Ann Zootech (1993) 42, 361-369

Original article

The effect of cimaterol and oestradiol-17β, given alone or combined, on ovine lipid metabolism, plasma metabolites and tissue cyclic AMP concentrations

JR Scaife, EM Alderson, H Galbraith

Department of Agriculture, University of Aberdeen, 581 King Street, Aberdeen AB9 1UD, UK

(Received 29 January 1993; accepted 19 April 1993)

Summary — The effect of cimaterol (2 mg/kg dry matter in the diet) and oestradiol-17β (OE, 15 mg implanted sc) given alone or combined for 62 or 63 d was studied in 24 male castrate sheep. Neither cimaterol nor OE treatment *in vivo* affected indices of lipid metabolism. However, *in vitro* studies suggested that added cimaterol: a) had little effect on lipogenesis; and b) increased lipolysis in subcutaneous adipose tissue (SCAT) at least as effectively as epinephrine. Plasma urea concentrations were reduced on d 7, 14 and 21 by cimaterol and on d 28 and 42 by OE. Plasma NEFA was reduced by OE treatment on d 7. Serum triacylglycerol concentrations were not affected by the treatment. Cimaterol reduced cyclic AMP concentrations in skeletal muscle (m gluteus) preparations. Treatment with OE reduced lipoprotein lipase activity of SCAT.

cimaterol / oestradiol-17β / lipogenesis / lipolysis / cyclic AMP

Résumé — Effet du cimatérol et de l'oestradiol-17β administrés seuls ou de façon combinée sur le métabolisme des lipides des ovins, les métabolites plasmatiques et les concentrés AMP cycliques des tissus. *Les effets sur le métabolisme lipidique et la concentration de cAMP dans les tissus d'un traitement avec cimatérol (2 mg/kg matière sèche dans l'aliment) et œstradiol-17β (OE, 15 mg implanté en sous-cutané) seul ou ensemble pendant 62 ou 63 jours, ont été étudiés dans 24 Finn x Dorset béliers castrés. Les vitesses de lipogenèse et de lipolyse ont été mesurées* in vitro *dans le tissu adipeux sous-cutané (SCAT) immédiatement après abattage, avec ou sans addition de cimatérol (0,15 et 1,5 μmol/l). Il n'a pas été trouvé de différences significatives dans les vitesses moyennes de lipogenèse* in vitro *entre les groupes. La vitesse moyenne de lipogenèse a eu tendance à diminuer avec l'augmentation de la concentration de cimatérol dans le milieu de couvraison (tableau I). Il n'y a pas eu de résultats significatifs des traitements sur la vitesse de base ni sur la vitesse stimulée sans addition de cimatérol. L'addition de cimatérol a augmenté significativement la vitesse de base de lipogenèse mais n'a pas changé la vitesse de lipogenèse stimulée par épinéphrine. La réponse de la vitesse de base de lipogenèse à l'addition de cimatérol (0,15 μmol/l) a été significativement réduite dans les animaux traités avec cimatérol (tableau II). Les concentrations de cAMP, dans l'ordre foie > muscle > (SCAT), ont été significativement plus basses dans le muscle des animaux traités avec du cimatérol (tableau IIIa). L'activité de lipoprotéine lipase en SCAT a été*

significativement réduite par le traitement avec OE (tableau IIIb). Les concentrations d'urée dans la plasmatique ont été réduites par cimatérol après 7,14 et 21 jours et par OE après 28 et 42 jours. NEFA dans le plasma a été réduit par le traitement avec OE sur jour 7. Les concentrations de triacylglycérol n'ont pas changé selon le traitement (fig 1). Les données suggèrent que les effets in vitro *de l'addition de cimatérol au milieu de couvraison sur la lipogenèse et la lipolyse dans les tissus adipeux d'ovins sont semblables aux autres substances β-adrénergiques. Les effets de traitement* in vivo *ne sont pas liés aux changements en lipogenèse et lipolyse mesurées* in vitro *et ne sont pas clairs.*

cimatérol / œstradiol-17β / lipogenèse / lipolyse / AMP cycliques

INTRODUCTION

The effects of oestrogenic or androgenic steroidal compounds in promoting growth and altering body composition in farm and laboratory animals has been well demonstrated (Galbraith and Topps, 1981). More recently there has been increasing interest in the effects of β-adrenergic sympathomimetic compounds ("β-agonists") in a number of species (Reeds and Mersmann, 1991).

Beta-agonists such as isoproterenol, salbutamol, racropamine, L-640-033 and BRL35135 are structural analogues of the catecholamines. A number of these synthetic compounds have shown considerable potential in the improvement of the growth performance and carcass characteristics of farm animals (Muir, 1988). Two such compounds, clenbuterol (5-[1-hydroxy-2(isopropyl-amino)ethyl]anthranilonitrile) and cimaterol (4-amino-[*t*-butyl amino methyl]-3,5-dichlorobenzyl alcohol) are orally active and have been shown to increase skeletal muscle accretion and reduce fat deposition in a number of species (Reeds and Mersmann, 1991).

The degree of similarity of the effects of anabolic steroids and those of β-agonists in altering body composition within animals is not clear and requires elucidation. It is possible that β-agonists act *via* adrenoreceptors and second messenger cyclic AMP (cAMP) production in fat depots and skeletal muscle tissue of animals, although this question has not yet been resolved (Reeds and Mersmann, 1991). Oestradiol-17β (OE) may act by altering responsiveness to somatotrophin (Buttery and Dawson, 1987). The objective of this experiment was to examine the effects of cimaterol given alone or in combination to castrate male sheep on lipogenesis and lipolysis *in vitro* in subcutaneous adipose tissue (SCAT), on lipoprotein lipase activity and on concentrations of cAMP in a variety of tissues. The effects of treatments on growth performance and body composition of sheep have been presented elsewhere (Galbraith *et al,* 1990). Briefly, both cimaterol and OE increased growth rate and protein deposited in the carcass, but cimaterol alone significantly reduced carcass fat accretion.

MATERIALS AND METHODS

A detailed description of animal selection, nutrition and experimental treatments has been given elsewhere (Galbraith *et al,* 1990). In summary, 24 Finn x Dorset male castrate sheep weighing on average 18.0 kg and aged ≈ 10 wk were offered a good quality diet to provide intakes of 38 g/kg live weight.

The sheep were blocked by weight into 6 blocks of 4 animals and randomly allocated to untreated controls (group U), implanted with 15 mg OE (one-third of the Compudose-365 implant ("Elanco Products Ltd") (group OE), which received cimaterol in the diet (Boehringer Ingleheim Vetmedica GmBh) (group C) or were implanted with 15 mg OE and received cimaterol in the diet (group OE + C). The concentration of

cimaterol in the diet was 2 mg/kg. To facilitate the processing of *post-mortem* samples, animals were implanted over a 2-d period and were slaughtered unfasted in blocks after 62 or 63 d over a period of 4 d.

Samples of liver, muscle (m gluteus) and SCAT were taken immediately *post-mortem*, frozen in liquid nitrogen and thereafter stored at –60°C prior to analysis. Measurement of the cAMP content of muscle, liver and SCAT was carried out by radioimmunoassay (Du Pont UK Ltd, Kit No NEK-033). Lipoprotein lipase (LPL) was prepared from SCAT as described by Rao and Hawkins (1976), and assayed using the incubation conditions described by Krauss *et al* (1973) and the fatty acids extracted by the procedure of Schotz *et al* (1970).

For practical reasons, comparison of lipogenic and lipolytic activity was made on the basis of wet weight of tissue rather than on an equivalence of cell numbers. Hu *et al* (1988) and Coleman *et al* (1988) have reported recently that adipocyte number per g of tissue was not altered in cimaterol-fed sheep, suggesting that satisfactory comparison of treatment effects may be made on a weight basis. Accordingly small pieces (≈ 30 g) of SCAT, used for the measurement of the *in vitro* rates of lipogenesis and lipolysis, were taken immediately *post-mortem* and transferred to physiological saline at 39°C. The rate of lipolysis (both basal and epinephrine-stimulated), and lipogenesis measured as described by Pothoven and Beitz (1973) and Pothoven *et al* (1975) respectively, was estimated in triplicate in the absence and in the presence of cimaterol added to the incubation medium to give a final concentration of 0.15 or 1.5 μmol/l.

Weekly blood samples (20 ml) were collected prior to morning feeding by jugular venipuncture for the preparation of plasma and serum. Samples of plasma were analysed for urea by the method of Marsh *et al* (1965) and non-esterified fatty acids (NEFA) by the method of Baird *et al* (1967) after extraction as described by Trout *et al* (1960). Serum samples were analysed for triglycerides (TG) by the Boehringer test combination kit (Boehringer Mannheim).

All data were analysed by analysis of variance using GENSTAT V (Lawes Agricultural Trust, 1977). There was no evidence of any influence of blocking on the analysis; thus the data used are unadjusted for missing values. Data for plasma NEFA and triglycerides were analysed using the mean value for samples taken on d 0 as covariate. Data for lipogenesis and lipolysis were analysed by split-plot ANOVA. In the analysis of variance the sum of squares of the treatments was partitioned into 3 orthogonal parts, each with one degree of freedom corresponding to the main effects of OE, cimaterol and the interaction between OE and cimaterol.

RESULTS

In vitro rates of lipogenesis determined by the incorporation of [^{14}C]-acetate into preparations of SCAT are shown in table I. In the absence of added cimaterol there was no effect of *in vivo* treatment with dietary cimaterol or OE implants on the rates of lipogenesis. Nor were there any significant effects of *in vivo* treatment of the animals

Table I. Rate of lipogenesis in SCAT in the presence or absence of cimaterol added to the incubation medium (μmol acetate incorporated/g tissue/h).

Cimaterol (μmol/l)	*Treatment groups*					*Overall means*
	U	*OE*	*C*	*OE + C*	*SED*	
0	5.3	5.5	6.7	4.8	1.19	5.6
0.15	4.1[a]	5.3[a]	4.5[a]	6.9[b]	1.19	5.2
1.5	4.2	5.2	4.0	4.2	1.30	4.4

SED for comparison of any 2 means in the same column = 1.84 (38 df); SED for overall means = 0.92; Treatment means in the same row with dissimilar superscripts are significantly different ($P < 0.05$).

on the response of SCAT to the addition of cimaterol to the incubation medium except in the presence of 0.15 μmol/l added cimaterol when the rate of lipogenesis in treatment OE + C was significantly greater ($P < 0.05$) than that in treatment U. However, comparison of orthogonal contrasts, comparing the effects of the presence *versus* the absence of the œstradiol-17β or cimaterol, indicated no significant effect of either cimaterol of oestradiol-17β on lipogenesis *in vitro*. Although overall treatment means decreased with increasing concentration of added cimaterol, these differences were not significant.

In the absence of cimaterol added to the incubation medium there was no significant effect of *in vivo* treatment of sheep with cimaterol and/or OE on the basal or epinephrine-stimulated rates of lipolysis (table II). Comparison of the overall treatment group means showed that, as expected, epinephrine significantly increased the rate of lipolysis from that shown for basal incubations with no added cimaterol. Added cimaterol also significantly increased the overall basal rate of lipolysis in a dose-dependent fashion (table IIa) but had no effect on the overall rates of epinephrine-stimulated lipolysis (table IIb). Comparison of orthogonal contrasts indicated that the basal lipolytic response to a cimaterol concentration of 0.15 μmol/l was significantly less ($P < 0.05$) in cimaterol-treated animals (groups C and OE + C) than in those which received no cimaterol in the diet (groups U and OE).

Cyclic AMP concentrations (table IIIa) were greatest in liver and lowest in SCAT. In these tissues treatment of sheep with cimaterol with or without OE or OE given alone had no significant effect upon cAMP concentrations. However, orthogonal contrasts indicated that in the skeletal muscle preparation cAMP concentrations were significantly lower ($P < 0.05$) in cimaterol-treated animals (groups C and OE+C).

Lipoprotein lipase activity in SCAT (table IIIb) was significantly lower ($P < 0.01$) in OE-treated animals (groups OE and OE + C) than in those animals not giv-

Table II. Effect of cimaterol on (a) basal and (b) epinephrine (2.7 μmol/l) stimulated rates on lipolysis in subcutaneous adipose tissue (μmol glycerol released/g/tissue/h).

Cimaterol (μmol/l)	*Treatment groups*					*Overall means*
	U	*OE*	*C*	*OE + C*	*SED*	
(a)						
0	330	363	414	370	103.5	370 [a]
0.15	1 738	1 698	1 267 *	1 224 *	375.9	1 484 [b]
1.5	1 822	1 767	2 007	1 850	308.9	1 866 [c]
(b)						
0	1 611	1 406	1 402	1 149	267.1	1 402 [b]
0.15	1 525	1 213	1 558	1 172	288.8	1 383 [b]
1.5	1 370	1 070	1 272	1 132	238.4	1 221 [b]

SED for comparison of any 2 means in the same column = 199 (92 df); SED for overall means = 99.6; overall means with dissimilar superscripts are significantly different ($P < 0.001$); * Main effect of cimaterol ($P < 0.05$).

Table III. Concentration of cAMP in subcutaneous adipose tissue, liver and skeletal muscle samples (nmoles/g) (a); and lipoprotein lipase activity in subcutaneous adipose tissue (μmol fatty acid released/g tissue/h) (b).

Cimaterol (μmol/l)	*Treatment groups*				
	U	*OE*	*C*	*OE + C*	*SED*
(a)					
Adipose tissue	0.16	0.27	0.20	0.20	0.027
Liver	2.24	2.08	2.04	1.85	0.326
Muscle	1.00	1.03	0.74*	0.78*	0.131
(b)					
Adipose tissue	12.4	6.07**	13.6	7.92**	2.40

* Main effect of cimaterol: $P < 0.05$; ** Main effect of oestradiol-17β: $P < 0.01$.

en OE (groups U and C). There was no significant effect of cimaterol treatment on LPL activity. Plasma urea, NEFA and serum triacylglycerols are shown in figure 1. Concentrations of plasma urea rose steadily throughout the experimental period and in treated animals were consistently lower than in the controls. However, treatment effects were variable. Comparison of orthogonal contrasts showed a significantly lower concentration due to a cimaterol effect on d 7 ($P < 0.01$), 14 ($P < 0.01$) and 21 ($P < 0.001$) and lower concentrations due to an oestradiol effect on d 28 ($P < 0.05$) and 42 ($P < 0.01$). Plasma NEFA concentrations fluctuated between 0.30–0.89 mmol/l and were lower in treated animals than in the control group. Treatment had no significant effect on plasma concentrations except for a reduction due to an oestradiol effect on d 7. Serum triacylglycerol concentrations showed no treatment-related change over the experimental period.

DISCUSSION

Previous reports have indicated variable *in vivo* effects of β-agonists on lipogenesis. Neither cimaterol nor oestradiol had any consistent effect on lipogenesis (table I). Coleman *et al* (1988) similarly reported that [^{14}C]-acetate incorporation into SCAT lipids was not affected in sheep given 2 mg clenbuterol/kg diet. Whereas a reduction in lipogenesis has been reported in SCAT from cattle fed 10 mg clenbuterol/head/day (Miller *et al*, 1986) and in rat epididymal fat pad incubated with an unspecified concentration of clenbuterol (Duquette and Muir, 1985). Thornton *et al* (1985) reported markedly reduced rates of lipogenesis, stearate uptake and incorporation in subcutaneous ovine adipocytes incubated with 0.1 μg clenbuterol. A similar though non-significant trend apparent for overall rates of lipogenesis was seen in SCAT (table I). In contrast, Hu *et al* (1988) reported stimulated rates of lipogenesis in growing lambs

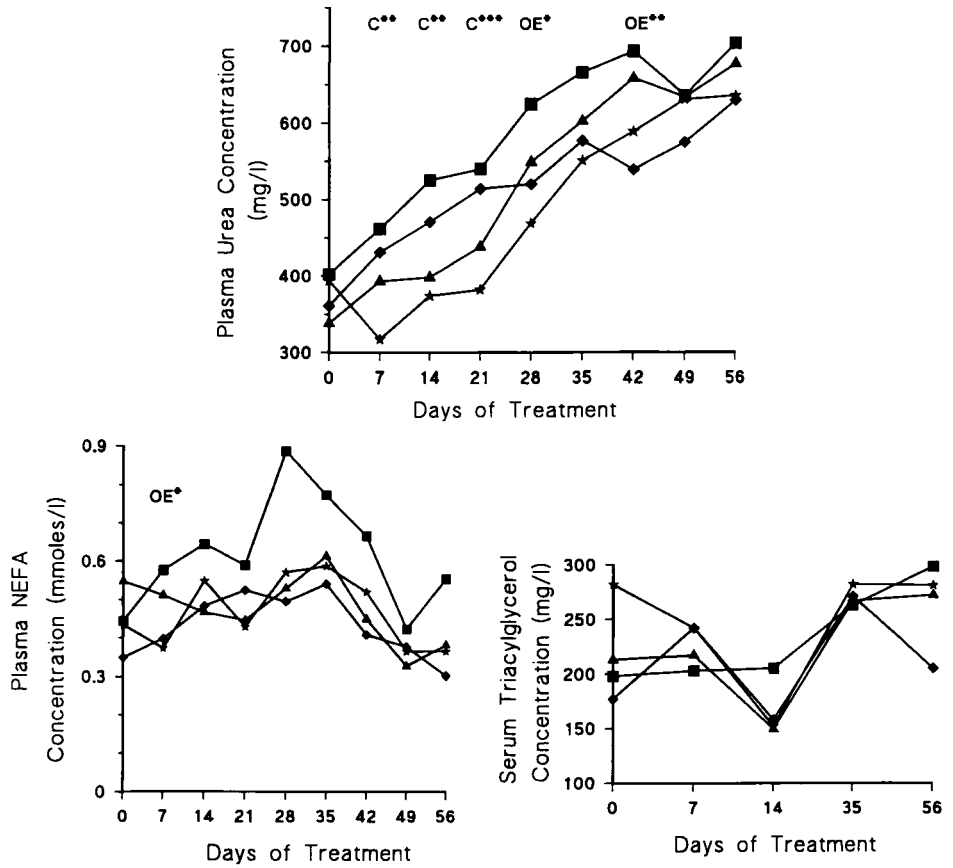

Fig 1. Concentration of plasma urea, NEFA and serum triacylglycerol through the treatment period. ■ : group U; ▲ : group OE; ◆ = group C, ★ = group OE + E. Significance of the main effects of cimaterol and oestradiol-17β are shown by the letters C and OE; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

fed clenbuterol. Coleman (1988) suggested that the inconsistent effects of clenbuterol on adipose tissue lipogenesis may indicate that its effect may be elicited *via* different mechanisms in sheep, cattle and rats. The same may also be true of cimaterol considering its similarity to clenbuterol both in structure and action on skeletal muscle. The effects of differences in tissue preparation and duration of exposure to hormonal preparations may also influence cellular response mediated *via* membrane receptors and may in part explain anomalies between *in vivo* and *in vitro* results.

There was no apparent effect of dietary cimaterol on *in vitro* lipolysis; however, when added to the incubation medium, cimaterol had a significant stimulatory effect on lipolysis (table II). The overall mean rates of lipolysis indicate that cimaterol (1.5 μmol/l) was a more potent stimulator of lipolysis than epinephrine (2.7 μmol/l).

There was no additive effect of cimaterol and epinephrine; indeed, addition of cimaterol tended to decrease the rate of epinephrine-stimulated lipolysis. The lack of any sustained lipolytic response to dietary cimaterol was supported by the absence of any consistently significant change in plasma NEFA (fig 1). The improved carcass characteristics (less fat and greater protein deposition) reported by Galbraith *et al* (1990) suggests that at the dose level used the main effect *in vivo* of cimaterol was to promote a repartitioning of nutrients away from fat synthesis and deposition rather than increasing body fat mobilisation.

Although short-term exposure of cultured L6 muscle cells to isoproteranol, zinterol and salmefamol has been shown to increase cAMP concentrations (Pittman and Molinoff, 1983), a decrease in the number of β-adrenergic receptors following chronic exposure to clenbuterol has been noted in rat skeletal muscle by Rothwell *et al* (1987) and Kim and Sainz (1990) following cimaterol treatment. Such a decrease or down-regulation in receptor numbers may account for the fall in muscle cAMP concentrations. However, it is unclear why similar effects were not seen in the other tissues studied. The data suggest that the long-term effects of cimaterol in muscle tissue may not be fully mediated *via* a cAMP-dependent process or indeed *via* a β-adrenergic receptor mechanism, a possibility suggested for clenbuterol by Reeds *et al* (1987).

The decrease in lipoprotein lipase activity in OE-treated animals is consistent with that reported in oestrogen-treated rat adipose tissue (Hamosh and Hamosh, 1975). Although cimaterol has been reported to increase lipoprotein lipase activity in rat skeletal muscle (Eadara *et al,* 1987), there was no evidence of any similar effect of cimaterol in adipose tissue.

Cimaterol has been previously been shown to cause a decrease in plasma urea concentration (Beermann *et al,* 1986 ; Galbraith *et al,* 1988). Similarly, MacVinish and Galbraith (1988) have reported a reduction in plasma urea levels in sheep following implantation with oestradiol-17β and trenbolone acetate. Several studies have reported elevated concentrations of plasma NEFA following administration of catecholamines or β-agonists (Basset, 1970; Beermann *et al,* 1985; Leenanuruska and McDowell, 1985). In the present study, blood samples were collected prior to feeding and the daily intake of cimaterol; thus any transient increase in NEFA would not have been detected. Scaife *et al* (1982) and Singh *et al* (1988) have reported little or no effect of a combined implant of oestradiol-17β and trenbolone acetate on plasma NEFA concentration, but marked changes in plasma triacylglycerol levels.

It would appear that cimaterol administered in the diet at 2 mg/kg had little effect on *in vivo* parameters of lipid metabolism. However, it did reduce the lipolytic response to cimaterol added *in vitro* and cyclic AMP concentrations in skeletal muscle. The effects of OE were confined to a reduction in lipoprotein lipase activity in SCAT. In general there were no significant interactions between the oestrogenic compound and cimaterol, nor evidence of similarity in the effects produced by the 2 compounds.

ACKNOWLEDGMENTS

The authors wish to thank J Struthers, N Lokke and TW Begg for the care of the experimental animals and C Jessiman, G Wetherall and M Franklin for advice on statistical methods and computing.

REFERENCES

Baird J, Black NW, Faulkner DE (1967) Semi-automated method for the estimation of free fatty acids in plasma. *J Clin Pathol* 20, 905-909

Basset JM (1970) Metabolic effects of catecholamines in sheep. *Aust J Biol Sci* 23, 903-914

Beermann DH, Butler WR, Hogue DE, Dalrymple RH, Ricks CA (1985) Plasma metabolic hormone, glucose and free fatty acid concentrations in lambs fed the repartitioning agent cimaterol (CL263,780). *J Anim Sci* 61 (suppl 1), 255

Beermann DH, Reeds PJ, DeB Hovell FD, Kyle D (1986) Cimaterol elicits rapid physiological responses in growing lambs wholly nourished by intragastric infusion. *J Anim Sci* 63 (suppl), 240

Buttery PJ, Dawson JM (1987) The mode of action of β-agonists as manipulators of carcass composition. *In: Beta-Agonists and Their Effects on Animal Growth and Carcass Quality* (Hanrahan JP, ed) Elsevier, London, 29-43

Coleman ME, Ereken PA, Smith SB (1988) Lipid synthesis and adipocyte growth in adipose tissue from sheep chronically fed a β-adrenergic agent. *J Anim Sci* 66, 372-378

Duquette PF, Muir LA (1985) Effect of the β-adrenergic agonists isoproteranol, clenbuterol, L-640,033 and BRL 35135 on lipolysis and lipogenesis in rat adipose tissue *in vitro*. *J Anim Sci* 61 (suppl 1), 265

Eadara J, Dalrymple RH, Delay R, Ricks DA, Rosmos DR (1987) Cimaterol, a novel α-agonist, selectively stimulates white adipose tissue lipolysis and skeletal muscle lipoprotein lipase activity in rats. *Fed Proc* 46, 1020

Galbraith H, Topps JH (1981) Effects of hormones on the growth and body composition of animals. *Nutr Abstr Rev Ser B* 51, 521-540

Galbraith H, Scaife JR, Hatendi PR, Alderson EM, Watt DC, Stewart DC (1988) Effect of oestradiol-17β and cimaterol alone or combined on corticosteroid status and other endocrine and body characteristics of male castrate lambs. *Proc Nutr Soc* 47, 107A

Galbraith H, Hatendi P, Alderson EM, Scaife JR (1990) The effect of cimaterol and oestradiol-17β alone or combined on growth and body composition of wether lambs. *Anim Proc* 51, 311-319

Hamosh M, Hamosh P (1975) The effect of oestrogen on the lipoprotein lipase activity of rat adipose tissue. *J Clin Inves* 55, 1132-1135

Hu CY, Suryawan A, Forskeng NE, Dalrymple RH, Ricks CA (1988) Effect of cimaterol on sheep adipose tissue lipogenesis. *Fed Proc* 46, 1177

Kim YS, Sainz RD (1990) Characterisation of beta-adrenoreceptors in rat skeletal muscle. *J Anim Sci* 68 (suppl 1), 251A

Krauss RM, Windmeuller HG, Levy RI, Fredrickson DS (1973) Selective measurement of two lipase activities in postheparin plasma of normal subjects and patients with hyperlipoproteinaemia. *J Clin Invest* 54, 1107-1124

Lawes Agricultural Trust (1977) *Genstat V, Mark 4.01*. Rothamsed Exptl Stat, Harpenden, Herts, UK

Leenanuruska D, McDowell GH (1985) Effects of prolonged intravenous infusion of adrenaline on glucose utilisation, plasma metabolites, hormones and milk production in lactating sheep. *Aust J Biol Sci* 38, 197-208

MacVinish LJ, Galbraith H (1988) The effect of implantation of trenbolone acetate and oestradiol-17β in wether lambs at two live weights on concentrations of steroidal residues and blood glucose, urea and thyroid hormones. *Anim Prod* 47, 75-85

Marsh WH, Fingerhut B, Miller H (1965) Automated and manual direct methods for the determination of blood urea. *Clin Chem* 11, 624-627

Miller MF, Garcia DK, Coleman ME, Ereken PA, Smith SB (1986) Nonesterified and glyceride-fatty acid synthesis in bovine adipose tissue from heifers fed clenbuterol. *J Anim Sci* 63, (suppl 1), 236

Muir LA (1988) Effects of β-adrenergic agonists on growth and carcass characteristics of animals. *In: Designing Foods: Animal Product Options in the Market Place*. Natl Acad Press, Washington, DC, 184-193

Pittman RN, Molinoff PB (1983) Interactions of full and partial agonists with β-adrenergic receptors on intact L6 muscle cells. *Mol Pharmacol* 24, 398-408

Pothoven MA, Beitz DC (1973) Effect of adipose tissue site, animal weight and long-term fast-

ing on lipogenesis in the bovine. *J Nutr* 103, 468-475

Pothoven MA, Beitz DC, Thornton JH (1975) Lipogenesis and lipolysis in adipose tissue of *ad libitum* and restricted fed cattle during growth. *J Anim Sci* 40, 957-962

Rao DR, Hawkins GE (1976) Activity of lipoprotein lipase in adipose tissue from steers. *J Dairy Sci* 59, 161-163

Reeds PJ, Hay SM, Dorwood PM, Palmer RH (1987) The effect of β-agonists and antagonists on muscle growth and body composition of young rats (*Rattus* sp). *Comp Biochem Physiol* 89C, 337-341

Reeds PJ, Mersmann HJ (1991) Protein and energy requirements of animals treated with β-adrenergic agonists: a discussion. *J Anim Sci* 69, 1532-1550

Rothwell NJ, Stock MJ, Sudera DK (1987) Changes in tissue blood flow and β-receptor density of skeletal muscle in rats treated with the β2-adrenoceptor agonist clenbuterol. *Br J Pharmacol* 90, 601-607

Scaife JR, Shehab-Eldin FM, Galbraith H (1982) The effect of subcutaneous implantation of trenbolone acetate and oestradiol-17β on plasma lipid concentrations in sheep. *Hormon Metab Res* 14, 589-592

Schotz MC, Garfinkle AS, Huebotter RT, Stewart JE (1970) A rapid assay for lipoprotein lipase. *J Lipid Res* 11, 68-69

Singh SB, Scaife JR, Galbraith H (1988) Blood and tissue lipid composition and lipase activity in wether lambs treated with trenbolone acetate combined with oestradiol-17β at two different live weights. *Anim Prod* 47, 87-96

Thornton RF, Tume RK, Payne G, Larsen TW, Johnson GW, Hohenhaus MA (1985) The influence of the β2-adrenergic agonist, clenbuterol, on lipid metabolism and carcass composition in sheep. *Proc N Z Soc Anim Prod* 45, 97-101

Trout DL, Estes EH, Freidberg SJ (1960) Titration of FFA in plasma: a study of current methods and a new modification. *J Lipid Res* 1, 199-202