for sheep are reached. There are several variations of this basic pattern. For instance, young suckling or milk fed animals are fatter than depicted in Fig.1 (Searle and Griffiths 1976). Breed effects are reflected by differing slopes of the fat accumulation relationships and/or the bodyweight at which rapid fat accumulation begins, i.e. larger, late maturing animals begin to fatten at heavier bodyweights than smaller, early maturing animals (Searle et al. 1982). However, to generalise about the relative leanness or fatness of different genera and species and breeds within species across the entire spectrum of ruminants is fraught with problems, some of which are well documented. For instance, dairy cattle are leaner but have more internal fat (omental, perirenal and channel) and less subcutaneous fat than beef cattle (see Truscott et al. 1983). Similarly, some breeds of sheep, those noted for both fecundity and milking ability in particular, have heavier internal fat deposits than breeds which are noted for their meat characteristics (Wood et al. 1980). Furthermore, Fig.1 is based on data from castrates and does not represent the accumulation patterns of males or females, which are generally considered to be leaner and fatter, respectively, than castrates. The bodyweight at which rapid fat accumulation begins (termed physiological maturity or the break point) is not associated with weaning or any other dietary transition, or puberty. Clearly this increased fat accumulation represents a marked repartitioning of dietary nutrients.

It should also be noted that ruminants can be extremely fat animals. When compared at similar bodyweights, sheep are much fatter than pigs (Searle et al. 1972). We have studied sheep which were apparently healthy, yet some 70% of their empty bodyweight was fat (Thornton et al. 1983). This compares with obese female humans whose bodies are of the order of 30% fat (Katch and McArdle 1977). Sheep, in particular, provide an excellent model for the study of obesity.

The fundamental aspects of adipose tissue growth in animals, such as distribution of adipose tissue, histogenesis of the fat cell, gross development of adipose tissue and the role and development of brown adipose tissue in the ruminant neonate have been reviewed by Leat and Cox (1980). Attempts to find brown adipose tissue cells in the white adipose tissue of adult ruminants using histological techniques have not been successful (Payne, personal communication). However, sensitive immunological

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Fig.1

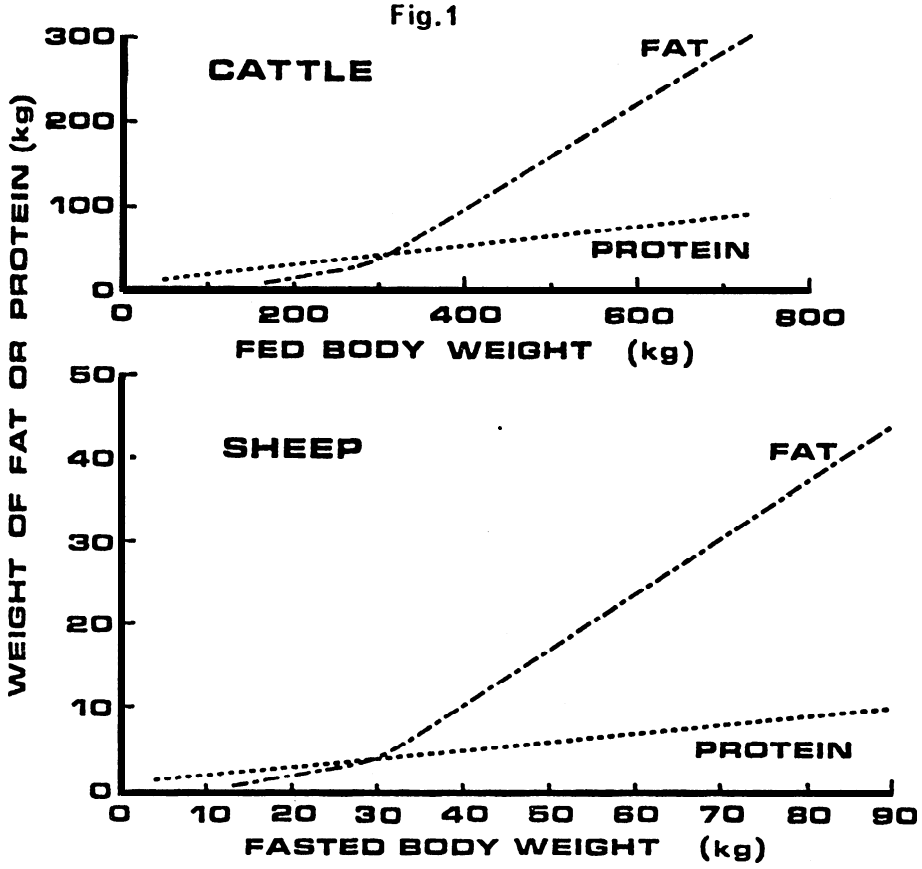

Figure 1 Plot of weight of fat and protein against fed (cattle) and fasted (sheep) bodyweights (from Searle et al. 1972).

ADIPOSE TISSUE METABOLISM

Fig. 2

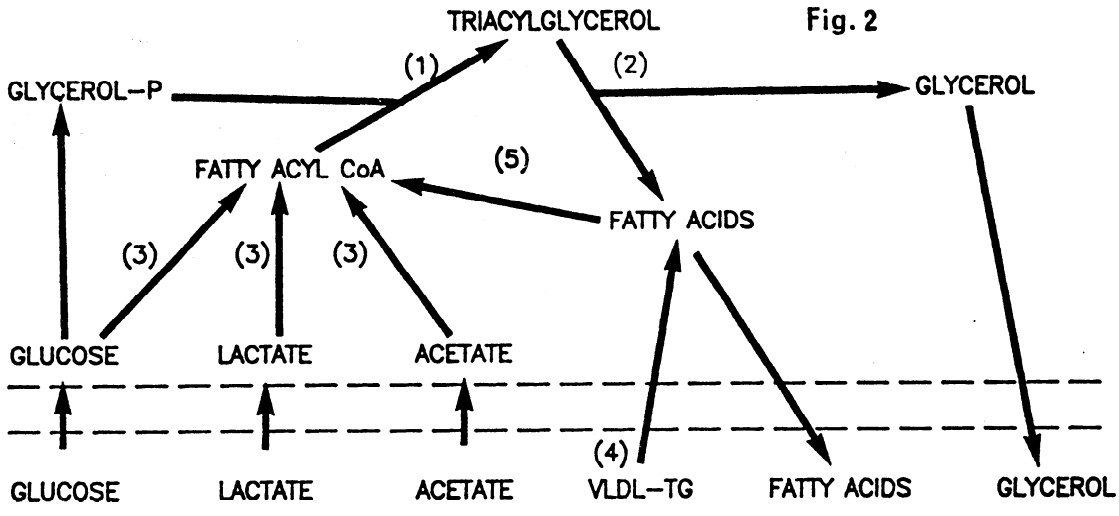

Figure 2 Schematic representation of the major pathways of lipid metabolism in ruminant adipose tissue.
(1) Esterification; (2) Lipolysis; (3) Fatty acid synthesis; (4) Lipoprotein lipase releases fatty acids from very low density lipoproteins (VLDL) triacylglycerols (TAG); (5) Re-esterification (adapted from Vernon and Flint 1984)

techniques for the detection of thermogenin, a protein exclusively associated with brown adipocytes, have not been applied to ruminant adipose tissue (Cannon and Nedergaard 1985). The present review is concerned with the control of regulatory mechanisms which determine fat accumulation in white adipose tissue, hereafter referred to as adipose tissue (see Figure 2).

CELLULAR DEVELOPMENT OF ADIPOSE TISSUE

As pointed out by Leat and Cox (1980), "the size of any organ or tissue can increase by (a) hyperplasia, or production of new cells by division; (b) hypertrophy, or enlargement of existing cells without a concomitant increase in number; and (c) a combination of both processes." The contribution of hyperplasia to adipose tissue development in mature ruminants remains conjectural. As a consequence of the development of the Coulter counter technique to size adipocytes (Hirsch and Gallian 1968) and its use in studies on man and laboratory rodents, a thesis developed which suggested that the number of adipocytes in an animal was determined early in life (see Faust et al. 1978). The Coulter counter technique and its modifications, both physio-chemical (Ether-ton et al. 1977a; Hood and Thornton 1980) and statistical (Cianzio et al. 1985; Cianzio et al. 1982; Whitehurst et al. 1981a), have been used to study adipocyte s-number and metabolism in sheep (Haugebak et al. 1974a) and cattle (Robelin 1981).

Hood and Allen (1973) reported that hyperplasia was complete in subcutaneous and perirenal adipose tissue, but was continuing in the intramuscular adipose tissue of 14 month old steers, weighing 470 kg. This conclusion was largely based on a lack of small cells in the subcutaneous and perirenal adipose tissue and an abundance of such cells in the intramuscular adipose tissue from four muscles. In a study on crossbred wethers, hyperplasia continued until the sheep were approximately 44 kg in weight and 11 months of age, and nutritional restriction and rehabilitation did not influence subcutaneous adipocyte number (Hood and Thornton 1979). In both of these studies, adipocytes were fixed with osmium tetroxide and counted in a Coulter counter. This method does not adequately measure the smaller cells (<20μ; see Hood 1982) but there is no indication in either of these studies, on sheep or cattle, that there was a significant hyperplasia and/or differentiation of adipocytes associated with fattening.

Some workers have reported bimodal distributions of adipocyte size in the adipose tissue of fat cattle (Allen 1976; Cianzio et al. 1985; Robelin 1981), which implies that there has been some hyperplasia during excess fat deposition. Both studies used the osmium fixation/Coulter counter technique, using statistical methods to remove "electrical noise? In contrast, by direct observation, we found mostly normal statistical distributions of adipocyte size in the perirenal, omental and subcutaneous adipose tissues of both thin and fat adult sheep (Thornton et al. 1984a).

Undoubtedly the suggestion that hyperplasia has occurred in the adipose tissues of cattle (Allen 1976; Cianzio et al. 1985; Robelin 1981) has been promoted by important new findings on rats by Hirsch's group (see Faust et al. 1978). This group reported that, contrary to their previous thesis, hyperplasia in adipose tissue was a general phenomenon which persisted into adulthood. It is clear that both hyperplasia and

hypertrophy of adipose tissue are responsible for the continued growth of adipose tissue into adulthood particularly in genetically obese strains of laboratory rodents (Hausman et al. 1980). The concept that new adipocytes are differentiated when existing adipocytes reach a certain size (Allen 1976; Faust et al. 1978) has gained general acceptance.

There is a need for further studies of adipocyte origins, differentiation, development and proliferation in ruminant adipose tissues. It remains to be established whether the primordial pre-adipocyte is a fibroblast, fibroblast-like or a reticulo-endothelial cell. Evidence of adipocyte hyperplasia in fattening cattle and sheep must come from techniques other than those based on frequency distributions of cells such as labelled thymidine. The identification of specific proteins associated with pre-adipocytes (Lee et al. 1986) could lead to their manipulation by immunological means (Flint et al. 1986), with consequent regulation of fat deposition.

LIPOGENESIS: lipid synthesis from carbohydrate.

Adipose tissue is the major site of fatty acid synthesis in adult, non-lactating ruminants. This contrasts with other animals in which the liver is the important organ of fatty acid synthesis, e.g. man and birds, or in which both adipose tissue and liver can contribute significantly to fatty acid synthesis, e.g. mice, rats and rabbits (reviewed by Vernon 1981).

That acetate rather than glucose is the major precursor for fatty acid synthesis in the adipose tissue of roughage-fed adult ruminants was first demonstrated by Hanson and Ballard (1967). In this classical study of ruminant lipogenesis, these workers showed that the activity of ATP-citrate lyase and NADP-malate dehydrogenase was minimal in adult ruminant adipose tissue. The utilisation of acetate as the major substrate for fatty acid synthesis in roughage-fed ruminants is clearly an adaptation to dietary circumstances in which the amount of glucose reaching the small intestine is minimal, because of rumen fermentation. Most of the glucose supply is synthesized in the liver by gluconeogenesis from propionate, amino acids, glycerol and lactate (Ballard et al. 1969). Thus glucose is being spared by virtue of acetate contributing to lipogenesis (Vernon 1981). Evidence that this is an adaptive mechanism was demonstrated in sheep by the infusion of glucose into the abomasum or intravenously (Ballard et al. 1972). In these experiments, the incorporation of glucose into fatty acids in adipose tissue increased 20 to 40 times, and the activities of both ATP-citrate lyase and NADP-malate dehydrogenase were also increased. The whole question of the role of glucose in lipogenesis has been revived by the finding that lactate can also be a significant precursor for fatty acid synthesis in ruminant adipose tissue (Prior 1978; Whitehurst et al. 1981b). This observation suggests that ATP-citrate lyase and NADP-malate dehydrogenase are not rate limiting (Smith and Prior 1981), as both glucose and lactate are metabolised through pyruvate.

There has been considerable controversy over which enzyme is rate limiting for glucose utilisation for fatty acid synthesis in ruminant adipose tissues (see Thornton and Tume 1984). Much of the controversy was generated by the fact that comparisons were often made on the basis that adipose tissues from both sheep and cattle were metabolically similar.

Recently, Smith and Prior (1986) have corrected this anomaly in a simultaneous study of lipogenesis and glucose metabolism in subcutaneous adipose tissue slices from both cattle and sheep fed the same diet. The results of this study were:

1. Total acetyl unit incorporation into fatty acids was significantly greater in adipose tissues from sheep than from cattle (e.g. 220 vs. 45 nmol/minute/g of tissue) .

2. Acetate provided about 80% of the acetyl units in both species but glucose provided less and lactate more in adipose tissue of cattle compared to that from sheep (82:14:4 acetate:lactate:glucose for sheep vs. 79:21:1 for cattle).

3. The addition of acetate (0 to 10 mM) to the media increased the utilisation of glucose in sheep adipose tissue (8-25 nmol/minute/g) but was without effect on glucose utilisation of bovine adipose tissue (9-12 nmol/minute/g). In both species about 50% of the glucose utilised was oxidised to CO, and about 13% went to glycerol synthesise However, considerably more label from glucose was recovered in fatty acids and less in lactate for sheep adipose than for bovine adipose,

4. Despite that considerably more glucose was utilised for fatty acid synthesis in sheep adipose than bovine adipose (35 vs. 6 nmol/ minute/g), the pentose cycle contributed about 50 to 60% of the NADPH for lipogenesis in both species. NADPH-malate dehydrogenase NADPH-malate dehydrogenase and NADP-isocitrate dehydrogenase provided the remainder of the NADPH for lipogenesis,

5. From the relative activities of ATP-citrate lyase, NADP-malate dehydrogenase, aconitate hydratase, NADP-isocitrate dehydrogenase, 6-P gluconate dehydrogenase and glucose-6-P dehydrogenase, it appeared that in ovine adipose tissue NADP-isocitrate dehydrogenase supplied a major proportion of the NADPH not supplied by the pentose cycle.

6. Acetyl-CoA carboxylase appeared to be rate limiting for lipogenesis of ovine adipose tissue, but fatty acid synthetase appeared to be rate limiting in bovine adipose tissue.

The subcutaneous adipose tissues studied by Smith and Prior (1986) were taken from steers and wethers fed a high energy diet (70% ground corn, 20% corn silage, 8.7% soybean meal, which yielded 3.05 Mcal of ME/kg and 13% crude protein; see Smith and Crouse 1984), presumably ad libitum, Such a diet could be expected to provide more glucose absorption from the small intestine and considerably higher propionate production in the rumen than a roughage based diet could provide (Ørskov 1986). Unfortunately, the circulating levels of glucose, acetate or lactate in the cattle and sheep studied by Smith and Prior (1986) were not reported. Furthermore, the in vitro incubations were all done in media containing 3 mM glucose, 33 mU/ml of insulin, acetate and lactate concentration ranged from 0 to 10 mM. The value of future comparative experiments would be enhanced by measurement of the circulating levels of insulin and the in vivo entry rates of glucose and acetate, and conducting the in vitro incubations across a range of substrate concentrations that encompass the in vivo values, Such an experiment would be particularly interesting when animals are fed a

roughage diet, i.e. when glucose supply is virtually all from gluconeogenesis.

Most studies on substrate utilisation have concentrated on subcutaneous adipose tissue as it is readily accessible by biopsy or immediately on slaughter. A recent study by **Smith and** Crouse (1984) demonstrates that different bovine adipose tissue deposits utilise substrates at different rates, These workers studied the utilisation of acetate, lactate and glucose in intramuscular and subcutaneous adipose tissues incubated in medium containing 5 mM of acetate, lactate and glucose, and **33** mU/ml of insulin, Relative rates of incorporation of substrates into fatty acids were 1, **1** and 3.5 nmol acetyl units/3 hours/10^5 cells for acetate, lactate and glucose into intramuscular tissue, and 65, 25 and 2 for subcutaneous adipose tissue,

Thus, intramuscular adipose tissue had much lower rates of lipogenesis than subcutaneous adipose, but intramuscular utilised more glucose than acetate **(3.46** vs.0.98) and its glucose utilisation was higher than that of subcutaneous adipose tissue (3.46 vs.1 ● ... . In terms of percentage of acetyl units, glucose provided **62%** in intramuscular but only 4% in subcutaneous, whereas acetate contributed 18% in intramuscular versus 70% in subcutaneous. In this study by Smith and Crouse (1984) the pentose cycle provided virtually all of the NADPH for lipogenesis. In a study of acetate and lactate utilisation by bovine subcutaneous, intermuscular and intramuscular adipose tissues) the relative rates of acetate utilisation were approximately 17:16:1 for subcutaneous, intermuscular and intramuscular adipose tissues respectively (Whitehurst et al. 1981b). Comparative studies on different adipose tissue from the same animal warrant further attention under a variety of dietary regimes. Such studies may provide an insight into methods of differentially manipulating the growth of adipose tissue deposi ts.

The potential glucose requirement for lipogenesis from acetate was highlighted in a review by Armstrong (1965). It has been hypothesised that glucose availability, from direct absorption and gluconeogenesis, could limit the efficient utilisation of acetate for fatty acid synthesis in roughage fed ruminants (MacRae and Lobley **1982).** These workers suggested that in these circumstances., acetate may need to be converted to heat by some substrate or futile cycle in order to prevent acetate accumulation, This hypothesis has gained indirect support from a simulation model of the metabolism of absorbed nutrients which, on the basis of the inherent assumptions in the model, suggested that NADPH production could limit fat synthesis (Gill et al. 1984). Other studies concerned with the utilisation of nutrients (MacRae et ale 1985; Lee et al.1987) have been interpreted along similar lines. However, these studies do not provide experimental proof of this hypothesis, and enzymatic and glucose flux studies on ruminant adipose tissues do not support this hypothesis (e.g. Smith and Prior **1986;** Smith and Crouse **1984).** Thus we again conclude that: "there appears to be sufficient capacity and flexibility in the NADPH generation systems to meet the demands of lipogenesis; indeed NADPH synthesis may be consequential rather than causal for lipogenesis" (Thornton and Tume **1984).** Furthermore, glucose is the major precursor of glyceride glycerol, and there is no evidence that fatty acid esterification is limited by glycerol-3-phosphate supply in ruminant adipose tissues (Vernon 1981).

There can be no doubt that grain based rations or high quality roughage diets promote greater growth rates and greater fat deposition (see Byers 1982). Such diets, grain based in particular, provide a greater glucose supply (from increased absorption from the small intestine or enhanced gluconeogenesi s from propionate, and perhaps lactate and protein) which can contribute more acetyl units, NADPH and glycerol-3-phosphate for fatty acid synthesis and triglyceride formation, i.e. fat deposition, Thus no singular role for glucose should be proposed, rather the role of glucose is part of a complex metabolic interaction between all substrates utilised directly or indirectly for triglyceride synthesis, i.e. acetate, lactate and glucose together with propionate, glycerol and glycogenic amino acids.

Consideration of the complex role of glucose in fat deposition raises the question of the regulatory role of insulin in ruminant adipose tissue metabolism, In vivo experiments on cattle have indicated that insulin does not have a major regulatory role in fat deposition, For instance, in experiments on Hereford and Friesian steers, serum insulin concentration rose in response to tolbutamide treatment, but the insulin response was not correlated with percentage body fat within or across breeds, Gregory et ale (1982) and Smith et ale (1983) used alloxan-diabetic cattle, insulin injections and glucose infusions to study the interrelationships between insulin and lipid metabolism, They concluded: "It appears that insulin may have only a limited effect on the processes (lipogenesis and 1 ipolysis) in the bovine." Recent, apparently conflicting, reports from Lincoln College, New Zealand have indicated: "infusion of insulin into early-weaned lambs caused a further reduction in the percent fat in the empty body" (Munro et al. 1985) and: "increasing the circulating levels of insulin is associated with an increase in the rate of backfat synthesis in lambs" (Davey et al. 1985). In this latter study, use of the euglycaemic insulin clamp technique increased the plasma insulin concentration from 13.5 to 160 mU/ml while glucose turnover and the amount of glucose converted to glyceride glycerol doubled. Glucose injection experiments have shown that relative to lean animals, "obese" heifers and "obese" anoestrous non-lactating ewes exhibit basal hyperinsulinaemia, insulin resistance and a greater glucose-induced increase in plasma insulin concentrations (McCann and Reimers 1986; McCann et ale 1986). However, such observations cannot be seen as causal of greater fat deposition as increased insulin secretioncould result from increased insulin resistance,

The effect of insulin on the metabolism of rat adipocytes was clearly demonstrated in a study by Green and Newsholme (1979). Increasing the insulin concentration in the media from 1 to 100 uU/ml increased glucose uptake almost fourfold and decreased glycerol release sixfold. However, when we repeated this experiment with ovine adipocytes, lipogenesis and lipolysis rates were unaffected by insulin concentration as high as 1000 uU/ml (Thornton et al.1982). These same preparations of ovine adipocytes responded to adrenaline, as lipolysis rates were markedly increased, Rat adipocytes, isolated and incubated under identical conditions to the ovine adfpocytes, had a similar response in glucose incorporation and glycerol release to those studied by Green and Newsholme (1979). These findings on ovine adipocytes, and similar observations on isolated bovine adipocytes (Vasilatos et al. 1983; Prior and Smith 1982), led to the conclusion that insulin has a negligible role in the regulation of ruminant adipose tissue metabolism, Recently, Etherton and Evock (1986) reported that insulin markedly stimulates lipogenesis of isolated bovine

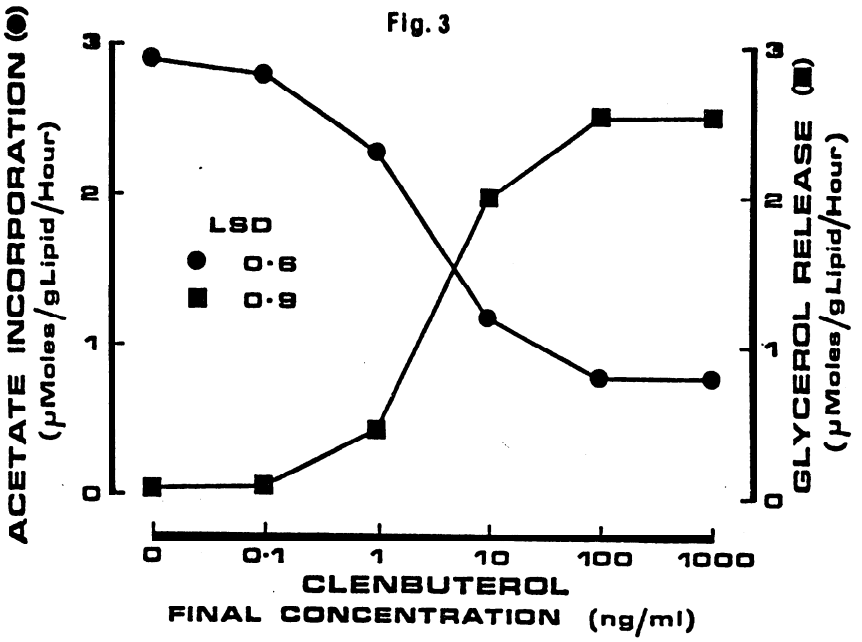

Figure 3

Plot of acetate incorporation (lipogenesis) and glycerol release (lipolysis) against the concentration of clenbuterol (ng/ml) in the media (pH 7.4, 3 mM glucose, 1 mM acetate, 0.5 mM lactate and 1% albumin). Each point is the mean of 8 experiments.

SERUM FREE FATTY ACID

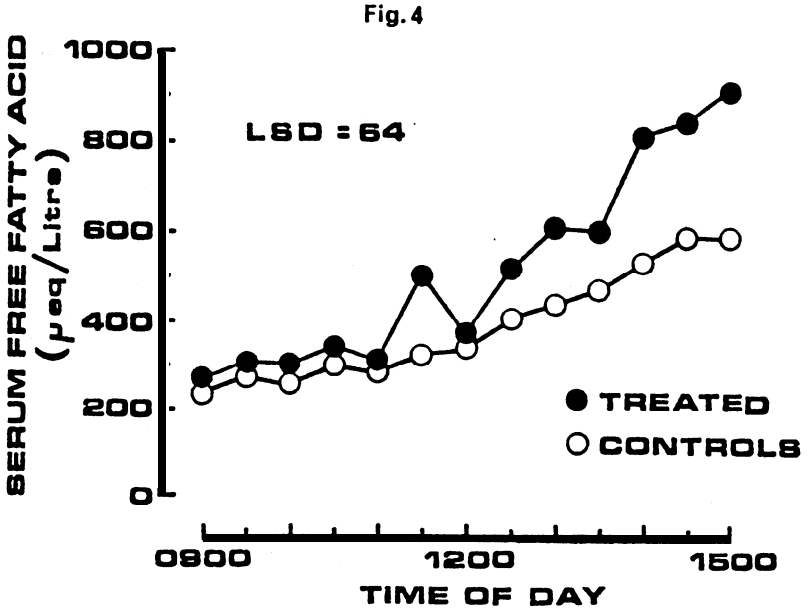

Figure 4

Serum free fatty acid concentration of control (o) and clenbuterol treated (●; 2.5 ug/kg liveweight/day) sheep. Each value is the mean of 5 sheep.

adipocytes and that this response was dependent upon the source of bovine serum albumin used in the medium. In that study, the increase in lipogenic rate was linear with insulin concentrations up to 10 ng/ml and maximally stimulated by 30 ng/ml. The potency of IGF-I was 76 times lower than that of insulin, and hydrocortisone (50 ng/ml) maintained the potency of insulin in bovine adipose tissue cultured for 48 hours (Ether-ton and Evock 1986). The lesser potency of IGF-I in this study on bovine adipose contrasts with a report on ovine adipose in which IGF-I (70 ng/ml) was more potent than insulin (2.7 ng/ml) in stimulating lipogenesis from acetate (Lewis et al. [illegible]. Lewis (personal communication) has also found that acetate incorporated into fatty acids by ovine adipose tissues was linearly related with insulin concentrations up to 5 ng/ml in medium 199 with no BSA present.

The production of large quantities of purified insulin(s) by recombinant DNA technology should permit long term studies to resolve the in vivo role of insulin, and insulin-like growth factors, in lipid metabolism of both sheep and cattle. This in turn should stimulate studies on the regulation of ruminant adipocyte insulin receptors in terms of both number and affinity. Bovine adipocytes have been shown to bind insulin, to internalise insulin, and their insulin receptors are subject to down regulation (Vernon et al. 1985).

LIPOLYSIS: the mobilisation of fat.

Relative to lipogenesis, there have been few studies of lipolysis in ruminant adipose tissues. Lipolysis of adipose tissue involves the activation of the hormone sensitive lipase-cyclic AMP phosphorylase enzyme system (see Vernon and Flint 1984). Lipolysis rates are usually referred to as basal or stimulated. The catecholamines stimulate lipolysis of ruminant adipose tissues but the responses to adrenalin of ruminant adipose tissues are not as great as those of laboratory animals (Jaster and Wegner 1981; Etherton et al. 1977b). Prostaglandin E2 did not affect basal or adrenalin stimulated lipolysis in subcutaneous adipose tissue from fasted or fed steers, but fasting decreased basal lipolysis by 40% (DiMarco et al. 1986).

The finding that β_2-adrenergic agonists, such as clenbuterol and cimaterol, cause a marked reduction in the fat content of ruminant meat, with a concomitant increase in lean, will undoubtedly stimulate research into β-adrenergic receptors of ruminant adipose tissues. This reciprocal exchange of fat and lean has led to these agonist compounds being described as "repartitioning agents" (Ricks et al. 1984). The potency of clenbuterol is such that less than 100 µg/head/day reduced the fat content and increased the lean by some 30% in pen fed weaners and suckling-grazing lambs (Thornton et al. 1985a).

β_2-adrenergic agonists have been shown to increase lipolysis rates of isolated ovine adipocytes (Thornton et al. 1984b; Thornton et al. 1985a; see Figure 3) and to increase circulating levels of free fatty acids (Thornton et al. 1987; Beerman et al. 1985; see Figure 4). However, clenbuterol also decreased lipogenesis and long chain fatty acid incorporation into lipids of isolated ovine adipocytes (Thornton et al. 1984b; Thornton et al. 1985a; Thornton et al. 1985b; see Figures 3 and 5). Recently) we have shown that clenbuterol (2.5 µg/kg liveweight/day)

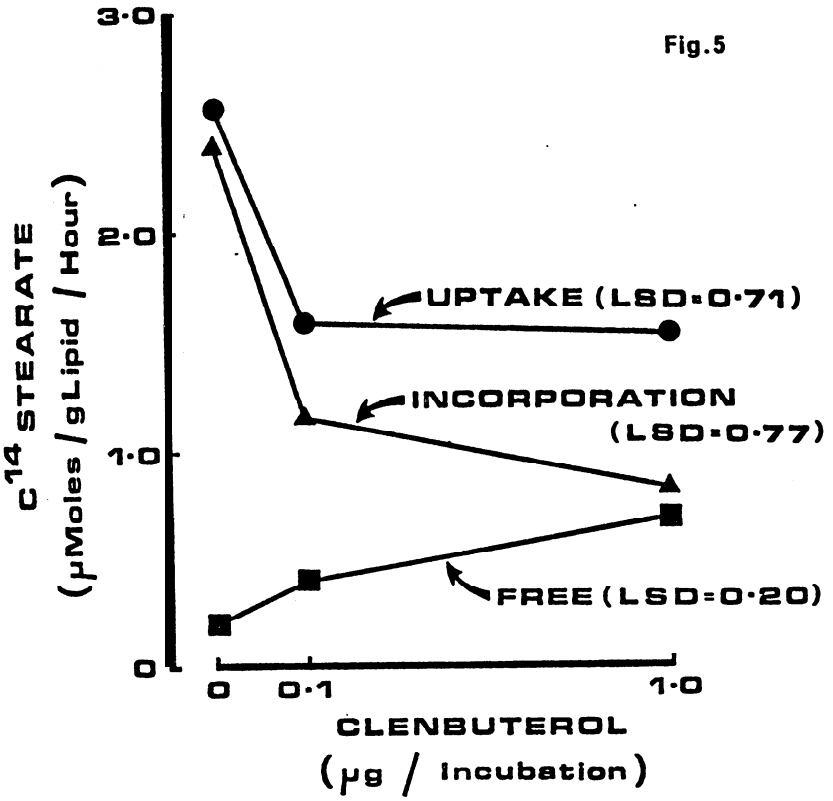

Figure 5 The effect of clenbuterol on the metabolism of C14 stearate by isolated ovine subcutaneous adipocytes.

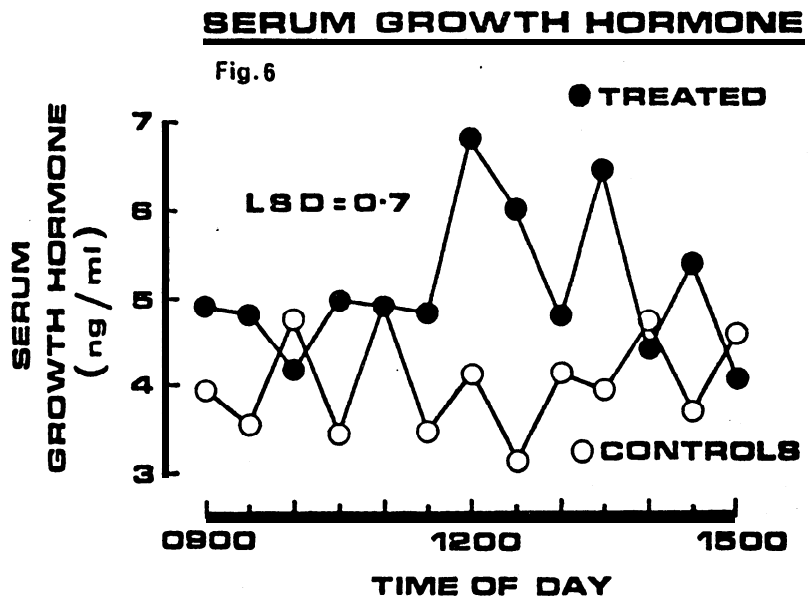

Figure 6 Serum growth hormone levels of control (o) and clenbuterol treated (●; 2.5 ug/kg liveweight/day) sheep. Each value is the mean of 5 sheep.

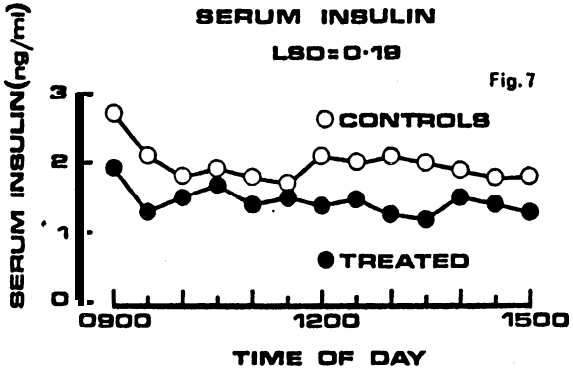

Figure 7 Serum insulin levels of control (o) and clenbuterol treated (●; 2.5 ug/kg liveweight/day) sheep. Each value is the mean of 5 sheep.

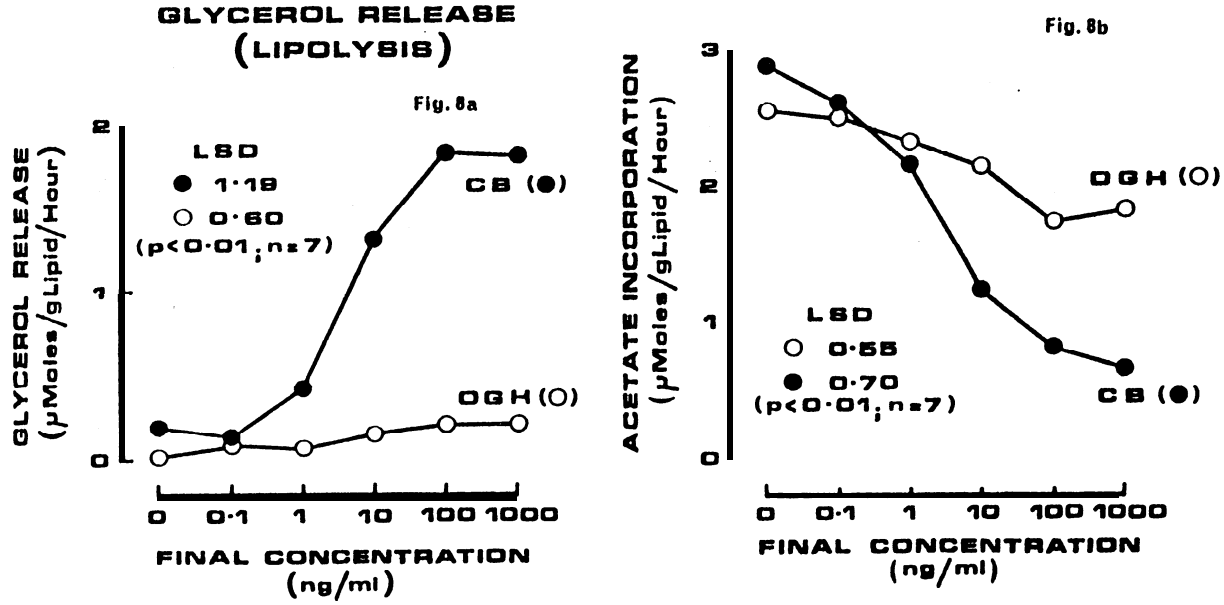

Figure 8 Plot of glycerol release (lipolysis; (a)) and acetate incorporation (lipogenesis; (b)) against the final concentration (ng/ml) of ovine growth hormone (o; OGH) and clenbuterol (●; CB) in the media (from Thornton et al. 1986). Each point is the mean of 7 experiments.

significantly ($P<0.001$) increased circulating growth hormone levels (5.1 vs. 4.0 ng/ml) and decreased insulin levels (2.1 vs. 1.4 ng/ml) of treated sheep relative to controls (see Figures 6 and 7). Thus it would appear that clenbuterol could exert its effects directly on the metabolism of the adipocyte or indirectly through altering the levels of circulating regulatory hormones (growth hormone and insulin). Growth hormone is generally considered to be lipolytic in ruminants. This conclusion is largely based on studies of lactating cows, in which an increase in growth hormone level is associated with increased fat mobilisation from adipose tissue depots and increased milk production (see Bauman and McCutcheon 1985). However, studies on isolated ovine adipocytes showed that ovine growth hormone (oGH), thyroid-stimulating hormone (TSH), lutenising hormone (LH) and adrenocorticotrophic hormone (ACTH), did not stimulate lipolysis (Duquette et al. 1984). The same concentrations of these hormones in isolated rat adipocyte preparations did stimulate lipolysis. Recently) we have also shown that ovine growth hormone at concentrations up to 1000 ng/ml did not stimulate lipolysis of isolated ovine adipocytes (Thornton et al. 1986; see Figure 8a and b). Discrepancies between in vivo and in vitro studies on lipolysis have been described by Bauman and McCutcheon (1985) and Mersmann (1986), meaning an equivocal conclusion that growth hormone is not lipolytic cannot be made. Furthermore, both naturally occurring growth hormone and recombinant growth hormone have been shown to reduce fat deposition in sheep (Johnsson et al. 1985; Pullar et al. 1986).

LONG-CHAIN FATTY ACID INCORPORATION AND LIPOPROTEIN LIPASE ACTIVITY

In a previous review (Thornton and Tume 1984) we emphasized the following:

1. The lipid intake of ruminants grazing high quality pastures and particularly of suckling animals , is much higher than generally recognized.

2. The amount of fatty acids reaching the small intestine (y) is highly related to fatty acid intake ($x; y = 5.2 + 1.17x, r = 0.98$) and the apparent digestibility of fatty acids in the intestine is very high (70-90%). Furthermore, because of the high calorific value of lipid and its high metabolisable energy value, long-chain fatty acids can make a substantial contribution to the net energy content, e.g. 22%, of high quality pasture diets. Dietary long-chain fatty acids have the potential to contribute significantly to fat deposition in ruminants and provide a source of energy that can readily be directed in accordance with tissue requirements.

3. Ruminant adipose tissues have the capability to utilize long-chain fatty acids and certainly do so under some dietary circumstances. For instance, in studies on isolated ovine adipocytes, stearate incorporation into triacylglycerol, in terms of carbon atoms incorporated, was greater than that of acetate or lactate, and adipose tissues from growing sheep had high lipoprotein lipase activity (see Thornton and Tume, 1984).

In this review we discuss the potential regulation of lipoprotein lipase (LPL) in ruminant tissues to reduce fat deposition.

LIPOPROTEIN LIPASE

The primary pathway for the removal of chylomicrons and very low density lipoprotein-triacylglycerol (VLDL-TAG) from plasma is by hydrolysis in the peripheral tissues (see Robinson 1970). LPL bound to the lumenal surface. of capillary endothelial cells, in association with its activator (plasma lipoprotein bound apolipoprotein CII) provides the rate limiting step in the removal of TAG-fatty acids from the circulation (Nilsson-Ehle 1981). LPL is not only important in determining the rate of TAG-fatty acid removal, but is able to direct those fatty acids to specific tissues. As LPL has a very short half life (Borensztajn et al. 1975; Wallinder et al. 1979) its presence at the capillary wall-dependent upon continual synthesis. Thus, in a fasting animal, synthesis of LPL in adipose tissue is very low, whereas synthesis continues or is even increased in skeletal and cardiac muscle. As a result TAG-fatty acids will be directed towards the muscle tissues for utilisation as a source of energy, rather than for deposition as storage fat in adipose tissue (Speake et al. 1985). LPL therefore has the ability to control the distribution of TAG-fatty acids according to the tissue demand, while the tissue uptake of free fatty acids (bound to plasma albumin) is dependent only upon the plasma concentration, and the blood flow through the tissue.

The low concentration of plasma TAG in ruminants has been suggested to result from either the low intake of dietary fat coupled with the continuous nature of the absorption process, insignificant synthesis of hepatic VLDL or the rapid rate of removal of TAG from the plasma by the various peripheral and hepatic systems (Bell 1981). The concentration of plasma TAG remains fairly constant with time and even fasting sheep for 5 days resulted in a reduction of less than 25% (from 0.29 to 0.22 mM) whereas TAG concentration in rats fell by nearly 50% during an overnight fast (Tume and Thornton 1985). The monogastric rabbit, al though consuming a diet similar to sheep or cattle, has a much higher plasma TAG concentration, some attaining levels similar to those found in man (≃ 1.6 mM; Lutton and Tsaltas 1965). It is likely therefore that it is the rapid removal of plasma TAG in the ruminant that is responsible for the low concentrations observed.

Various workers have studied LPL activity from ruminant adipose tissue and their findings are summarized by Vernon (1981). However, apart from the work of Chilliard et al. (1977) on LPL of goat omental tissue, most studies have reported only specific activities of various tissues in relation to nutritional or physiological states (Shirley et al. 1973; Haugebak et al. 1974b; Rao et al. 1973; Cryer and Jones, 1979).

We have shown that LPL preparations from adipose tissues of ad libi turn fed sheep can hydrolyse TAG at a high rate, even at relatively low TAG concentrations (Tume et al. 1983). The Km (apparent) of ovine LPL for TAG (0.4 mM) was consider-lower than that measured for rat adipose tissue LPL (≃5 mM), indicating that the enzyme from ovine adipose tissue has a greater potential to remove TAG from the plasma (Tume et al. 1983). LPL is dependent upon the presence of plasma components (the C-group of apolipoproteins) and we have shown that not only is serum from sheep a particularly. good source of activator for in vitro experiments but also that serum from fasted sheep has a greater activating effect than that from ad libitum fed sheep (Tume and Thornton 1985). The reason for this

increased activating effect of serum from fasted animals is not known, but the enhanced activity promoted by such serum would ensure that those tissues which continue to synthesize LPL during the fasting state (eg. cardiac and skeletal muscle) are able to assimilate the relatively low amounts of circulating TAG. In studies with humans, Hallberg(1965) observed that the elimination of intravenously infused artificial fat emulsions (Intralipid) was much greater in patients who had fasted for 39 hours compared with those fasted for only 15 h. Presumably TAG was rapidly directed to those tissues in need of a readily utilizable energy source. Therefore) a combination of the increased activating effect observed for serum from fasting animals and the low Km of cardiac muscle (Fielding 1976) and skeletal muscle (Lithe11 and Boberg1978) LPL for TAG may explain how these tissues continue to metabolise TAG under fasting conditions at rates similar to those of fed animals.

Further investigation of the total plasma apolipoproteins by SDS polyacrylamide gel electrophoresis were unable to show any gross differences between the fasted and ad libitum fed sheep, but by studying only those apoproteins that would bind to TAG emulsions following short incubation, we have been able to show that a greater amount of the apoproteins from fasted serum were bound to TAG. Further, when these apoprotein-containing washed TAG emulsions were used as substrates for LPL, those preparations from fasted sheep serum gave a 2-fold higher activation. In an attempt to determine whether there is more activator present in fasted sheep serum, or whether the activator present is more readily transferred to the substrate emulsi on, we have isolated the activator protein(s) in pure form from sheep plasma. Using essentially the method of Astrup and Bengtsson (1982), the activator protein was bound to an artificial TAG emulsion (Intralipid), along with certain other apoproteins. Gel filtration and ion exchange chromatography resulted in the isolation of two distinct activator proteins for LPL (Tume et al. **1987).** Although isoelectric variant forms of activator proteins have been observed within other species (Lim and Scanu **1976;** Clegg **1978;** Have1 et al. 1979) they are about the same molecular size (Mr 8-9,000). The ovine activators, present in about equal amounts, were well separated by isoelectric focussing and were readily separated on the basis of size (Mr about **8,000** and 5,000). Each of the ovine activator proteins was as effective as human apolipoprotein CII in activating LPL, with 1 ug/ml giving near to maximum activation.

In man and other animals, LPL is under hormonal control, being dependent upon various nutritional., environmental, physiological and genetic-pathological conditions. It is also possible to inhibit LPL in vivo in the short term, by a number of techniques. LPL is strongly inhibited following injection of the non-ionic detergent, Triton WR1339, resulting in the rapid accumulation of TAG-rich lipoproteins in the plasma (Fiser et al. **1974).** Whereas in monogastrics the plasma Triton is rapidly removed, Triton concentrations in ruminants remained high over a period of weeks (Mamo et al. 1983;O'Kelly, personal communication), resulting in an elevated plasma TAG concentration throughout this period. LPL activity has also been blocked in chickens by the injection of highly purified antibodies to LPL (Kompiang et al. **1976;** Behr et al. 1981). However, because the half-life of functional LPL is only a few minutes (Wallinder et al. 1979), repeated injections of antibodies are required to maintain LPL inhibi tion. On the other hand, the activator of LPL, apolipoprotein CII,

has a half-life of a number of days (Huff et al. 1981) and therefore is likely to be more suitable for immunologic-getting and thus maintain reduced LPL activity for a longer period.

Certain genetically-linked lipid disorders have been described in humans where either LPL or its activator, apolipoprotein CII, are absent. Such patients are unable to remove plasma TAG through the normal pathways and consequently, high plasma TAG results. Although a deficiency of LPL usually has more severe clinical manifestations than does a deficiency of activator, it is possible by restricting dietary fat intake to reduce plasma TAG and maintain a patient free of symptoms. It therefore follows that manipulation of the plasma TAG removing system can have a profound effect on the deposition of fat in various tissues. In the relatively short time required for growth of meat animals, particularly lambs, to market size, the effects of high plasma TAG are unlikely to have any serious effect on the health and welfare of the animals.

It has been known for some time that LPL activity is suppressed and plasma TAG concentrations increased when patients acquire certain infections and malignancies (see Torti et al. 1985). The resulting catabolic state can also be induced in experimental animals following injection of live gram-negative bacteria or their endotoxins (Bagby and Spitzer 1981; Kawakami and Cerami 1981). Recently, such trauma has been shown to cause the release of a protein, cachectin, from macrophages into plasma (Beutler et al. 1985) which binds to specific receptors in muscle, liver and adipose tissue. This low molecular weight monokine (subunit 17,000 daltons) has been purified and shown to be responsible for the inhibition of LPL synthesis and other lipid synthesizing enzymes in 'vivo, and in 3T3-L, cells in tissue culture. Cachectin is believed to be identical to tumour necrosis factor (Price et al. 1986), a cytotoxic protein present in the plasma of certain cancer patients. This is supported by studies on amino acid sequencing, monoclonal antibodies and similarity of function, and suggests that both the anti-tumour activity and inhibition of lipid synthetic activity reside in the same molecule. It is therefore interesting to speculate on the possibility of isolating that fragment of cachectin which is responsible for the inhibition of lipid synthetic activity as distinct from the intact protein which also contains anti-tumour activity. Such a polypeptide fragment should be specific for inhibition of lipid synthesis and free of any of the cytotoxic effects of the whole molecule.

IMMUNOLOGICAL CONTROL OF FAT DEPOSITION

We have mentioned the possible short term control of LPL and its activator(s) by immunological techniques as a means of reducing fat deposition in adipose tissues of meat animals. Whereas this technique allows fairly specific targetting of a fat accumulation pathway, others have used a broader approach, attacking fat already deposited in the fat tissue. Flint and co-workers (1985, 1986) prepared antiserum in sheep by injecting them with purified rat adipocyte plasma membranes. When al i quota of sheep anti-rat adipocyte plasma membrane serum, or the purified antibodies, were injected into rats daily for 4 days and then killed 4 days later, there was evidence of a massive infiltration of lymphocytes into adipose tissue accompanied by a considerable breakdown of fat cells. The cytotoxic effect of the antiserum was still in evidence at 2 months after

the initial injections, as essentially no recovery of fat cells or growth of adipose tissue had occurred. Analysis of the carcass at this time indicated a 30% reduction in fat content and a 5% and 7% increase in protein and water content respectively (Flint and Futter 1985).

We have repeated the experiments of Flint et al. (1986), but using horse anti-sheep adipocyte plasma membrane serum for interperi tonal injection into lambs. Horse antiserum gave strong precipitation lines against Triton solubilized sheep plasma membrane and we have attempted to identify, by immunoprecipitation, those membrane proteins which are strongly antigenic. However, using a similar protocol to that used by Flint et al. (1986), repeated injections of the Y-globulin fraction of the antiserum into sheep, failed to produce any significant reaction to fat tissue. Adipose tissue LPL was the same in control and treated animals (56 vs. 54 nmol FFA/min/g tissue), and plasma TAG (379 vs. 370 μmol/ℓ) and FFA concentrations (323 vs. 351 μeq/ℓ) were unaltered. No gross differences were observed in the carcass fat content, but there was evidence of cytotoxicity in those lambs receiving the "immune" Y-globulin, as a significant increase in plasma lactate dehydrogenase was found (0.52 vs. 0.65 μmol lactate/min/ml plasma for controls vs. treated respectively).

The reason for our failure to observe any alteration to gross carcass fat composition and lipid synthetic activity may well have resulted from the short time course used, considering lambs (≈ 20 kg) are much larger than the rats (100-120g) used by Flint et al. (1986) in their studies. Current experiments are addressing this aspect.

CONCLUSIONS

There is an increasing demand for leaner meat throughout western societies , including Australia. It is clear that β_2-adrenergic agonists have the capacity to allow these demands of the market to be met using existing breeds of sheep and cattle and current husbandry practices. Implants could readily be inserted at marking time. The commercial application of β_2-adrenergic agonists for use in ruminants will be constrained by political regulatory considerations rather than technical or economic factors.

Immunological control of fat deposition, either by regulating the differentiation of adipocytes, reducing the activity of adipose tissue LPL directly or indirectly through its apoprotein activators, or through the use of adipose tissue plasma membrane proteins as antigens, would seem to be a promising approach for lean lamb production.

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