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MODES OF ACTION OF REPARTITIONING AGENTS IN SHEEP

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SUMMARY

Experiments were conducted on growing lambs and isolated ovine subcutaneous adipocytes to study the regulatory mechanisms of the β_2-adrenergic agonist clenbuterol on ovine lipid metabolism.

The growth rate (~ 200 g/day), carcass weight (~ 19.0 kg) and ultimate pH of the L.dorsi muscle (~ 5.6) were not different when comparing clenbuterol treated (2.5 μg/kg liveweight/day) and control animals. However, clenbuterol treatment significantly reduced the fat content of the carcass meat (28.2% vs. 35.2%; 5.4 vs. 6.6 kg) and increased the weight of lean from the carcass (13.6 vs. 12.2 kg). Clenbuterol treatment resulted in increased serum levels of free fatty acids (518 vs. 387 μeq/l) and growth hormone (5.1 vs. 4.0 ng/ml) and decreased insulin levels (1.4 vs. 2.0 ng/ml). Lipogenesis (acetate incorporation) was reduced and lipolysis (glycerol release) was stimulated in isolated adipocytes when clenbuterol was added to the incubation medium. However, isolated adipocytes from clenbuterol treated animals showed similar rates of lipogenesis and lipolysis to those from control animals. Ovine growth hormone at levels up to 1000 ng/ml did not markedly affect lipogenesis or lipolysis of isolated adipocytes.

It is suggested that the repartitioning effects of clenbuterol could be ascribed to either direct effects on the lipid metabolism of adipocytes and/or indirect effects through the alteration of circulating levels of regulatory hormones (growth hormone and insulin) known to affect both lipid and protein metabolism.

INTRODUCTION

There is increasing interest in the efficient production of lean meat from domestic livestock. The long term approach to this goal has been the development of genetic selection and crossbreeding programmes. The advent of transgenic animals could increase the rate of progress of such genetic programmes. However, the development of certain pharmaceutical compounds, known as repartitioning agents, presents a possible short term solution to the production of lean meat from existing breeds of domestic livestock raised by conventional livestock husbandry practices. The term "repartitioning agents" describes the shift of dietary nutrients away from fat deposition and towards protein deposition in animals treated with, or ingesting, β_2-adrenergic agonists (1).

In our previous in vivo studies on pen-fed weaners and on suckling/grazing lambs, small quantities (50-100 μg/day) of the β_2-adrenergic agonist, clenbuterol, were injected subcutaneously. Control animals received an equal volume of physiological saline. Clenbuterol treatment had no significant effects on growth rate and carcass weight, but the amount of fat was decreased and the amount of lean increased by as much as 30%, respectively, in the carcass meat of treated sheep relative to controls (2).

To further study the regulatory mechanisms of clenbuterol on ovine lipid metabolism, in vivo and in vitro (isolated adipocyte) experiments were conducted. Preliminary accounts of this work have been presented (3, 4).

METHODS

The in vitro studies on isolated ovine subcutaneous adipocytes were conducted as previously described (2), using cells isolated from biopsied adipose tissue. The isolated cells were incubated in medium containing 3 mM glucose, 1 mM acetate, 0.5 mM lactate, 1% albumen at pH 7.4 and 39°C. Each incubation contained about 10^5 cells in a final volume of 1.2 ml. The concentrations of clenbuterol and ovine growth hormone ranged from 0 to 1000 ng/ml. Acetate incorporation into lipid and glycerol release into the medium were estimated using previously described methods (5, 6).

The in vivo study was on a group of 12 crossbred lambs weighing 33 kg fed ad libitum a diet of 70% lucerne chaff and 30% crushed grain. Six were injected subcutaneously with clenbuterol (2.5 μg/kg/day) in physiological saline (treated) and the remainder (controls) received an equivalent volume of physiological saline. On the day prior to slaughter the sheep were bled from the jugular vein each half hour from 0900 to 1500 hrs. Serum growth hormone and serum free fatty acid concentrations were measured by established methods (7, 8). The ultimate pH of the L.dorsi muscle was measured using the technique of Bouton et al (9). Other relevant procedures have been described (2).

RESULTS AND DISCUSSION

In the in vitro studies clenbuterol was found to markedly reduce acetate incorporation into lipid (lipogenesis) and to stimulate glycerol release into the medium (lipolysis). At clenbuterol concentrations of 100 ng/ml lipogenesis was at basal levels (1 μmol/g lipid/hr) and lipolysis rates were high (3 μmol/g lipid/hr; see Figure 1). These results are in good agreement with our previous reports in which the lowest clenbuterol

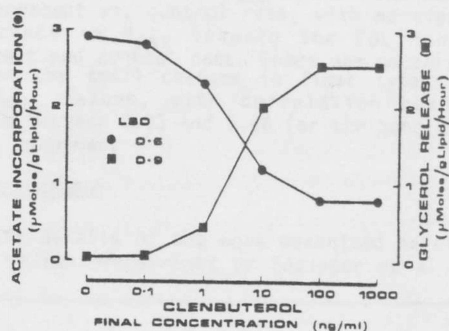

Figure 1: Plot of acetate incorporation (lipogenesis) and glycerol release (lipolysis) against the concentration of clenbuterol (ng/ml) in the media. Mean of 8 experiments.

concentration employed was 100 ng/incubation (10, 2). β_2-adrenergic agonists presumably stimulate the activity of the hormone sensitive lipase/cyclic AMP-phosphorylase enzyme system within the adipocyte and thus promote increased lipolysis. The mechanism by which clenbuterol reduces lipogenesis from acetate, or glucose (17), is unknown. Clenbuterol also decreases the uptake and incorporation of long chain fatty acids into lipid of isolated ovine adipocytes (18).

Adipocytes prepared from control and clenbuterol treated animals showed similar levels of both lipogenesis and lipolysis in control medium (zero clenbuterol) and responded to added clenbuterol in a similar way (see Figure 2a and b). On the basis of our previous in vitro experiments (10, 2) we expected adipocytes from clenbuterol treated animals to exhibit lower rates of lipogenesis and higher rates of lipolysis. This was clearly not the case, but similar findings have been reported (11). High, unphysiological concentrations of ovine growth hormone (greater than 10 ng/ml), marginally depressed lipogenesis but were without significant effect on lipolysis rates. (See Figure 2a and b). This was an unexpected finding as growth hormone is commonly regarded as lipolytic, particularly in the lactating animal (12). However, a similar lack of lipolytic response of isolated ovine adipocytes to growth hormone has been reported (13).

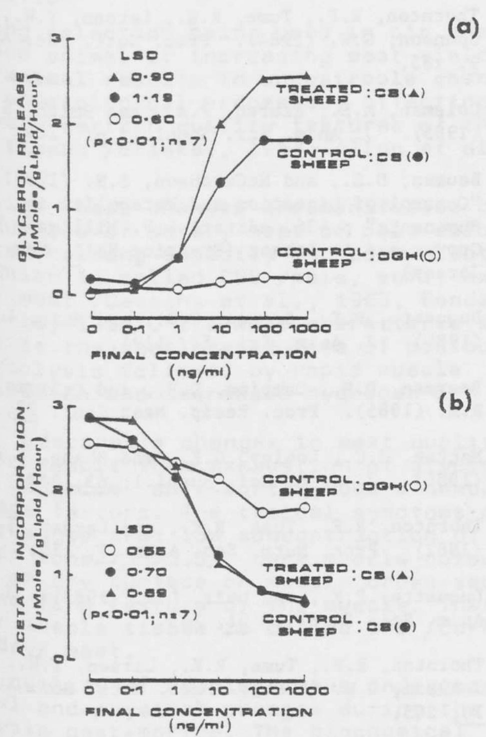

Figure 2: (a) Plot of glycerol release (lipolysis) and (b) acetate incorporation (lipogenesis) against the final media concentration (ng/ml) of ovine growth hormone (o; OGH) and clenbuterol (•; CB) for control (o, •), and for clenbuterol treated sheep (▲). Mean for 7 experiments.

The growth rates, final liveweight, carcass weight and boned out meat weight of both the groups of sheep were similar, but clenbuterol treatment reduced the fat content of meat by some 20% whilst the lean increased by a similar amount (Table 1). Similar findings have been reported previously (2). There was no difference in the ultimate pH (5.6) of the L.dorsi muscle between the control and treated animals. This contrasts with the higher muscle pH (6.2) of cimaterol (analogue of clenbuterol) treated lambs, when compared to controls (muscle pH 5.9), previously reported (14). Clearly, pH and other attributes of meat quality, such as colour, tenderness and flavour, from animals treated with β_2-adrenergic agonists, warrant further study.

Treatment with clenbuterol significantly increased the serum levels of free fatty acids and growth hormone, but significantly decreased the levels of insulin in serum (Table 1; Figure 3a, b and c). Similar results have been reported (14). Thus the repartitioning effects of clenbuterol could be ascribed to either direct effects on the lipid metabolism of the adipocytes, and/or indirect effects through the alteration of circulating levels of regulatory hormones known to affect lipid metabolism. Clenbuterol has been shown to reduce muscle protein catabolism and leucine oxidation, but to have little effect on whole body protein synthesis in sheep (15).

Table 1. Growth, Carcass and Serum Characteristics of Clenbuterol Treated and Control Sheep.

	Control	Clenbuterol Treated	Signif.
Growth Rate (g/day)	205	198	NS
Liveweight (kg)	46.6	47.6	NS
Carcass Wt. (kg)	24.2	24.3	NS
Meat Wt. (kg)	18.8	19.0	NS
Fat Thickness (mm)	7.7	6.0	NS
Meat pH	5.6	5.6	NS
Meat Fat (%)	35.2	28.2	*
Meat Fat Wt. (kg)	6.6	5.4	*
Lean Wt. (kg)	12.2	13.6	*
Growth Hormone (ng/ml)	4.0	5.1	**
Insulin (ng/ml)	2.0	1.4	***
FFA (μeq/l)	387	518	***

Similarly these responses in muscle protein metabolism could be direct effects on muscle cells, and/or indirect effects induced through changes in circulating levels of regulatory hormones, as described in the present study on sheep.

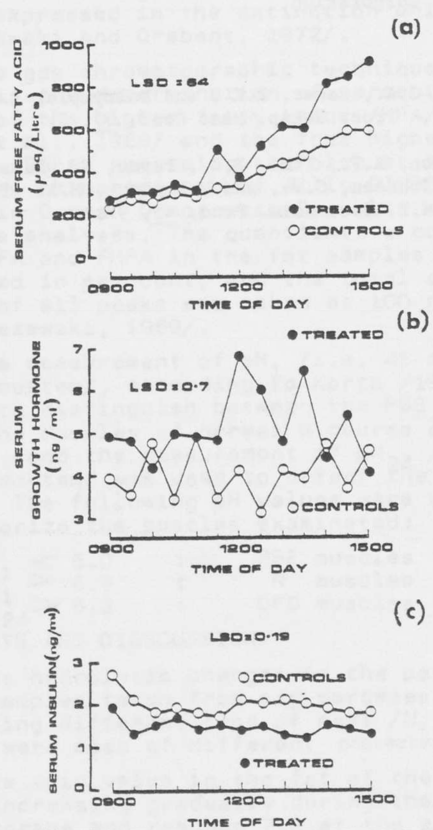

Figure 3: (a) Serum free fatty acid, (b) growth hormone and (c) insulin levels of control (o) and clenbuterol treated (•) sheep. Mean of 5 sheep.

On the basis of the negligible in vitro responses of adipocytes to growth hormone reported here, and to insulin (16), clenbuterol-induced changes to circulating levels of growth hormone (+ 25%) and insulin (- 30%) would not appear large enough to exert major changes to lipid metabolism of ovine adipocytes. However, such extrapolations can be dangerous. The advent of large scale production of recombinant growth hormone and insulin should permit in vivo experiments on sheep to test such a hypothesis.

CONCLUSIONS

These studies on isolated ovine adipocytes and growing lambs indicate that the β_2-adrenergic agonist, clenbuterol (a) was not detrimental to growth rate and carcass yield, (b) markedly reduced the fat content, and increased the lean, of carcass meat, (c) did not affect the ultimate pH of muscle, (d) increased lipolysis and decreased lipogenesis in adipocytes and (e) increased growth hormone and decreased insulin levels in serum.

Both the direct effect of clenbuterol on the lipid metabolism of adipocytes and its indirect effects of altering the circulating levels of regulatory hormones, contribute to the repartitioning of dietary nutrients away from fat deposition and towards protein deposition in the carcass meat of growing lambs.

The commercial application of β_2-adrenergic agonists for lean meat production from ruminants will be constrained by political regulatory considerations rather than technical or economic factors.

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