

The effect of acute and chronic administration of the β -agonist, cimaterol, on protein synthesis in ovine skin and muscle

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The action of intravenous infusion of the β -agonist cimaterol (2.5 mg/d) on whole-body N retention and protein synthesis in peripheral tissues was examined in growing sheep. Wool growth was determined from skin patch clippings and adjusted to total fibre production. Protein synthesis was measured, using sequential large dose injections of [^{13}C]valine, leucine and phenylalanine and then [ring- d_3]phenylalanine, on biopsy samples from skin and *m. longissimus dorsi* taken before β -agonist administration, at day 3 and day 15 of cimaterol infusion, and 15 d after withdrawal of the drug. Cimaterol increased total N retention by 1.9–2.3 g N/d ($P < 0.01$) over three successive 5 d periods. In contrast, wool growth was significantly reduced by 0.7 g N/d ($P < 0.001$) and the proportion of total N retained in wool declined from 0.71 to 0.25 ($P < 0.01$). The reduction in wool growth was accompanied by a decrease in protein fractional synthesis rate (FSR) in skin (11.6 v. 6.3%/d, $P < 0.01$). Muscle protein FSR, on the other hand, was markedly stimulated during cimaterol infusion (1.45 v. 3.01%/d, $P < 0.001$) as was RNA concentration ($P < 0.001$), RNA:protein ($P < 0.001$) and protein:DNA ($P < 0.05$). The estimated increase in total protein synthesis in muscle (+24 to 30 g/d) due to cimaterol administration was counterbalanced by reductions for skin (–25 to 27 g/d); this may account for the lack of changes in whole-body protein synthesis following β -agonist administration reported in other studies. Although N retention rapidly returned to control values following withdrawal of the drug, both wool growth and skin protein synthesis remained depressed, while muscle protein FSR declined, but not to pre-treatment values. These results suggest a persistent action of cimaterol, but whether this is a function of residue concentrations or long-term metabolic responses is not known.

β -Agonists: Cimaterol: Protein synthesis: Wool growth: Sheep

Certain β -agonists (e.g. clenbuterol, cimaterol, ractopamine) improve protein retention in both laboratory (e.g. Emery *et al.* 1984; Reeds *et al.* 1988) and commercial species (e.g. Baker *et al.* 1984; Beerman *et al.* 1986; Borohov *et al.* 1987; Bergen *et al.* 1989), with the net action directed towards increased muscle gain (e.g. Borohov *et al.* 1987; Williams *et al.* 1987; Bergen *et al.* 1989). Despite the consistent myotrophic response, considerable controversy still surrounds the mechanism of action of these β -agonists, with the competing claims that protein synthesis is elevated (Emery *et al.* 1984; Bergen *et al.* 1989; Maltin *et al.* 1989) and/or that protein degradation is depressed (Reeds *et al.* 1986; Borohov *et al.* 1987; Higgins *et al.* 1988; Wang & Beerman, 1988; Weikard *et al.* 1992).

Several factors may contribute to this controversy, including the species and β -agonists used and possible differences between acute and chronic effects. In rodents, for example, transient stimulation of muscle protein synthesis occurs (2–4 d; Maltin *et al.* 1989; Hesketh *et al.* 1992), whereas prolonged hypertrophy appears to involve decreased degradation

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(Reeds *et al.* 1986; Weikard *et al.* 1992). The situation is more confused with the commercial species because, despite muscle hypertrophy being more prolonged, reports suggest that clenbuterol reduces protein degradation in sheep (Borohov *et al.* 1987; Higgins *et al.* 1988) while ractopamine stimulates protein synthesis in pigs (Bergen *et al.* 1989) and possibly cattle (Smith *et al.* 1989). Both actions have been reported following cimaterol administration to cattle (Dawson *et al.* 1991).

Protein metabolism studies on larger species are complicated because the usual methodologies of protein synthesis estimation involve single measurement times, such that when comparisons are made inter-animal variability may mask the effect of treatment. This uncertainty can be reduced by intra-animal measurements, based on sequential biopsy of accessible tissues. When the biopsy approach is allied to the large dose procedure, which eliminates differences in isotopic activity between the various free amino acid pools, subtle alterations in tissue protein synthesis can be detected. This combination of techniques has been applied to study both short- and longer-term effects of cimaterol on protein synthesis in ovine muscle and skin. The latter tissue was examined because of reports that the muscle hypertrophy may be associated with a reduction in the growth rate of other tissues (Reeds *et al.* 1986; Williams *et al.* 1987; Sainz & Wolff, 1988), including lamb wool production (Sainz & Wolff, 1987) and mink pelt quality (Slayden *et al.* 1991), which may have profound effects on net S-amino acid availability for carcass gain. The mechanisms underlying reduced anabolism in non-muscle tissues following β -agonist administration have not been examined in the farm species, and such information may be important to understand the basis of the induced N repartitioning.

MATERIALS AND METHODS

Animals

Six Suffolk cross-bred wether lambs (6 months old, 35 kg initial live weight) were housed in individual metabolism crates and offered daily 1200 g lucerne (*Medicago sativa*) pellets (10.5 MJ metabolizable energy and 25 g N/kg dry matter; water content 120 g/kg), supplied hourly in equal portions. Animals were maintained under these conditions for 3 weeks before the cimaterol infusion commenced. They were harnessed throughout the study for collection of excreta, urine by suction into 4 M-H₂SO₄ and faeces by bag, over 5 d periods. Polyvinyl chloride catheters (0.8 mm i.d., 1.2 mm o.d.; Dural Plastics and Engineering, Dural, NSW, Australia) were inserted into each external jugular vein under local anaesthesia 3 d before the start of experimental studies.

Design

This involved (a) a pre-cimaterol period of 10 d, including two N balance periods, measurement of wool growth (10 d), and a single determination of protein synthesis in skin and skeletal muscle biopsies; (b) a 15 d continuous intravenous infusion of cimaterol (26 μ g/ml in sterile saline (9 g NaCl/l) at 4 ml/h; total 2.5 mg cimaterol/d), with three 5 d N balance periods, measurement of wool growth between days 5 and 15, and two protein synthesis determinations (day 3 and day 15); and (c) a 15 d post-cimaterol period with three 5 d N balance periods, wool growth determined between days 5 and 15 and finally a protein synthesis measurement on day 15.

Wool growth

The fleece was clipped to a uniform 20 mm length at the start and finish of the experiment to determine total wool growth. To allow estimation of wool production during each period two 100 mm \times 100 mm patches were marked and shaved to skin level, one on each

flank at the start of the pre-cimaterol period. After 10 d one patch ('treatment') was re-clipped and the shavings collected. For the measurement of wool growth during the plus- and post-cimaterol treatments, wool was not clipped from the 'treatment' patch during the first 5 d of each period to allow for the emergence time (Downes & Sharry, 1971) of fibres from the follicle to a height above the skin that is removed by clipping. The wool was clipped, therefore, on days 5 and 15 of these periods. The patch on the alternate side was shaved at the end of the experiment and the clean wool weight compared with the total clippings. Treatment patch weights when multiplied by this ratio provided an estimate of total fleece changes during each period. The individual wool collections were weighed and then degreased with aqueous Tween 20 (25 ml/l), followed by drying at room temperature and reweighing. Clean wool N was determined by Kjeldahl analysis.

N balance

The lucerne pellets and collected excreta were analysed for N content by Kjeldahl analysis using procedures approved by the Association of Official Analytical Chemists (1980).

Protein synthesis measurements

The four serial measurements of protein synthesis followed the procedures described by Rocha *et al.* (1993), using [^{13}C] valine, leucine and phenylalanine and [L-ring- $^2\text{H}_5$] phenylalanine (all 98–99 atoms %; Tracer Technologies Inc., Somerville, MA, USA). The enriched amino acids were mixed with appropriate quantities of the naturally abundant form for each injection; the proportions (g:g) were valine 1.5:8.5; leucine 0.8:4.2; phenylalanine 0.6:2.4. The respective volumes of sterile water (39°) were 200 ml, 250 ml and 200 ml. The amounts injected were chosen to elevate the total body respective free amino acid concentration by approximately 10–12 times and thus establish 'flood' conditions over the period of measurement (Rocha *et al.* 1993). Biopsies were taken from *m. longissimus dorsi* and shaved skin from the back of each sheep before the experiment to establish the natural abundance of the appropriate protein-bound amino acids. Immediately before each injection a blood sample (5 ml) was withdrawn to provide natural abundance of the relevant free amino acid.

The amino acids were injected intravenously over a 10–15 min period. Blood samples (3 ml) for plasma free amino acid enrichment analysis were taken at 5 min intervals during the first 30 min, at 10 min intervals between 30 and 60 min, then at 75 and 90 min and immediately after the biopsies. At 90 min tissue single biopsies (approximately 200 mg) were removed under local anaesthesia from a small area of skin (approximately 50 × 30 mm) on the back (shaved immediately before biopsy) and *m. longissimus dorsi* and treated as described previously (Rocha *et al.* 1993). Care was taken to ensure that effects of previous biopsies on subsequent tissue protein synthesis were minimized. Samples were removed from areas at least 200 mm distant from the previous biopsy site and both sides of the backbone were used. For the final post-cimaterol measurement the animals were killed 90 min after the start of the large-dose injection with an overdose of pentobarbitone and then the tissue samples rapidly removed (less than 2 min). The order of injections was valine, leucine, phenylalanine (^{13}C then ^2H). Concomitant studies (Rocha *et al.* 1993) had shown that similar rates of protein synthesis were obtained with these three amino acids but, because both leucine and phenylalanine can provoke insulin release similar to the acute actions of the β -agonists, it was decided to use these two amino acids during the period of cimaterol infusion. Use of [L-ring- $^2\text{H}_5$]phenylalanine for the terminal measure provided a direct comparison between the chronic and post-drug effects using the same amino acid with a corresponding evaluation between the branch-chain amino acids for the pre- and acute-cimaterol treatments.

Calculation of tissue protein synthesis rate (k_s , % per d; g protein synthesized/100 g tissue protein per d) was as described previously (Lobley *et al.* 1990). Translational efficiency (k_{rna} , g protein synthesized/d per g total RNA) was obtained from the division of k_s by the g total RNA per 100 g protein.

Isotope and chemical analyses

Plasma and tissue free and protein-bound [^{13}C] amino acids were prepared and processed for gas-liquid chromatography-mass spectrometry (GCMS) in electron impact and chemical ionization modes, and isotope-ratio mass spectrometry as described previously (Lobley *et al.* 1990; Rocha *et al.* 1993). Isotope analyses of protein-bound [L-ring- $^3\text{H}_5$]phenylalanine was by GCMS of protein hydrolysates, as described by Calder *et al.* (1992).

Protein, RNA and DNA separations and measurements followed the procedures outlined by Lobley *et al.* (1990). Particular care was needed to ensure homogeneity of the skin samples, with frozen samples initially chopped on surfaces cooled with liquid N_2 and then homogenized in 3 ml cold water. The homogenizer rotor was then washed with water which was combined with the sample and treated with sulphosalicylic acid (final concentration 70 g/l). The subsequent stages were as described earlier (Lobley *et al.* 1990) except that double extraction steps were included for RNA (warm alkali) and DNA (hot perchloric acid).

Statistical analyses

Results were examined by analysis of variance for effects of period (treatment), with animals treated as blocks.

RESULTS

Nitrogen retention

All animals gained weight during the study, and no apparent adverse reactions to either treatment or biopsy were observed, except for slight redness of the eye during the first 24 h of cimaterol infusion. Excreta collections were also trouble-free and this is shown by the low variabilities (Table 1). N digestibility was improved by 3 percentage units ($P < 0.01$) during the β -agonist administration. N retentions were similar between the pre- and post-drug periods, with slightly lower values during the latter measurements, as might be expected for the larger animals maintained on a fixed intake. Cimaterol improved overall N retention by more than 2 g N/d with approximately 70% of this due to reduced urinary-N elimination (Table 1).

Wool growth

Cimaterol significantly reduced clean wool growth ($P < 0.01$) and N concentration ($P < 0.001$; Table 2). During 5–15 d of the post-cimaterol period wool growth did not return to pre-drug values, although N concentration was partially restored (Table 2). There was no apparent effect of the repeat clipping procedure because recovery of wool from the 'control' patch on the other side matched the time-adjusted sum of the three shavings. Based on the assumption that the patch wool was typical of the general response, the contribution of fibre growth to total N retention (Table 1) was estimated. Before β -agonist infusion the wool represented 71% of total N gain, but this declined to 25% during cimaterol treatment and was only partly restored (to 40%) during the drug-withdrawal phase (Table 2).

Table 1. *Effect of intravenous cimaterol infusion (2.5 mg/d) on apparent dry matter (DM) and N digestibilities and N retention for lambs offered 1200 g lucerne (Medicago sativa) pellets (25.5 g N) daily**
(Means for six animals)

Period†...	Pre-cimaterol			+ Cimaterol			Post-cimaterol			SED	P‡
	1	2	3	4	5	6	7	8			
DM digestibility	0.56	0.56	0.58	0.58	0.60	0.58	0.58	0.59	0.013	NS	
N digestibility	0.52 ^a	0.54 ^{ab}	0.55 ^{ab}	0.56 ^b	0.57 ^b	0.54 ^{ab}	0.55 ^{ab}	0.56 ^b	0.012	< 0.01	
Urinary N output (g/d)	10.2 ^a	10.3 ^a	8.9 ^b	8.8 ^b	8.8 ^b	10.2 ^a	11.0 ^a	11.3 ^a	0.58	< 0.05	
N retention (g/d)	3.2 ^a	3.5 ^a	5.2 ^b	5.5 ^b	5.7 ^b	3.6 ^a	3.0 ^a	3.0 ^a	0.52	< 0.01	

* For details of procedures, see pp. 502–503.

† Successive 5 d N balance measurements were conducted throughout the study incorporating days 1–10 of the pre-cimaterol, days 1–15 of cimaterol infusion and days 1–15 after cessation of the cimaterol administration.

‡ Based on analysis of variance for 35 residual df. Differences between means of individual periods determined by use of the *t* statistic.

^{a, b} Values with different superscripts are significantly different ($P < 0.01$ for N digestibility and N retention; $P < 0.05$ for urinary N output).

Table 2. *Effect of intravenous cimaterol infusion (2.5 mg/d) on clean wool growth, wool N concentration and retention for lambs offered 1200 g lucerne (Medicago sativa) pellets daily**

(Means for six animals)

	Pre-control	+ Cimaterol	Post-cimaterol	SED	<i>P</i>
Clean wool growth‡ (g/d)	14.7 ^a	10.4 ^b	9.9 ^b	0.95	< 0.01
Wool N concentration (g/100 g)	14.01 ^a	12.86 ^b	13.47 ^c	0.186	< 0.01
Wool N retention‡ (g/d)	2.06 ^a	1.35 ^b	1.33 ^b	0.141	< 0.001
% total N retained as wool N	70.9 ^a	25.1 ^b	40.1 ^{ab}	12.52	< 0.01

* For details of procedures, see pp. 502–503.

^{a, b, c} Values with different superscripts are significantly different ($P < 0.01$).

† By analysis of variance for 10 residual df. Differences between means assessed by use of the *t* statistic.

‡ Values based on weight and composition of wool from 100 × 100 mm clipped patches scaled to the whole fleece.

Skin metabolism

Cimaterol treatment did not cause any significant change in either protein, RNA or DNA concentrations (Table 3), although the difficulties of preparing consistent extracts increased the variability of the determinations and may have masked real changes. There were trends for the drug to decrease DNA and also, during the acute phase (3 d), RNA. In consequence, protein:DNA was increased by the β -agonist, which might indicate reduced cell proliferation.

Estimates of skin protein fractional synthesis rate (FSR) for the pre-cimaterol period (mean 11.6%/d; Table 3) were similar to values reported both using other amino acids in large-dose procedures (Lobley *et al.* 1992; Rocha *et al.* 1993) and based on continuous infusion studies in lambs, with the plasma free amino acid isotopic activity selected as representative of the true precursor (Davis *et al.* 1981; Lobley *et al.* 1992). Protein synthesis was significantly reduced ($P < 0.01$) during both the chronic and post-cimaterol periods (Table 3). The reductions of 46% and 43%, compared with pre-drug values, were similar to the 36 and 35% decreases in wool N retention (Table 2). Based on the assumptions that: first, the protein content of total shaved skin of 35–40 kg sheep is 500–570 g (MacRae *et al.* 1993); second, the skin and wool samples taken are representative of the whole; and, third, that short-term measurements of protein synthesis, made within each period, can be related to the wool N retention of that period, then the proportion of skin k_s attributable to wool protein gain can be calculated to remain similar (25–28%) before, during and after administration of the β -agonist (Table 3), despite the substantial alterations in metabolism and fibre production. Translational efficiency (k_{rna} ; g protein synthesized/d per g total RNA) declined during chronic cimaterol treatment ($P < 0.05$) and had not increased by the end of the 15 d withdrawal period (Table 3).

Muscle metabolism

As a result of chronic cimaterol infusion (Table 4) the concentration (w/w) of DNA was reduced by 28% ($P < 0.001$) and that of protein by 15% ($P < 0.05$). In contrast, total RNA was significantly increased (12%, $P < 0.001$). These effects persisted during the drug

Table 3. Responses in protein, RNA and DNA concentrations (mg/g), protein synthesis (k_s) and translational activity (k_{rna} , g protein synthesized/d per g total RNA) in ovine skin with intravenous infusion of cimaterol (2.5 mg/d)*

(Means for six animals)

	Pre-cimaterol - 5 d	Plus-cimaterol		Post-cimaterol + 15 d	SED	P
		3 d	15 d			
Concentrations						
Protein	161	157	157	147	11.0	NS†
RNA	3.34	2.25	2.94	2.69	0.546	NS†
DNA	2.75	1.79	2.11	1.86	0.372	NS†
Ratios						
Protein:DNA	61	91	91	94	19.5	NS†
RNA:DNA	1.23	1.31	1.64	1.48	0.322	NS†
RNA:protein (mg:g)	21.1	14.9	19.2	19.3	2.66	NS†
Protein metabolism‡						
k_s (% per d)	11.6	10.9	6.3	6.6	1.59	< 0.01‡
k_{rna}	6.01 ^{ab}	7.9 ^a	3.5 ^b	3.7 ^b	0.79	< 0.05‡
% Skin protein synthesis deposited as wool	25	—	28	26	5.5	NS§

* For details of procedures, see pp. 502–504.

† By analysis of variance but due to technical difficulties there are missing values with, in consequence, only 11 residual df and with incomplete animal blocks.

‡ By analysis of variance based on 15 residual df. Row values with unlike superscripts are significantly different ($P < 0.05$).

§ By analysis of variance based on 12 residual df. Because wool growth measurements were made over 5–15 d for each period no calculations are presented for the 3 d plus-cimaterol time point.

¶ Protein synthesis measured by the large-dose procedure with [^{1-13}C] valine, leucine and phenylalanine used successively for each of the first three measurements and [ring- 3H_s]phenylalanine for the post-cimaterol study.

withdrawal period (Table 4) and, as a consequence, protein:DNA, often used as an index of cell hypertrophy, increased markedly throughout the chronic and post-drug periods (by 18 and 27% respectively, $P < 0.05$; Table 4). Similarly, RNA:DNA, an index of cellular activity, was also elevated over the same periods (55 and 95% respectively, $P < 0.01$) as was RNA:protein, a marker of the translational capacity of the tissue, by 32% and 47% respectively ($P < 0.001$; Table 4).

Cimaterol caused substantial increases in k_s ($P < 0.001$; Table 4). Within 3 d of the start of infusion k_s was increased 90% compared with pre-drug values and this was maintained at 15 d. Withdrawal of the β -agonist resulted in a significant reduction ($P < 0.05$) in the rate of protein synthesis but not to the pre-treatment values ($P < 0.05$; Table 4). Translational efficiency also increased significantly during infusion of the drug ($P < 0.01$; Table 4) but returned to control values during the post-treatment phase. Based on the assumption that muscle protein gain is similar to whole-body protein accretion (from N retention; Table 1) and that the sheep contains 145 g protein/kg live weight then fractional protein growth rates increased from 0.37 to 0.72%/d as a result of cimaterol treatment. The incremental improvement (0.35% per d) was only 22% of the increase in muscle k_s and, even allowing for preferential accretion of muscle during cimaterol treatment, estimated protein fractional degradation in the tissue must also have increased as a result of action of the drug.

Examination of the earlier muscle and skin biopsy sites at the end of the study indicated that any damage was local, and restricted, where present, to within 5 mm of the wound site.

Table 4. Responses in protein, RNA and DNA concentrations (mg/g), protein synthesis (k_s) and translational activity (k_{rna} , g protein synthesized/d per g total RNA) in ovine m. longissimus dorsi with intravenous infusion of cimaterol (2.5 mg/d)*

(Means for six animals)

	Pre-cimaterol - 5 d	Plus-cimaterol		Post-cimaterol + 15 d	SED	P†
		3 d	15 d			
Concentrations						
Protein	157 ^a	144 ^{ab}	134 ^b	138 ^b	7.3	< 0.05
RNA	0.78 ^a	0.76 ^a	0.87 ^b	1.01 ^c	0.042	< 0.001
DNA	0.54 ^a	0.50 ^a	0.39 ^b	0.36 ^b	0.024	< 0.001
Ratios						
Protein:DNA	294 ^{ab}	286 ^a	345 ^{bc}	382 ^c	28.5	< 0.05
RNA:DNA	1.46 ^a	1.52 ^a	2.23 ^b	2.79 ^c	0.139	< 0.01
RNA:protein (mg:g)	4.96 ^a	5.30 ^a	6.49 ^b	7.30 ^c	0.362	< 0.001
Protein metabolism‡						
k_s (% per d)	1.45 ^a	2.76 ^{bc}	3.01 ^b	2.20 ^c	0.332	< 0.001
k_{rna}	2.89 ^a	5.23 ^b	4.63 ^b	2.97 ^a	0.704	< 0.01
k_p §	0.43 ^a	0.66 ^b	0.72 ^b	0.37 ^a	0.057	< 0.001

* For details of procedures, see pp. 502–504.

† By analysis of variance based on 15 residual df. Row values with unlike superscripts are significantly different ($P < 0.05$).

§ Whole body fractional rates of N retention; based on N balance data (Table 1) and assumed N content for sheep of 23 g N/kg liveweight.

¶ Protein synthesis measured by the large-dose procedure with [$1\text{-}^{13}\text{C}$] valine, leucine and phenylalanine used successively for each of the first three measurements and [ring- $^2\text{H}_5$]phenylalanine for the post-cimaterol study.

Effects of local reactions on the other biopsy sites were considered, therefore, to be minimal, but systemic effects could not be eliminated although the general health and well-being of the animals makes any deleterious responses unlikely, especially as measurements were made at intervals of between 7 and 15 d.

DISCUSSION

The improvement in N retention during the period of cimaterol infusion confirms the many observations that certain β -agonists produce substantial and prolonged improvements in protein gain for farm species (e.g. Baker *et al.* 1984; Beerman *et al.* 1987; Borohov *et al.* 1987; MacRae *et al.* 1988; Bergen *et al.* 1989; Dawson *et al.* 1991). Because the animals were maintained on a fixed intake throughout the study, and N digestibility was only slightly enhanced by cimaterol, a large improvement in the net efficiency of utilization of absorbed amino acids almost certainly occurred. Similar improvements in total protein gain in milk-fed calves treated with clenbuterol included a repartitioning between lean tissues, such that the extra accretion in muscle exceeded that for the whole animal, with the rates of gain in other tissues reduced (Williams *et al.* 1987). In the current study part of such repartitioning included depression in wool growth and, presumably, the net production of cyst(e)ine-rich keratins. For ruminants supplied with forage diets rumen microbial protein provides the bulk of absorbed amino acids and many studies have indicated that methionine is often first limiting under such conditions (e.g. Nimrick *et al.* 1970; Storm & Ørskov, 1984). One of the fates of methionine is donation of S to cysteine synthesis and, if the demands for fibre production are considerable, then this may limit the amount of

methionine available for protein gain in other tissues. Indeed, provision of extra methionine to sheep often increases wool growth (Reis *et al.* 1990).

One underlying mechanism in the repartitioning of protein to muscle appears to involve a decrease in skin synthesis. The current study did not distinguish between skin constitutive protein and fibre synthesis but, because wool protein accretion represents a small proportion of total integument protein metabolism during both control and treatment phases, then, even allowing for the processing losses of the prokeratins, a general reduction of skin protein synthesis appears more probable. The reduction may be due to reduced availability of essential amino acids, particularly methionine and cysteine, diverted to improved muscle protein gain. If this resulted in lowered production of the high-S protein in the fibre matrix it would also account for the lowered N content of the fibre, because the matrix proteins determine the variation in fibre N contents (Gillespie, 1983). The decrease in skin protein synthesis associated with cimaterol treatment may also have resulted from reduced integument cell proliferation rates, which have been reported to decline in response to adrenaline treatment (Rao *et al.* 1971; Aoyagi *et al.* 1980), possibly acting via a negative feedback mechanism (Cameron, 1971). This may be a general feature of sympathomimetics, including cimaterol, and is supported by the trend towards reduced DNA concentrations in the current study.

The mechanisms underlying the myotrophic response to β -agonists are still the subject of controversy. Data are available supporting increases in protein synthesis (Emery *et al.* 1984; Bergen *et al.* 1989; Maltin *et al.* 1989; Dawson *et al.* 1991; Hesketh *et al.* 1992) and decreases in degradation (Reeds *et al.* 1986; Borohov *et al.* 1987; Maltin *et al.* 1987; Dawson *et al.* 1991; Weikard *et al.* 1992). Furthermore, ractopamine has been shown to increase the abundance of mRNA for muscle proteins in both pigs (Helferich *et al.* 1990) and steers (Smith *et al.* 1989), indicative of a pre-translational potential for stimulation of protein synthesis. In contrast, both clenbuterol and cimaterol when given to lambs have reduced the activity of certain proteolytic enzymes, especially the calpains (Higgins *et al.* 1988; Wang & Beerman, 1988; Kretchmar *et al.* 1989), responsible for loosening the myofibrillar network, with the implication that protein degradation is inhibited. The current results do not support this latter concept, at least under the experimental conditions employed, since the substantial changes in protein synthesis observed more than exceeded the calculated response in muscle growth. This would imply a probable increase in proteolysis, similar to the situation observed in many nutritional studies, where synthesis and degradation alter in the same direction, but to different extents (Millward *et al.* 1976; Harris *et al.* 1992). Changes in enzyme activity do not necessarily presume that metabolic responses follow since both substrate availability (possibly linked to compartmentalization of proteases) and sensitivity may also need to be altered. Why such consistent changes in apparent proteolytic activity occur following β -agonist treatment remains a mystery, but may be linked to a required remodelling of the myofibrillar structure. Indeed, while catheptic activities can be reduced by cimaterol *in vitro*, net anabolism is not observed (Bechet *et al.* 1990).

The increased muscle protein synthesis provoked by cimaterol had associated changes in cellular RNA, and the marked rise in both absolute concentration and RNA:protein are supportive of increases in the synthetic capacity of the tissue. These findings are not in agreement with those of Beerman *et al.* (1987), where no change in either variable was observed after 7 or 12 weeks of cimaterol administration to lambs. Beerman *et al.* (1987) did, however, report a lowered DNA concentration and elevated RNA:DNA, findings confirmed in the current study. The reason for the lowered DNA is unclear, but may involve reduced satellite cell numbers, particularly if differentiation, followed by incorporation into the myofibre, forms part of the anabolic process. In the present study

the proportional chronic increases observed in synthesis exceeded those in RNA, such that translational efficiency was also elevated. While k_{rna} measurements are rather simplistic, both in practical and theoretical terms, they are often associated with stimulatory events, such as the transitions in the fed-fast cycle (Millward *et al.* 1976; Garlick *et al.* 1983). In muscle (and other tissues) of fattening ruminants translational activity is usually much lower than for the preruminant and young nonruminant and, therefore, has the capacity to respond to appropriate stimuli (Lobley, 1993).

There is evidence from cell culture studies that certain β -agonists can stimulate muscle protein synthesis directly (Young *et al.* 1987; Anderson *et al.* 1990), probably through actions at a β_2 -receptor (Sillence *et al.* 1991), although interactions with humoral factors cannot be excluded at present. The fact that β -agonists have been shown to work in adrenalectomized, hypophysectomized and gonadectomized animals appears to eliminate many of the major systemic hormones (see Yang & McElligott, 1989). A direct action on muscle would be expected to lead to rapid reversal of effects and the restoration of whole-body total N retention to pre-drug values (McRae *et al.* 1988; Hovell *et al.* 1989; present investigation) has tended to support this view. The current results cast doubt on this simplistic interpretation as muscle protein synthesis is maintained above control rates while wool growth is still markedly depressed during the 5 to 15 d period after withdrawal of cimaterol. One possible explanation for the persistent effect on muscle may involve neural interactions. It has been observed that clenbuterol is effective in reducing the atrophy associated with nerve damage (Maltin *et al.* 1987) and suggestions have been made that nerve-derived growth factors (NDGF) may play an important role in myotrophic growth (Davis *et al.* 1985). Recovery from nerve damage or metabolic modifications can be lengthy and stimulated production of NDGF or associated regulatory proteins may continue for some time following termination of drug treatment. The residual effect of cimaterol on wool growth may be linked to possible lowered rates of cell division in the follicle, resulting in reduced follicle size. Evidence from nutritional studies suggests that, in circumstances where follicle size is reduced, periods as long as 3 to 5 weeks are required before size and, therefore, productivity are restored (Hynd, 1982). Alternative explanations may involve either very low concentrations of cimaterol being required to maintain metabolic changes after the initial responsive dose or the drug remaining within tissues at active doses. There is some evidence for accumulation of β -agonists in the eye of calves but, in general, residue levels in most tissues are reduced rapidly to low concentrations (Meyer & Rink, 1991).

Measurements of whole-body amino acid flux, corrected to total protein synthesis, have often shown little or no change in response to β -agonists (Borohov *et al.* 1987; MacRae *et al.* 1988) and the current findings offer an explanation. Based on the assumptions that the skin and muscle samples taken are representative, that total protein values in skin and muscle are 500 g and 1900 g respectively for sheep of 35 kg (MacRae *et al.* 1993) and that the responses observed to cimaterol are typical of β -agonists, then the increased synthesis in muscle (+24 to 30 g/d) during short- and long-term administration of cimaterol is counterbalanced by reduced integument activity (-25 to 27 g/d).

This study again demonstrates the considerable protein repartitioning effect of β -agonists towards muscle and reveals that the effects are aided by reduced gain, through lowered protein synthesis, in the integument. Whether similar metabolic effects occur in other, non-muscle tissues that show decreased rates of protein accretion remains to be investigated. The disturbance of the normal distribution of lean protein mass induced by β -agonists provides an important approach to understanding the mechanisms which regulate body composition and which may be manipulated either for animal production or for reversal of protein wasting ailments.

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