The influence of the β_2-adrenergic agonist, clenbuterol, on lipid metabolism and carcass composition of sheep

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ABSTRACT

β_2-adrenergic agonists have been described as "repartitioning agents" and "potential anti-obesity drugs" because of the decreased fat deposition they have induced in laboratory rodents and some domestic animals.

The influences of clenbuterol, a β_2-adrenergic agonist, on lipid metabolism of isolated ovine adipocytes and the carcass meat composition of suckling/grazing lambs and pen-fed weaner lambs are reported here.

Clenbuterol decreased rates of lipogenesis and increased rates of lipolysis in isolated adipocytes. This suggests that clenbuterol may have induced an increased rate of the triacylglycerol-fatty acid substrate (futile) cycle in these cells.

Growth rate of clenbuterol treated lambs was not different to that of controls in either growth experiment. Suckling/grazing lambs were slaughtered at a mean carcass weight of 16.0 kg which yielded 12.5 kg of boned out meat, containing 24.6% fat in control animals *v* 17.2% in clenbuterol treated animals or 3.2 *v* 2.1 kg of fat respectively. The pen-fed weaners were slaughtered at a carcass weight of 23 kg which yielded 19.3 kg of meat containing 38.3% fat in the controls *v* 27.5% in clenbuterol treated animals or 7.5 *v* 5.3 kg of fat respectively. In both experiments the ~30% reduction in fat was replaced with an equivalent amount of lean tissue. Responses to clenbuterol were similar in ewe, wether and ram lambs and clenbuterol caused no apparent local or general ill effects in any of the lambs.

Clearly the metabolic consequences of treating lambs with clenbuterol are such that carcass fat deposition is decreased and protein deposition is enhanced. These responses are discussed in terms of the synthetic and catabolic biochemical mechanisms which underly both fat and protein accretion in the meat of lambs.

Keywords Clenbuterol; β_2 agonists; adipocytes; lipogenesis; lipolysis; lambs; carcass composition; growth rate.

INTRODUCTION

That there is an economic need to produce larger leaner lamb carcasses is undeniable. The report of the Lean Meat Production Working Party (MAF, 1983) states "NZ lamb is perceived by many consumers as lacking versatility, sometimes tough and as being excessively fatty, with an unattractive appearance compared to poultry and other red meats. Excess fat is seen as the main problem". The per capita consumption of lamb in Australia has dropped from 23-24 kg in the early 1970's to 15-16 kg in the early 1980's (see Thornton and Larsen, 1985). Although market forces e.g., price, packaging, presentation and promotion, have undoubtedly influenced consumer preference, the underlying factor that sheep meat is an energy dense food (Thornton and Larsen 1985) has been detrimental to lamb consumption in affluent Western societies. Furthermore, the increasing Middle East markets demand, for largely cultural reasons, larger leaner lamb carcasses. All indicators suggest that these trends will continue.

For many years researchers interested in the regulation of lipid metabolism, and particularly those working in the area of human obesity, have been searching for compounds, synthetic or naturally occurring, which would reduce fat deposition and/or promote fat mobilisation. Very recently the compounds known as β_2-adrenergic agonists have been shown to significantly reduce fat deposition in laboratory rodents (Arch *et al.*, 1984; Emery *et al.*, 1984) and in domestic animals (Ricks *et al.*, 1984). In all of these studies lean tissue deposition in the treated groups has been greater or similar to that of control animals and skeletal muscle protein synthesis was increased by some 30% in the rats studied by Emery *et al.* (1984). These responses have invoked the term "repartitioning agents" (Ricks *et al.*, 1984) and these compounds have been promoted as potential "anti-obesity drugs" (Arch *et al.*, 1984). This paper reports the influence of the β_2-adrenergic agonist, clenbuterol, on the lipid metabolism of isolated ovine adipocytes and on the composition of the meat from the carcasses of lambs run with their

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mothers at pasture, and from pen-fed weaner lambs.

METHODS

Isolated Adipocyte Studies

Subcutaneous adipose tissue was biopsied from near the base of the tail of anaesthetised (Nembutal) sheep (weighing approximately 26 kg live weight and fed a diet of 50% lucerne chaff and 50% cattle feedlot pellets *ad libitum* to ensure positive growth). Adipocytes were isolated using procedures similar to those outlined by Rodbell (1964). The biopsied tissue was immediately placed in a collagenase solution (40 mg of collagenase (Boehringer Mannheim) in 20 ml of buffer (Hepes, 25 mM, 2 ml; Krebs buffer, ½ Ca^{++}, pH 7.4, 2 ml; glucose 0.5 M, 20 μl; and H_20, 16 ml containing 4% w/v albumin)) at pH 7.4, thoroughly diced and digested for approximately 90 minutes. The digested tissue was filtered through nylon gauze and the cells washed with incubation medium (glucose 3 mM, acetate 1 mM, and lactate 0.5mM in Krebs buffer, ½ Ca^{++}, containing 1% albumin, pH 7.4). All steps were conducted at 39°C, with gentle agitation in polythene containers. Some 10^5 cells (50 to 100 mg of lipid) were incubated (2 ml) with [1-^{14}C]-acetate for 2 hours in disposable polythene transfer pipettes at 39°C for the estimation of rates of acetate incorporation into lipid (lipogenesis), as described by Hood and Thornton (1980) and glycerol release into the incubation medium, by the method of Eggstein and Kuhlmann (1974).

Pen-fed Weaner Lambs

A group of 30 crossbred lambs (Dorset Horn x Border Leicester/Merino) weighing approximately 28 kg live weight were divided, at random, into 3 groups of 10 (5 ewes and 5 wethers) and offered a diet of 50% lucerne chaff and 50% cattle feedlot pellets *ad libitum*. They were injected subcutaneously every second day with either 0.9% saline (controls), 50 μg, or 100 μg of clenbuterol/d (treated). The site of injection was rotated around the 4 bare skin areas (leg pits) of each sheep. Two sheep from each treatment, 1 ewe and 1 wether, were slaughtered on days 1, 35, 76, 132 and 152.

Suckling Grazing Lambs

A group of 36 crossbred single lambs (Dorset Horn x Border Leicester/Merino) aged 4 to 8 weeks was subdivided into 3 sex groups, rams, wethers and ewes (12/group). They were ear tagged, weighed and marked (tails docked and 12 rams surgically castrated) and allocated to treatment groups. Half of each group (6 lambs) were subcutaneously injected with 0.9% saline (controls) and the other half with clenbuterol (2.5 μg/kg live weight/d (treated)). Their initial live weights ranged from 10 to 16 kg and the lambs grazed with their mothers on irrigated ryegrass-berseem clover pastures. Every second day the lambs were brought into yards and injected as described for the pen-fed lambs. The lambs were weighed at fortnightly intervals and the dosage of clenbuterol adjusted to their increased live weight. They were slaughtered in two groups (16 on day 85 and 20 on day 106) at live weights ranging from 30 to 38 kg. Clenbuterol used in both these growth studies was donated by Boehringer Ingleheim and was 30 μg/ml of 0.9% saline. Thus the maximum volume subcutaneously injected was ~7 ml. Procedures used to slaughter the animals, dress the carcasses, bone out the carcasses, grind and sample the meat from the carcasses and for estimating its moisture, fat and protein content, have been previously described (Thornton *et al.*, 1979).

RESULTS

Isolated Adipocyte Studies

Clenbuterol, at the level of 0.1 μg/incubation (2 ml) reduced lipogenesis rates (acetate incorporation) from control values of ~160 (μg/g lipid/h) down to basal levels of ~30. Increased levels of clenbuterol up to 10 μg/incubation had no further influence on lipogenesis (see Fig. 1). On the other hand lipolysis rates (glycerol release : μg/g lipid/h) increased from 350 to 700 with increasing levels of clenbuterol (0.1 μg to 10 μg/incubation) and 0.1 μg of clenbuterol increased lipolysis by a factor of 3 fold over control levels (150 to 350 see Fig. 1).

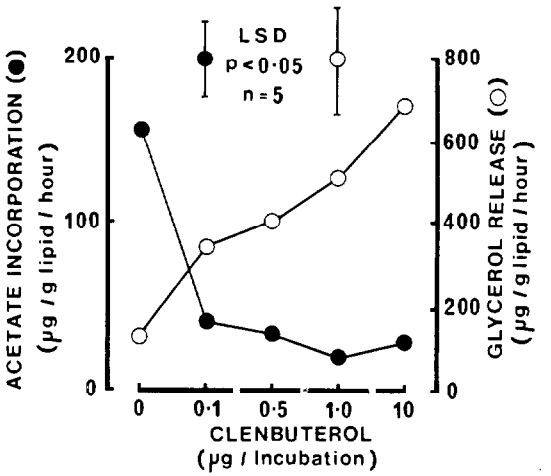

FIG. 1 Rates of lipogenesis (acetate incorporation) and lipolysis (glycerol release) from isolated ovine adipocytes incubated with clenbuterol.

Pen-fed Weaner Lambs

None of the sheep, controls or treated, suffered any infections, localised necrosis or any ill effects from the injections. These sheep had an average growth

rate of ~140 g/d and clenbuterol treatments did not significantly influence growth rates. Similarly carcass weight gain (~72 g/d) was not influenced by treatment and carcass weights ranged from 10.7 to 24.5 kg. Clenbuterol treatment resulted in a significant ($P < 0.01$) reduction in the percentage of fat in the meat (see Fig. 2). Consequently clenbuterol treated animals had significantly ($P < 0.01$) less fat (see Fig. 3), and significantly more ($P<0.01$) lean than did controls. The level of clenbuterol (ie., 50 or 100 μg/d) had no significant ($P<0.05$) effect on any of the carcass meat parameters. Regression analysis indicates that for a 12 kg gain in carcass weight, some 11.3 kg of meat was gained of which 42% was lean and 58% was fat in the control animals *v* 62% lean and 38% fat in the 100 μg treatment group. These findings are summarised in Table 1 which contains data from the last 4 animals killed in each treatment group. Carcasses weighing 23 kg contained 19.3 kg of meat which was 38% fat in the control and 27% fat in the treated animals; control animals yielded 12 kg of lean, 7.5 kg of fat and 2.5 kg of protein *v* 14 kg of lean, 5.3 kg of fat and 2.8 kg of protein from the meat of treated animals. Sex did not significantly ($P<0.05$) influence any carcass composition variable although the ewes tended to be fatter than the wethers.

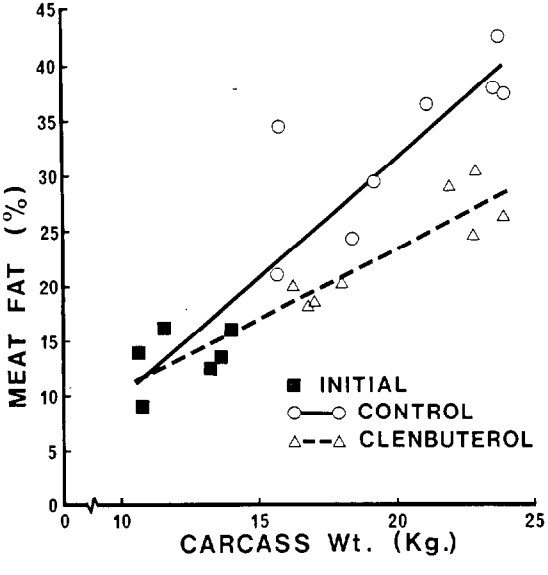

FIG. 2 Plot of percentage fat in carcass meat *v* carcass weight (kg) of pen-fed weaner lambs.

The fatty acid composition of the subcutaneous adipose tissue of the treated animals contained less palmitate (23 *v* 26%) and more stearate plus oleate (67 *v* 63%; see Table 1).

Suckling Grazing Lambs

These lambs gained live weight at ~200 g/d and treatment had no significant effect on live-weight gain (see Fig. 4). Repeated subcutaneous injections caused no infections or localised necrosis and none of the lambs showed any ill effects from the treatment. Carcass weights ranged from 13.40 to 19.20 kg (mean 16.0) and the weight of boned out meat ranged from 10.25 to 15.35 (mean 12.5) but neither variable was significantly influenced by treatment or sex ($P>0.05$

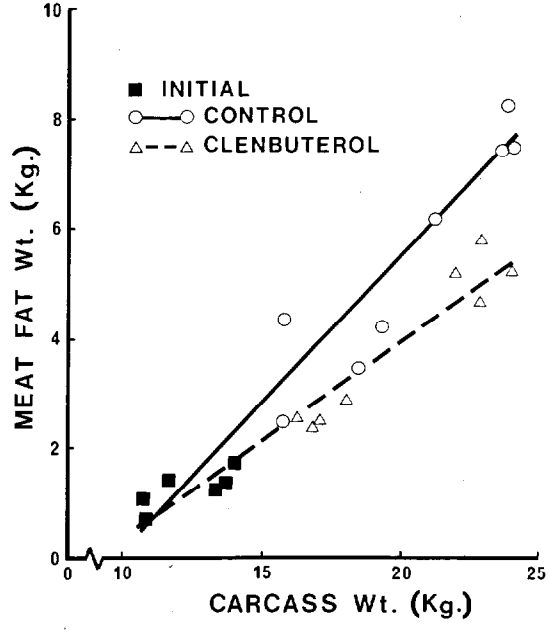

FIG. 3 Plot of the weight of fat (kg) in carcass meat *v* carcass weight (kg) of pen-fed weaner lambs.

TABLE 1 The effect of clenbuterol for 132 to 152 days on carcass components and fatty acid composition of the fat of pen-fed weaner lambs (n = 4).

Variable	Clenbuterol (μg/day) 0	50	100	LSD ($P<0.05$)
Carcass wt (kg)	23.15	23.08	22.93	1.97
Meat wt (kg)	19.45	19.13	19.26	2.05
Meat fat (%)	38.3	27.8	27.3	5.25
Meat fat wt (kg)	7.46	5.32	5.24	1.50
Lean meat wt (kg)	11.99	13.81	14.02	1.50
Meat protein (%)	12.8	14.6	14.8	0.75
Meat protein wt (kg)	2.50	2.79	2.85	0.55
Palmitate (16:0,%)	26.1	23.3	23.5	1.45
Stearate (18:0,%)	27.9	28.2	30.0	1.42
Oleate (18:1,%)	35.2	37.0	37.8	1.68
Stearate + oleate (%)	63.1	65.2	67.8	1.41

TABLE 2 The effect of clenbuterol (2.5 μ g/kg live weight /d) of suckling-grazing lambs. (C = control; T = treated)

Variable	Ewes		Wethers		Rams		LSD
	C	T	C	T	C	T	($P<0.05$)
Carcass wt (kg)	16.2	15.8	16.9	15.8	15.7	15.6	1.12
Meat wt (kg)	12.9	12.4	13.4	12.4	12.2	12.0	0.99
Meat fat (%)	30.1	20.2	24.3	17.4	19.5	14.0	3.35
Meat fat wt (kg)	3.91	2.59	3.31	2.16	2.38	1.71	0.57
Lean meat wt (kg)	8.94	9.79	10.09	10.20	9.77	10.30	0.61

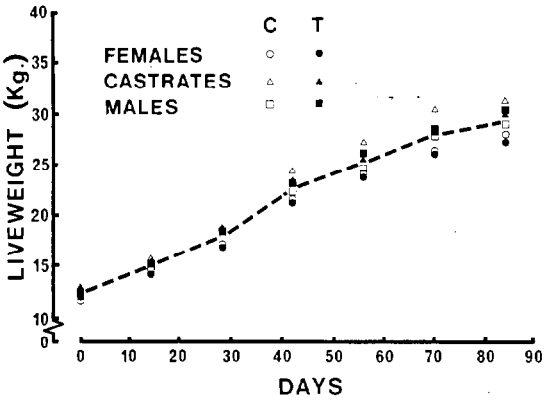

FIG. 4 Live weight gains of suckling-grazing lambs.

see Table 2). However, both treatment ($P<0.001$) and sex ($P<0.01$) significantly influenced the percentage fat in the meat and consequently the amount of fat in the meat, but the interaction (treatment x sex) was not significant for either parameter. Treatment reduced the fat content of the meat from 24.6 to 17.2% or 3.20 to 2.15 kg. Meat from ewes contained 25.2% fat (3.25 kg), from wethers 20.8% (2.74 kg) and from rams 16.7% (2.04 kg; see Table 2).

DISCUSSION

Clenbuterol clearly has the capacity to markedly reduce fat accumulation in the meat of growing lambs. Regardless of sex, age or diet the carcass meat of clenbuterol treated lambs contained some 30% less fat than that of controls in both the *in vivo* studies reported here. Because clenbuterol treated pen-fed lambs had less palmitate and more stearate and oleate in their depot fat we erroneously thought that clenbuterol may not exert such a great effect on depot fat accumulation in suckling-grazing lambs. Dietary long chain fatty acids could have been expected to contribute the greater proportion of fat accumulation in the suckling-grazing lambs as these animals would have had a high fat intake from ewes' milk and pasture. Lipogenesis, from acetate, would have been a major contributor to fat accumulation in the pen-fed lambs which had a lower dietary fat intake than the suckling-grazing lambs (reviewed by Thornton and Tume, 1984). These findings compare favourably with the 40% reduction in fat depth over the 12th rib of finishing lambs eating a diet containing 2 ppm clenbuterol reported by Ricks *et al.* (1984). However there was no indication, in either of the present experiments, that clenbuterol increased growth rate as claimed by Ricks *et al.* (1984). These workers have also claimed improved food conversion in lambs fed diets containing clenbuterol.

Furthermore, lean tissue mass and protein accumulation were promoted by clenbuterol treatment in the present experiments on growing lambs, to the extend that lean tissue (largely muscle) quantitatively substituted for the fat which was not laid down. This result supports the concept of clenbuterol acting as a "repartitioning agent" as defined by Ricks *et al.* (1984) ie., "an agent which will direct substrates away from adipose tissue towards muscle accretion". Such a definition can only be supported in terms of net effects and not in terms of mechanisms or processes. In the present experiments on lambs, there was no evidence of massive muscular hypertrophy (41.5%), described by the Cyanamid group (Ricks *et al.*, 1984; Ingle, personal communication). In contrast to all of these findings on sheep, Emery *et al.* (1984) found that in rats both clenbuterol and fenoterol (another β_2-adrenergic agonist) increased body protein and water mass but body fat was not significantly different from that of controls.

The mechanism(s) by which β-agonists, eg., clenbuterol, influence changes in body composition through alteration to both energy and protein metabolism are not clear. Ricks *et al.* (1984) have proposed a simple theoretical overview in which β-agonists reduce fat synthesis and protein catabolism while stimulating fat mobilisation and protein synthesis. Clenbuterol reduced lipogenesis (acetate incorporation) and stimulated lipolysis in our studies on isolated ovine adipocytes. We have previously shown that adrenalin markedly depresses acetate incorporation into lipid of isolated ovine adipocytes (Thornton *et al.*, 1982) so this similar effect of clenbuterol was not unexpected. Similarly, β-agonists have been shown to stimulate lipolysis

rates of both white adipose tissue (Mersmann, 1979) and brown adipose tissue (Arch *et al.*, 1984). That lipogenesis was depressed and lipolysis stimulated by clenbuterol could indicate that the rate of the triacylglycerol-fatty acid substrate (futile) cycle in these isolated ovine adipocytes was increased. Brooks *et al.* (1982) have shown that both fenoterol and noradrenalin promote high increases in the rate of this substrate cycle in isolated rat adipocytes. We have studied the effect of clenbuterol on stearate incorporation into lipid of isolated ovine adipocyte (data not shown) and found variable responses. On balance, clenbuterol seems to have little influence on stearate incorporation, but this area needs further research before conclusions can be made. Both clenbuterol and fenoterol increased fractional muscle protein synthesis rate and total protein mass in rats (Emery *et al.*, 1984). These workers suggest, as an explanation of the measured fractional protein synthesis rates being higher than the rate of protein accumulation, that muscle protein degradation may also have been slightly increased.

The fact that repeated subcutaneous injections of clenbuterol caused no local infections, tissue necrosis or any other visible ill effects suggests that it could be delivered to the lamb's tissues from a subcutaneous implant. This warrants further research if clenbuterol, or related compounds are to be developed for use in grazing animals. An implant could easily be inserted at lamb marking time. Clenbuterol is used as a therapy by asthmatics and to assist respiration in coughing horses; its use for lean lamb production lies in the hands of regulatory bodies.

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