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Increased body-weight gain and body protein in castrated and adrenalectomized rats treated with clenbuterol

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1. Daily injection of the β_2-adrenergic agonist clenbuterol (1 mg/kg body-weight) increased weight gain by 12% in young (35 d) male rats and by 18% in castrated rats, but had no effect on energy intake, expenditure or efficiency in either group.

2. Body fat content was not affected by clenbuterol or castration, but water and protein content were significantly increased by clenbuterol treatment in both intact and castrated rats. The ratio, body protein:fat was increased by 13 and 16% in these two groups compared with their respective, untreated controls.

3. Bilateral surgical adrenalectomy (ADX) of young (45 d) male rats significantly reduced body-weight, and energy intake, expenditure and efficiency. Carcass energy and fat contents were also reduced in ADX rats compared with age-matched controls.

4. Clenbuterol injections stimulated weight gain (% increase: intact 15, ADX 35), and increased body protein content (% increase: intact 12, ADX 8) and the ratio, carcass protein:fat (% increase: intact 34, ADX 23).

5. These findings demonstrate that the effects of clenbuterol on body-weight gain and composition in male rats occur in the absence of either gonadal or adrenal hormones. Together with other studies, these results provide further evidence to suggest that clenbuterol probably exerts its effects by a direct action on lean body mass.

Anabolic steroids have been used for many years to stimulate body growth and protein deposition, and have proved to be of considerable value, both clinically and in animal production. However, attention has recently turned towards a new class of compounds, the β_2-adrenergic agonists. Most of the work in this field has concentrated on clenbuterol, a selective β_2-agonist which causes marked increases in body-weight gain (27%) and carcass protein content (50%) in young rats (Emery *et al.* 1984), and comparable improvements in live-weight gain or carcass quality, or both, in larger animals (e.g. Dalrymple *et al.* 1983; Ricks *et al.* 1983, 1984*a*, *b*; Baker *et al.* 1984; Jones *et al.* 1985).

The mechanism of action of clenbuterol is at present unknown, and studies on its effects on muscle protein synthesis are somewhat conflicting. Emery *et al.* (1984) described acute increases in in vivo protein synthesis in the skeletal muscle of rats treated with clenbuterol, whereas Reeds *et al.* (1986) observed no change in synthesis and ascribed differences in muscle protein deposition to reduced degradation rates. These apparent differences in the mode of action may depend on the dose, route and timing of clenbuterol administration, and it is possible that both effects contribute to the long-term effect of clenbuterol on lean body mass. Whatever its effects on protein synthesis and degradation, it is still not known whether clenbuterol affects protein deposition by a direct action on muscle, or whether it acts indirectly via other anabolic influences. An obvious category of hormones which could mediate the anabolic actions of clenbuterol are the steroids, and the present studies were undertaken to assess the involvement of gonadal and adrenal hormones in the changes in body composition induced by chronic clenbuterol treatment.

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MATERIALS AND METHODS

All animals were male, Sprague-Dawley rats aged 35–45 d obtained from Charles River (Marston, Kent). They were allowed free access to a standard pelleted diet (PRD, Christopher Hill Group Ltd, Poole, Dorset) and were housed at $24 \pm 1°$ with a 12 h light–12 h dark cycle. Adrenalectomized rats received normal saline (9 g sodium chloride/l) instead of drinking water. Daily injections of either clenbuterol (1 mg/kg body-weight in 0·2 ml medium-chain triglyceride, subcutaneously) or vehicle (medium-chain triglyceride) were administered at 09.00 hours.

Metabolizable energy (ME) intake was calculated from the weight of food consumed and its ME density (12·0 kJ/g as fed; obtained in previous feeding trials). At the end of the experiments the carcasses were frozen, chopped and freeze-dried to constant weight to determine body water content. The dry carcasses were homogenized and samples were taken for determination of energy content by ballistic bomb calorimetry, fat content by Soxhlet extraction and protein by Kjeldahl analysis. In the two experiments to be described, six weight-matched animals were killed on the 1st day, and analysed for energy content. Using the energy density values for these animals it was possible to estimate body energy gain of the experimental animals and to calculate total energy expenditure (ME intake minus body energy gain), gross energetic efficiency (body energy gain per unit ME intake) and net energetic efficiency (body gain per unit ME intake above maintenance), assuming a constant value for maintenance in all groups of 420 kJ/kg body-weight$^{0·75}$ per d).

Experimental

Expt 1. *Effect of castration.* Thirty-two rats at 35 d of age (122 g) were anaesthetized (halothane (20 ml/l) in oxygen–nitrous oxide) and subjected to castration (surgical removal of both testes) or sham operations. Half the castrated and half the sham-operated rats were injected with clenbuterol daily for 21 d; the remainder received vehicle alone (controls). The animals were killed on day 22 and the carcasses analysed.

Expt 2. *Effect of adrenalectomy.* Thirty-two rats at 45 d of age (183 g) were anaesthetized (as described previously) and bilateral adrenalectomy (ADX) or a sham operation performed. After allowing 1 d for recovery, groups of ADX and sham-operated rats were treated with either clenbuterol or vehicle (as described previously) for 14 d, after which they were killed and the carcasses analysed.

Values have been presented as means with their standard errors. Statistical differences were assessed using Scheffe's critical range analysis with a probability of less than 5%

RESULTS

In Expt 1, castration caused a slight inhibition of weight gain (Table 1), but clenbuterol treatment significantly enhanced weight gain in intact (12%) and castrated (18%) rats. ME intake, body energy gain and energy expenditure were all slightly, but not significantly, increased in clenbuterol-treated rats, and both gross and net energetic efficiencies were unaffected by any of the treatments.

The percentage of water, fat and protein in the carcasses at the end of the experiment was unaffected by castration or clenbuterol treatment (Table 2), and total body fat content was also comparable for all groups. Total water content was increased by clenbuterol treatment in intact (8%) and castrated (10%) rats, and total body protein was also significantly elevated in drug-treated groups compared with their controls (intact 6%, castrated 13%). Protein content was reduced in vehicle-injected castrated rats compared with intact

Table 1. *Expt 1. Effect of clenbuterol on energy balance in intact and castrated rats**

(Mean values with their standard errors for eight rats)

	Intact				Castrated			
	Vehicle†		Clenbuterol		Vehicle		Clenbuterol	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wt gain (g)	139[a]	6	155[b]	3	127[c]	5	150[ab]	4
ME intake (kJ)	5500[a]	150	5725[a]	110	5290[a]	155	5650[a]	165
Body energy gain (kJ)	1095[a]	35	1140[a]	15	1070[a]	55	1159[a]	45
Energy expenditure (kJ)	4400[a]	50	4585[a]	70	4220[a]	65	4490[a]	70
Gross energetic efficiency (%)	20[a]	1	20[a]	1	20[a]	1	21[a]	1
Net energetic efficiency (%)	38[a]	1	37[a]	1	38[a]	1	37[a]	1

ME, metabolizable energy.

* Mean body energy content of animals killed at start of experiment was 740 (SEM 8) kJ.

[a, b, c] Values in horizontal rows sharing the same superscript letter were not significantly different.

† Medium-chain triglyceride.

Table 2. *Expt 1. Effect of clenbuterol on body composition of intact and castrated rats*

(Mean values with their standard errors for eight rats)

	Intact				Castrated			
	Vehicle*		Clenbuterol		Vehicle		Clenbuterol	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body water								
g	173[a]	1	186[b]	1	169[a]	2	186[b]	1
%	66·5[a]	4	67·1[a]	0·3	66·5[a]	0·3	67·1[a]	0·2
Body protein								
g	52[a]	1	55[b]	1	48[c]	1	54[ab]	1
%	19·5[a]	2	19·8[a]	0·2	19·4[a]	0·6	19·7[a]	0·3
Body fat								
g	26[a]	1	25[a]	1	27[a]	2	26[a]	2
%	10·0[a]	0·5	9·0[a]	0·3	10·6[a]	0·3	9·4[a]	2
Protein:fat (g/g)	1·98[a]	0·04	2·23[b]	0·07	1·79[c]	0·7	2·08[a]	0·6

ME, metabolizable energy.

[a, b, c] Values in horizontal rows sharing the same superscript letter were not significantly different.

* Medium-chain triglyceride.

controls. The ratio, body protein:fat mass was slightly reduced in castrated rats injected with vehicle and elevated in clenbuterol-treated animals (intact 13%, castrated 10%).

In Expt 2, adrenalectomy caused large reductions in ME intake, body-weight and energy gains, energy expenditure and gross energetic efficiency (Table 3). Net energetic efficiency, assuming a constant maintenance requirement, was also slightly reduced by adrenalectomy. Clenbuterol treatment stimulated body-weight gain by 15% in intact and 35% in ADX rats and did not affect significantly energy balance or gross energetic efficiency. Net efficiency, however, was slightly elevated.

Adrenalectomy caused quite marked changes in body composition, resulting in lower values for total water, protein and fat contents (and percentage body fat), but these animals

Table 3. *Expt 2. Effect of clenbuterol on energy balance of intact and adrenalectomized (ADX) rats**

(Mean values with their standard errors for eight rats)

	Intact				ADX			
	Vehicle†		Clenbuterol		Vehicle		Clenbuterol	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wt gain (g)	122[a]	4	140[b]	3	74[c]	4	100[d]	5
ME intake (kJ)	5615[a]	110	5300[a]	175	3790[b]	115	3860[b]	135
Body energy gain (kJ)	1030[a]	60	1020[a]	35	510[b]	30	580[b]	60
Energy expenditure (kJ)	4585[a]	80	4370[a]	105	3290[b]	70	3320[b]	120
Gross energetic efficiency (%)	18[a]	1	19[a]	1	13[b]	1	15[b]	1
Net energetic efficiency (%)	29[a]	1	32[b]	1	26[c]	1	31[ab]	1

ME, metabolizable energy.

* Mean body energy content of animals killed at start of experiment was 1160 (SEM 10) kJ.

[a, b, c, d] Values in horizontal rows sharing the same superscript letter were not significantly different.

† Medium-chain triglyceride.

Table 4. *Expt 2. Effect of clenbuterol on body composition of intact and adrenalectomized (ADX) rats*

(Mean values with their standard errors for eight rats)

	Intact				ADX			
	Vehicle*		Clenbuterol		Vehicle		Clenbuterol	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body water								
g	209[a]	1	224[b]	1	180[c]	1	201[d]	1
%	68·5[a]	0·3	69·6[b]	0·1	70·2[b]	0·3	71·1[c]	0·2
Body protein								
g	59[a]	1	66[b]	1	52[c]	1	56[a]	2
%	19·5[a]	2	20·3[a]	0·8	20·1[a]	0·1	19·0[a]	0·1
Body fat								
g	24[a]	2	20[a]	1	15[b]	1	14[b]	1
%	8·0[a]	0·4	6·1[b]	3	5·5[bc]	5·1	5·2[c]	2
Protein:fat (g/g)	2·46[a]	0·07	3·30[b]	0·08	3·50[b]	0·8	4·30[c]	0·9

ME, metabolizable energy.

[a, b, c, d] Values in horizontal rows sharing the same superscript letter were not significantly different.

* Medium-chain triglyceride.

had a greater percentage body water and a higher protein:fat ratio compared with intact controls (Table 4). The slightly lower percentage body fat content of the intact animals in this experiment (cf. the younger rats in Expt 1) may have reflected the greater trauma of the sham operation for the adrenalectomy experiment. Clenbuterol treatment increased the body water and protein contents in intact and ADX rats, and also produced a lower percentage of body fat in intact animals. The ratio protein:fat was elevated by 34% in intact rats and by 23% in ADX rats given clenbuterol, relative to their vehicle-injected controls.

DISCUSSION

We have previously shown that adrenalectomy and castration inhibit weight gain in the rat, partly by reducing food intake, but also because of lower levels of energetic efficiency (Rothwell & Stock, 1984, 1986; Rothwell *et al.* 1984). These changes in weight gain are due mainly to lower rates of fat deposition and are associated with enhanced levels of thermogenesis, particularly in older and obese animals (Rothwell & Stock, 1984, 1986; Rothwell *et al.* 1984). In the present study, as in our previous experiments, adrenalectomy exerted much more potent effects than castration on energy balance, causing marked reductions in body-weight and fat content. Adrenalectomy also markedly reduced gross efficiency, but the interpretation of these energy balance results is complicated by the marked suppression of energy intake which resulted from the operation. Since only the energy consumed above maintenance requirements will contribute to body energy gain, it is more valid to consider net energetic efficiency. This calculation assumes constant and similar maintenance requirements in all groups and reveals a small, but nevertheless significant reduction in ADX rats. We have previously found that changes in energetic efficiency in ADX rats are due to increased activity of the sympathetic nervous system, and can be reversed by replacement with corticosterone (Rothwell & Stock, 1984).

In intact animals, clenbuterol stimulated body-weight gain without significantly affecting energy intake, expenditure or gross efficiency (Tables 1 and 3), although net energetic efficiency was significantly enhanced in Expt 2. This was presumed to be due to an increase in the efficiency of protein deposition. Clenbuterol injection did not affect total fat content and all of the extra weight gained was due to increases in body water and protein (Tables 2 and 4). These effects of clenbuterol on body composition and weight gain were sustained in both castrated and ADX rats. Once again it is impossible to avoid the complicating effects of changes in food intake, since body composition will be influenced by hypophagia as well as by the direct actions of the drug. This effect of food intake is particularly important in adrenalectomized rats when comparing them with controls on a considerably higher energy intake, although direct comparisons can be made between ADX rats receiving vehicle or clenbuterol.

In castrated animals, clenbuterol produced slightly greater increases in body-weight gain (18 *v.* 12% for intact rats) and protein content (13 *v.* 6% for intact animals). The increases in body protein content were slightly attenuated in ADX rats treated with clenbuterol, but total weight gain was substantially greater. Calculations of feed efficiency are often used by animal nutritionists, particularly when describing the effects of growth-promoting agents, although they may be of limited value in the interpretation of energy balance data. In these experiments, feed efficiency (g gain/MJ eaten) was increased by 33% in clenbuterol-treated ADX rats (25·9 (SE 0·7)) compared with untreated ADX rats (19·5 (SE 1·1), $P < 0.05$). Clenbuterol produced a 21% increase in feed efficiency in the intact animals in this adrenalectomy experiment, whereas in Expt 1, clenbuterol produced only 8–10% increases in feed efficiency in intact and castrated rats. Variations in feed efficiency can obviously reflect changes in energetic efficiency or the composition of gain, or both, as well as alterations in intake. Clenbuterol, like most β-adrenergic agonists, stimulates thermogenesis (see Stock & Rothwell, 1986), but because its thermogenic actions are relatively small and short-lived in the rat (Winter, 1983) its overall effects on energy balance are mainly determined by its anabolic actions.

At present, the mechanisms by which β_2-selective adrenoceptor agonists increase body protein deposition are unknown. In earlier studies, we found no effect of clenbuterol on circulating concentrations of growth hormone, 3, 5, 3′-triiodothyronine or insulin (Winter, 1983; Stock & Rothwell, 1986). Furthermore, the stimulation of protein deposition by

clenbuterol is sustained in hypophysectomized (James & Barker, 1987) and diabetic (N. J. Rothwell and M. J. Stock, unpublished results) animals, and still occurs in denervated muscles (Maltin *et al.* 1986, 1987). The present results now suggest that adrenal and gonadal steroids are also not required for clenbuterol to exert its repartitioning effects on body composition.

When considered together, all these studies on the mode of action of clenbuterol on protein deposition indicate that the compound is probably having direct effects on lean tissue (i.e. muscle), and does not act indirectly via the permissive effects or other effects of most hormones. We (Rothwell *et al.* 1987) have recently found that chronic treatment of rats with clenbuterol results in a reduced blood flow to skeletal muscle, and it is possible that this could influence muscle protein deposition by inhibiting protein degradation.

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