Effects of the β-agonist, cimaterol, on growth, body composition and energy expenditure in rats

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1. Male Sprague–Dawley rats weighing 146·5 (SE 4·3) g were fed on a semi-synthetic diet containing 0, 25 or 150 mg cimaterol/kg for 12 d. Net changes in weight and composition of carcass, liver, heart, gastrointestinal tract, gastrocnemius plus plantaris muscles, skin and remainder were estimated by comparative slaughter.

2. Cimaterol increased protein gains in gastrocnemius plus plantaris muscles from 0·09 g in controls to 0·14 and 0·12 g in 25 and 150 mg cimaterol/kg groups respectively. Carcass protein gains increased from 6·27 g in controls to 8·00 and 7·05 g in 25 and 150 mg cimaterol/kg groups respectively.

3. Rats treated with cimaterol either gained less fat or actually lost fat from all tissues studied, whilst control rats gained fat. These changes were reflected in lower energy retention in cimaterol-fed rats.

4. Energy intake was not affected by treatment. Cimaterol increased heat production from 776 kJ/kg body-weight$^{0.75}$ in controls to 863 kJ/kg body-weight$^{0.75}$ in both treated groups. Gross efficiency was reduced from 17·4% in controls to 8·0 and 7·7% in rats fed on 25 and 150 mg cimaterol/kg diets respectively.

5. These results indicate that cimaterol increases protein gain at the expense of fat in rats. In addition, subcutaneous adipose tissue appears to be more sensitive than abdominal fat, whilst protein gains are particularly enhanced in skeletal muscle relative to other body tissues.

Some β-adrenergic agonists have the ability to increase lean gain at the expense of fat, which has led to the use of the term repartitioning agents (Asato *et al.* 1984). Clenbuterol (4-amino-α-(*t*-butylamino-methyl)-3,5-dichlorobenzyl alcohol) and cimaterol (5-(1-hydroxy-2-(isopropylamino)-ethyl)anthranilonitrile) were identified by their ability to increase body-weight gain and decrease uterine fat pad weight in mice (Asato *et al.* 1984). Both compounds have been shown to increase carcass protein and decrease fat in sheep (Baker *et al.* 1984; Thornton *et al.* 1985; Wolff *et al.* 1987), cattle (Ricks *et al.* 1984), poultry (Asato *et al.* 1984; Dalrymple *et al.* 1984) and pigs (Jones *et al.* 1985). These changes are desirable in view of consumer demand for leaner meat. In addition, β-agonists provide a powerful tool for understanding the factors determining body composition in general and muscle growth in particular. Accordingly, several studies have been aimed at elucidating the mechanism of action of these compounds (Emery *et al.* 1984; Thornton *et al.* 1985; Reeds *et al.* 1986; Bohorov *et al.* 1987). However, none of the studies to date have included detailed information on initial and final weights of body components, as well as feed intakes. Here we report on quantitative effects of cimaterol on changes in the weight and composition of various tissues in growing rats and on feed intake and energetic efficiency.

MATERIALS AND METHODS

Male Sprague–Dawley rats, 6–7 weeks of age and mean body-weight 146·5 (SE 4·3) g, were housed in individual stainless-steel cages and fed on a semi-synthetic diet (obtained from Applied Biochemistry Division, New Zealand Department of Scientific and Industrial Research, Palmerston North). Diet composition (g/kg) was: lactalbumin 120, starch 690, maize oil 80, cellulose 100, mineral mix 50, vitamin mix 50 (James & Treloar, 1981). Metabolizable energy (ME; 17·75 kJ/g dry matter) content of the diet was determined using a separate group of similar rats. Following a 7 d period of adaptation to the diet, a

Table 1. *Initial tissue and component weights (g) of rats, with regression coefficients used for prediction*

(Model fitted was $y = b_0 + b_1 \times$ body-weight)

Tissue	Component	Mean	SEM	b_0	b_1	R
Body	Wt	146·5	4·3	—	—	—
	Protein	25·16	0·80	−1·94	0·185	0·98
	Fat	15·44	0·57	−3·90	0·132	0·88
Carcass	Wt	63·69	2·17	−9·56	0·500	0·99
	Protein	11·47	0·38	−1·35	0·0875	0·98
	Fat	5·66	0·25	−2·82	0·0579	0·80
Liver	Wt	6·98	0·23	−0·68	0·0523	0·80
	Protein	1·18	0·04	−0·200	0·00942	0·85
	Fat	0·24	0·009	−0·061	0·00205	0·68
GI tract	Wt	9·04	0·12	4·95	0·0279	0·57
	Protein	1·08	0·02	0·459	0·00424	0·82
	Fat	0·50	0·01	0·149	0·00239	0·45
Heart	Wt	0·70	0·017	0·111	0·00400	0·74
	Protein	0·13	0·003	0·0243	0·000718	0·67
Muscle	Wt	0·80	0·02	0·124	0·00460	0·87
	Protein	0·17	0·004	0·0197	0·00101	0·83
Skin	Wt	22·45	0·68	−0·55	0·157	0·95
	Protein	5·07	0·20	−1·83	0·0471	0·94
	Fat	5·42	0·16	0·17	0·0358	0·74
Remainder	Wt	34·01	0·82	6·18	0·190	0·97
	Protein	5·89	0·15	0·913	0·0340	0·89
	Fat	3·56	0·15	−1·35	0·0335	0·91

GI, Gastrointestinal; muscle, gastrocnemius plus plantaris (average of both sides).

representative group of twelve rats was killed to provide estimates of initial body composition, and six rats were assigned to each treatment group. Treatments consisted of the basal diet with cimaterol (American Cyanamid Co., Princeton, New Jersey) added at 0, 25 or 150 mg/kg. All animals were fed *ad lib.*

After 12 d of treatment, experimental rats were bled, killed and dissected; liver, empty gastrointestinal (GI) tract, heart, gastrocnemius plus plantaris muscles, skin (with hair), carcass and remainder (head, feet and tail plus remaining organs) were weighed and frozen pending analysis. Water and fat contents were determined as the weight losses following freeze-drying and Soxhlet extraction with light petroleum (b. p. 40–60°) respectively. Crude protein was calculated as Kjeldahl nitrogen × 6·25. Due to insufficient tissue mass, fat contents of gastrocnemius plus plantaris muscles and heart were not measured.

Initial tissue weights and compositions were estimated from body-weight, using regression equations derived from the initial slaughter group (Table 1). Net tissue weight, protein and fat gains were calculated from the final composition of each animal and its estimated initial composition. Results were analysed by analysis of covariance (Steel & Torrie, 1981). We originally intended to adjust data for initial body-weight, however, it was not a significant covariate. Adjustment of tissue and component gain data for differences in weight gain during the week before treatment produced significant reductions in residual error. In addition, all slopes were positive, indicating that the effect of covariance adjustment was to correct for true differences in growth rate. Significant differences ($P < 0·05$) were examined further using Duncan's new multiple-range test (Steel & Torrie, 1981).

Table 2. *Effects of cimaterol on rat body and tissue gains, adjusted for differences in initial weight gain during the week before treatment*

Cimaterol (mg/kg diet)	Body	Carcass	Liver	GI tract	Heart	Muscle	Skin	Remainder
				Wt gains (g/12 d)				
0	62·9[a]	30·9	2·55	0·17	0·18	0·37[b]	9·79[a]	12·53[a]
25	57·5[ab]	32·7	1·31	0·14	0·21	0·55[a]	4·12[b]	9·91[b]
150	49·4[b]	28·7	1·43	−0·45	0·26	0·49[a]	2·10[b]	8·57[b]
SEM	3·1	1·6	0·37	0·36	0·03	0·03	0·87	0·72
Statistical significance	*	NS	†	NS	NS	**	***	**
				Protein gains (g/12 d)				
0	11·81	6·27[b]	0·37	0·07	0·03	0·09[b]	2·60	2·29
25	13·32	8·00[a]	0·30	0·03	0·04	0·14[a]	2·41	2·27
150	11·17	7·05[ab]	0·27	−0·02	0·05	0·12[a]	1·47	2·13
SEM	0·70	0·33	0·05	0·04	0·005	0·006	0·35	0·13
Statistical significance	NS	*	NS	NS	NS	***	†	NS
				Fat gains (g/12 d)				
0	6·03[a]	2·41[a]	0·09[a]	0·07[a]	ND	ND	1·60[a]	1·91[a]
25	−0·14[b]	0·34[b]	−0·01[b]	−0·09[b]	ND	ND	−0·66[b]	0·34[b]
150	−1·92[b]	−0·59[b]	0·02[b]	−0·14[b]	ND	ND	−1·29[b]	0·15[b]
SEM	0·93	0·38	0·04	0·04	ND	ND	0·36	0·28
Statistical significance	***	***	***	**	—	—	***	**

GI, Gastrointestinal; muscle, combined gastrocnemius plus plantaris muscles (average of both sides): ND, not determined; NS, not significant.

[a, b] Means in the same column with different superscript letters were significantly different ($P < 0·05$).

Statistical significance: † $P < 0·10$; * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Body-weight gains included blood and GI-tract contents, therefore were greater than the sum of tissue gains.

Table 3. *Effects of cimaterol on daily metabolizable energy intake, energy retention and heat production (kJ/kg body-weight$^{0·75}$) in growing rats*

Cimaterol (mg/kg diet)	Metabolizable energy intake	Retained energy	Heat production
0	939	163[a]	776[b]
25	938	75[b]	863[a]
150	934	72[b]	863[a]
SEM	11	13	13
Statistical significance	NS	***	**

NS, Not significant.

[a, b] Means in the same column with different superscript letters were significantly different ($P < 0·05$).

Statistical significance: ** $P < 0·01$; *** $P < 0·001$.

In order to evaluate changes in energy utilization accompanying the altered growth pattern in cimaterol-fed rats, the amounts of energy ingested, retained in body tissues and released as heat by each animal were calculated. ME intake was estimated from individual feed intake and the ME content of the diet. Retained energy (RE) was calculated assuming that body protein contains 23·85 kJ/g (Kleiber, 1961) and using a measured value of 39·15 kJ/g for fat. Heat production (HE) was estimated as the difference between ME intake and RE.

RESULTS

Initial tissue and component weights, along with the appropriate regression coefficients, are given in Table 1. Body-weight gains decreased with cimaterol treatment in a dose-dependent fashion, largely due to lower gains in skin and remainder (Table 2). On the other hand, muscle weight gain was increased by cimaterol from 0·37 g in controls to 0·55 and 0·49 g (i.e. 49 and 32% higher) in 25 and 150 mg cimaterol/kg groups respectively. Protein gain was increased in muscle at both dosages and in carcass (25 mg cimaterol/kg group only). Other tissues, notably skin, liver and GI tract tended to gain less protein in treated groups ($P > 0·05$), resulting in non-significant changes in whole-body protein gain. Fat gains were significantly affected in all tissues (Table 2); whole-body fat gain was 6·03 g in controls, whilst cimaterol-fed rats lost 0·14 and 1·92 g (25 and 150 mg cimaterol/kg groups respectively).

Actual empty body compositions for the 0, 25 and 150 mg cimaterol/kg groups respectively were (g/kg): water, 660, 680 and 686 (SEM 5, $P < 0·05$); protein, 189, 206 and 201 (SEM 2, $P < 0·001$); fat, 110, 79 and 74 (SEM 6, $P < 0·01$). Ratios of body water:protein were 3·49, 3·31 and 3·41 for the 0, 25 and 150 mg cimaterol/kg groups respectively (SEM 0·04, $P < 0·05$).

ME intake was not affected by cimaterol (Table 3). However, RE was sharply reduced ($P < 0·001$) in cimaterol-fed rats, and calculated HE was about 863 kJ/kg body-weight$^{0·75}$, i.e. 11% higher in both treated groups (Table 3). As a result, gross efficiency was reduced from 17·4% in controls to 8·0% and 7·7% in the 25 and 150 mg cimaterol/kg groups respectively.

DISCUSSION

The aim of the present study was to determine quantitatively the effects of cimaterol on rat growth, body composition and efficiency of energy utilization. Responses observed in the present study were generally in agreement with those reported previously. Cimaterol demonstrated clear repartitioning effects in growing rats, increasing protein gain at the expense of fat. Previous reports of the repartitioning effects of β-agonists have concentrated on changes in whole-body protein and fat. Our results define more precisely the tissues affected by cimaterol.

Cimaterol treatment resulted in a loss of whole-body fat (Table 2). At the tissue level, this was due to either decreased gain or actual loss of fat; these effects were highly significantly in all tissues. Fat in skin, reflecting mainly subcutaneous adipose depots, was most sensitive to the drug, whereas fat in remainder, largely comprising internal adipose depots, was least sensitive. This selectivity may have practical importance, since subcutaneous fat is more likely to accompany the carcass of meat animals than abdominal fat.

The effects of cimaterol on protein gains were also not uniform among tissues. Treated animals gained significantly more protein in carcass and gastrocnemius plus plantaris muscles, and tended to gain more protein in heart ($P > 0·05$). Protein gains in other tissues were lower in treated rats, although these differences were not significant. Carcass protein gain was not significantly increased in the 150 mg cimaterol/kg group relative to controls. Coupled with decreased gains in other non-muscle tissues, this resulted in a slight (non-significant) decrease in whole-body protein gain at the highest dose of cimaterol. The reason for this loss of activity is not clear. Possibly, there is some down-regulation of receptors in muscle at very high dosages. On the other hand, the effects of cimaterol on fat tended to be greater at 150 mg/kg than at 25 mg/kg. This supports the conclusion of Sainz & Wolff (1987) that cimaterol must have specific effects in both muscle and adipose tissue to produce the observed changes in body composition.

Body water increased from 660 g/kg in controls to 680 g/kg in cimaterol-treated rats, in parallel with the increase in body protein from 190 to 200 g/kg. However, body protein content increased proportionately more than water content, resulting in a lower water:protein ratio (WPR) in the 25 mg cimaterol/kg group (3·31 compared with 3·49 in controls, a 5% reduction). The ratio of body water:protein is not constant, and indeed has been proposed as an index of physiological maturity (Bailey *et al.* 1960). In addition, WPR can be altered by nutritional state (Mendez, 1966; Suzuki *et al.* 1975). However, β-agonists do not appear to alter WPR in lambs (Baker *et al.* 1984), calves (Williams *et al.* 1986) or steers (Ricks *et al.* 1984). In contrast, WPR decreased 3·1% from 3·81 in controls to 3·69 in clenbuterol-treated rats (Rothwell *et al.* 1984). Emery *et al.* (1984) reported a 9·3% reduction of WPR, from 3·45 in controls to 3·13 in rats treated with clenbuterol, whilst fenoterol had no effect on WPR. Similarly, clenbuterol produced 5·5 and 11·3% reductions in WPR in normal and dystrophic mice respectively (Rothwell & Stock, 1985). Possibly, differences in tissue components other than protein which bind water (e.g. glycogen) may contribute to observed changes in WPR. The present study is in agreement with demonstrated reductions of WPR in rodents treated with clenbuterol. The reason for the different response of WPR to β-agonists observed in ruminant livestock species and laboratory rodents is not clear.

In the present study, cimaterol did not affect energy intake, while decreasing energy retention and increasing heat production (Table 3). These results contrast with those of Emery *et al.* (1984), who reported that clenbuterol increased ME intake and energy expenditure, without affecting energy gain in rats. The major difference appears to be that in the present study, rats did not increase food intake to compensate for increased energy expenditures, resulting in mobilization of fat reserves to provide substrates for oxidation. Differences in dosage are probably not responsible for these discrepancies. Emery *et al.* (1984) used a daily dose of 2 mg clenbuterol/kg body-weight; in the present study, rats consumed about 2·3 and 13·8 mg cimaterol/kg body-weight in the 25 and 150 mg/kg diets respectively. Possibly, these differences are due to the method of delivery, since in the Emery *et al.* (1984) study clenbuterol was injected twice daily, whereas we incorporated cimaterol in the diet. Also, these drugs may differ in their metabolic effects in one or more tissues. Without information about receptor and tissue specificities, second messengers or changes in energy-utilizing processes due to cimaterol and clenbuterol, these differences must remain unresolved.

In conclusion, the present study has demonstrated that cimaterol produces similar effects in rats as in sheep, i.e. protein gain is increased at the expense of fat. In addition, muscle growth was particularly enhanced by this β-adrenergic agonist, in agreement with reports of increased yields of saleable meat in livestock. As was pointed out by Reeds *et al.* (1986), β-agonists may provide an important tool in the elucidation of factors limiting muscle growth and therefore controlling meat production. Identification of the mechanism(s) of action of β-agonists will undoubtedly improve our understanding of the factors controlling growth and body composition.

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REFERENCES

Asato, G., Baker, P. K., Bass, R. T., Bentley, T. J., Chari, S., Dalrymple, R. H., France, D. J., Gingher, P. E., Lences, B. L., Pascavage, J. J., Pensack, J. M. & Ricks, C. A. (1984). *Agricultural and Biological Chemistry* **48**, 2883–2888.

Bailey, C. B., Kitts, W. D. & Wood, A. J. (1960). *Canadian Journal of Animal Science* **40**, 143–155.

Baker, P. K., Dalrymple, R. H., Ingle, D. L. & Ricks, C. A. (1984). *Journal of Animal Science* **59**, 1256–1261.

Bohorov, O., Buttery, P. J., Correia, J. H. R. D. & Soar, J. B. (1987). *British Journal of Nutrition* **57**, 99–107.

Dalrymple, R. H., Baker, P. K., Gingher, P. E., Ingle, D. L., Pensack, J. M. & Ricks, C. A. (1984). *Poultry Science* **63**, 2376–2383.

Emery, P. W., Rothwell, N. J., Stock, M. J. & Winter, P. D. (1984). *Bioscience Reports* **4**, 83–91.

James, K. A. C. & Treloar, B. P. (1981). *Journal of Nutrition* **111**, 1797–1804.

Jones, R. W., Easter, R. A., McKeith, F. K., Dalrymple, R. H., Maddock, H. M. & Bechtel, P. J. (1985). *Journal of Animal Science* **61**, 905–913.

Kleiber, M. (1961). *The Fire of Life*. New York: John Wiley.

Mendez, J. (1966). *Journal of Nutrition* **89**, 513–519.

Reeds, P. J., Hay, S. M., Dorwood, P. M. & Palmer, R. M. (1986). *British Journal of Nutrition* **56**, 249–258.

Ricks, C. A., Dalrymple, R. H., Baker, P. K. & Ingle, D. L. (1984). *Journal of Animal Science* **59**, 1247–1255.

Rothwell, N. J. & Stock, M. J. (1985). *Bioscience Reports* **5**, 755–760.

Rothwell, N. J., Stock, M. J. & Winter, P. D. O'B. (1984). *Proceedings of the Nutrition Society* **43**, 71A.

Sainz, R. D. & Wolff, J. E. (1987). In *Proceedings of the 2nd International Symposium on the Nutrition of Herbivores*, pp. 153–154. Brisbane: Australian Society of Animal Production.

Steel, R. G. D. & Torrie, J. H. (1981). *Principles and Procedures of Statistics: A Biometrical Approach*. New York: McGraw-Hill.

Suzuki, H., Goshi, H. & Sugisawa, H. (1975). *Journal of Nutrition* **105**, 90–95.

Thornton, R. F., Tume, R. K., Payne, G., Larsen, T. W., Johnson, G. W. & Hohenhaus, M. A. (1985). *Proceedings of the New Zealand Society of Animal Production* **45**, 97–101.

Williams, P. E. V., Pagliani, L. & Innes, G. M. (1986). *Livestock Production Science* **15**, 289–293.

Wolff, J. E., Dalrymple, R. H. & Ingle, D. L. (1987). In *Proceedings of the 4th Asian–Australasian Association of Animal Production Animal Science Congress*, p. 490. Hamilton, New Zealand: AAAP Organising Committee.

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