Protective effect of clenbuterol on duodenal epithelium during food restriction in rats

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ABSTRACT

The aim of the study was to examine the effect of the β_2-adrenoceptor, clenbuterol, on the duodenal epithelium of food-restricted rats. Clenbuterol was administered as a dietary admixture (4 mg/kg diet) to three groups of male Wistar rats (n = 8) housed individually in metabolic cages and fed *ad libitum* for 15 days at 110 % and 160 % of the estimated requirement for energy maintenance. Untreated groups at each energy intake level were also included. Samples of the duodenum were examined by light microscopy. Compared with control animals, clenbuterol treatment significantly increased body mass in all diet groups, although it induced no changes in mean food intake. Gastrointestinal (GIT) dry mass was increased by clenbuterol only in the most severely-restricted-diet group. In this group, clenbuterol treatment increased GIT tissue nitrogen (23 %), more than it did in the *ad libitum* group (13 %). In all treated groups, clenbuterol induced significant hypertrophy of duodenal enterocytes and circular muscle layers, and the diameter of lymphatic vessels increased. In the clenbuterol-treated, restricted-diet groups the height of the brush borders of enterocytes increased. It is concluded that clenbuterol has a protective effect on the intestinal structure in rats on restricted as well as *ad libitum* diets.

Key words: β-adrenergic agonists, food restriction, intestinal epithelium.

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INTRODUCTION

The selective β_2-adrenergic agonist clenbuterol has anabolic and body repartitioning effects in rats[13,14] and in farm animals.[3,9] These effects are mainly due to increases in body protein (mainly skeletal muscle) and decreases in fat content. Most studies on the effects of clenbuterol and similar repartitioning agents such as cimaterol have been done in laboratory and on farm animals under *ad libitum* feeding conditions.[17,1]

For public health reasons, the use of anabolic agents in meat production is no longer allowed in certain countries. However, they may have veterinary clinical uses and there is growing interest in the potential use of anabolic β_2-adrenergic agonists in the treatment of some human muscle-wasting diseases[10]. Muscle wasting also occurs naturally in livestock suffering from seasonal decreases in food availability, and in many parts of the world loss of protein is a major constraint on animal production. Methods for minimising the seasonal decrease in live mass and the loss of protein should therefore be of interest. In energy-restricted rats, β-adrenoceptor anabolics cause an increase in growth, protein accumulation, nitrogen and energy balance compared with rats fed *ad libitum*.[4,5]

Since clenbuterol has its major effect on anabolic protein metabolism, duodenal samples were studied because of the important role of this intestinal area in amino acid absorption. The aim of the present study was to evaluate the action of clenbuterol on the structure of the duodenal wall in diet-restricted animals. The work described here refers to the histological changes of duodenal epithelium of rats fed different amounts of diet for 15 days, with and without the addition clenbuterol.

MATERIALS AND METHODS

Adult male (200 g) rats were obtained from a Wistar colony maintained at the Gulbenkian Foundation Science Institute (Lisbon, Portugal) and divided into six weight-matched groups (n = 8). The experimental groups were allocated to three dietary treatments (two groups per treatment).

In one of the diets the rats were fed *ad libitum* (235 % of estimated energy maintenance requirements) and the other two involved restricting energy intake to 160 % and 110 % of estimated maintenance requirements that were assumed to be 460 kJ/kg$^{0.75}$/day[11]. From the manufacturer's figures for the metabolisable energy of the stock diet (CRM, Biosure, UK, metabolisable energy density 12.8 kJ/g, 17.5 crude protein, 2.4 % fat and 5.3 % ash) it was calculated that 160 and 110 % of maintenance per rat would respectively be provided by 17 and 12 g of food daily. These amounts were then fed throughout the 15-day experiment.

Within each feeding treatment, one group received the diet alone while the other group received clenbuterol (Boehringer Ingleheim, Bracknell, UK) incorporated into the diet at a concentration of 4 mg/kg. Thus, there were six experimental groups:: *ad libitum* without clenbuterol (ADL); *ad libitum* with clenbuterol (ADLC); 17 g restricted without clenbuterol (R17); 17 g restricted with clenbuterol (R17C); 12 g restricted without clenbuterol (R12); and 12 g restricted with clenbuterol (R12C). The rats were housed individually in metabolic cages in a room at 24 ± 1 °C, 60–65 % humidity and with a 12 h light/dark cycle. Food intake (corrected for spillage) was recorded daily and body mass every other day.

On day 15, all rats were anaesthetised using sodium pentothal and killed by cardiac puncture and exsanguination. The gastrointestinal tract (GIT), considered from the distal oesophagus to the anus, was dissected, cleaned, the contents removed, cleared of other tissue, weighed and frozen (–20 °C) until analysed. GIT dry mass was determined by freeze-drying (Modulyo, Edwards, UK) to constant mass, and after homogenisation the protein content of samples was determined by Kjeldahl digestion (Buchi 315, Schweiz, Germany).

Small samples of duodenum were removed from the experimental animals, fixed in formalin, dehydrated in a graded alcohol series and embedded in paraffin. Sections (5 μm thick) were stained with

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Table 1: **Mass, and protein and histological features of the duodenum of rats fed various diets with and without clenbuterol.**[a]

Feature measured	Dietary group[b]												*F*-test[j]		
	R12		RC12		R17		RC17		AD		ADL[c]		LSD[i]	Diet	Trt.	Diet × Trt
Food intake (g)	175.7	(2.8)	178.7	(0.5)	252.7	(1.1)	248.9	(1.4)	363.6	(9.5)	379.3	(6.8)	30.3	171.4[z]	0.26	0.504
Weight gain (g)	-0.6	(2.1)	25.4***	(3.2)	37.8	(2.4)	56.3***	(3.5)	97.6	(5.2)	128.4**	(7.4)	12.5	272.9[z]	49.2[z]	1.008
GIT[d] mass (g)	1.02	(0.02)	1.29**	(0.09)	1.53	(0.07)	1.53	(0.05)	2.52	(0.19)	2.85	(0.36)	0.13	40.7[x]	1.9	0.49
GIT[d] protein (%)	72.32	(1.79)	89.02***	(2.77)	62.95	(1.85)	63.77	(1.03)	48.37	(1.28)	54.52*	(1.93)	5.43	125.6[x]	22.5[x]	11.6[x]
Musc. thickness[e] (μm)	107.64	(8.44)	120.12*	(4.75)	113.52	(4.22)	130.34*	(6.33)	106.92	(11.61)	129.88**	(6.97)	14.52	1.42	7.47[z]	0.20
Enteroc. height[f] (μm)	18.30	(1.08)	22.82*	(3.21)	20.04	(1.36)	25.84*	(2.31)	23.46	(1.63)	33.32**	(2.17)	3.02	18.32[z]	37.64[z]	239
Brush b. height[g] (μm)	1.72	(0.15)	2.24***	(0.12)	1.74	(0.16)	1.90*	(0.18)	1.77	(0.13)	1.71	(0.24)	0.02	16.3	40.3[z]	16.3[z]
Diam. ax. lacteal[h] (μm)	0.5	(0.08)	24.2***	(5.24)	0.64	(0.15)	14.88***	(2.42)	6.02	(1.3)	16.32***	(2.4)	3.56	4.63[y]	158.24[z]	9.67[z]

[a]Values are means (SEM, $n = 8$).
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *vs* respective control group (Student's unpaired *t*-test).
[b]Diets: R12, R17 fed at 12 and 17 g daily; ADL available *ad libitum.*
[c]Indicates added clenbuterol.
[d]GIT = gastrointestinal tract.
[e]Musc. thickness = circular muscle thickness.
[f]Enteroc. height = enterocyte height.
[g]Brush b. height = brush border height.
[h]Diam.ax. lacteal = diameter of axial lacteal.
[i]LSD = least significant difference between groups (ANOVA).
[j]For *t*-test results: x = $P < 0.05$; y = $P < 0.01$; z = $P < 0.001$ for *F*-ratio significance levels.

hematoxylin and eosin (HE), for light microscopy.

The thickness of smooth muscle layers, diameter of lacteals, height of enterocytes and brush borders were measured. For each study animal, five duodenal histological sections were evaluated and the results analysed by ANOVA to determine the effects of feeding level, clenbuterol treatment and their interaction. *Post hoc* significant differences between various treatment groups were identified by the least significant difference test (LSD, $P < 0.05$). Within each feeding level, significant differences between untreated and clenbuterol-treated rats were determined using Student's *t*-tests for unmatched data. All probabilities are two-tailed.

RESULTS

At all levels of food intake, clenbuterol treatment resulted in greater body mass gains than in the respective control groups (Table 1). By the end of the experimental period, mass gains had increased by 32 % and 49 % respectively in the ADLC and R17C groups. The most severely-restricted-diet rats (R12) lost mass (0.6 g), but the effect of clenbuterol was to reverse this and produce a mass gain of 25 g. Table 1 also shows that GIT dry mass was significantly increased by clenbuterol treatment only in the severely-restricted-diet rats (RC12), which were 26 % heavier than the controls. GIT protein content (percentage of dry matter), relative to the control values, was much more increased in the RC12 group (23 %) than in the *ad libitum*-fed, clenbuterol-treated rats (12.8 %). Clenbuterol treatment also induced significant increases in circular muscle thickness, enterocyte height and the diameter of axial lacteals in rats on all diets. Brush border height was increased by clenbuterol treatment in both restricted-diet groups.

Fig. 1**: Duodenum of diet-restricted rat, with clenbuterol treatment (group RC12). Compared to Fig. 2, there is an increase in blood and lymphatic vessel diameters and hypertrophy of enterocytes and their brush borders.** Scale bar = 20 μm. H&E, ×400.

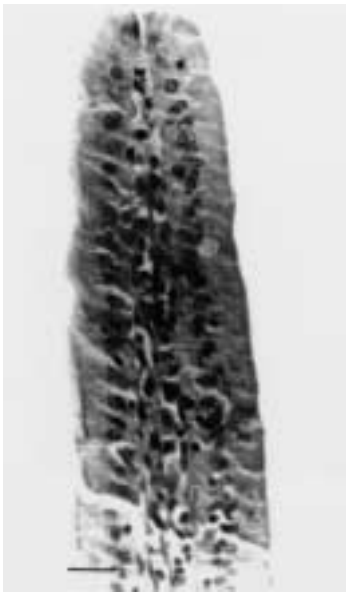

Fig. 2: **Duodenum of diet-restricted rat, without clenbuterol treatment.** Scale bar = 20 μm. H&E, ×400.

Oedema was present in the intestinal villi of the clenbuterol-treated severely-restricted-diet group (Fig. 1) but not in the control animals (Fig. 2). These figures also show that the clenbuterol-treated, severely-restricted-diet group maintained better anatomical and functional integrity than did the control group.

DISCUSSION

Clenbuterol's anabolic effect on protein metabolism could be responsible for the increase in GIT dry mass that was observed in the treated, severely-restricted-diet group and is probably related to the increase in protein concentration. Increased food absorption can be associated with increased lymphatic vessel diameter. In fact, it has been shown that clenbuterol increases protein digestibility in rats on a very restricted diet[6].

The oedema that was observed in the intestinal villi of clenbuterol-treated severely-restricted-diet rats (RC12) could be due to increased local blood pressure caused by the β-agonist. The oedema can also be due to an increased permeability of enterocyte 'tight junctions' which is inversely related to intracellular calcium concentration[12]. Reduction of intercellular calcium is one of the β-adrenoceptor effects[15]. Compared to the control group,

the RC12 group showed increases in GIT mass and protein content, circular muscle thickness, enterocyte and brush border height and diameter of the axial lacteal. In this diet group, the changes can be related to increased anatomical and functional integrity of the intestinal epithelium and to a better performance of enterocyte and brush border enzymes involved in the terminal steps of protein and carbohydrate digestion. The hypertrophy of circular muscle layers observed in the treated rats can be due to β-adrenoceptor adrenergic stimuli, providing an increase in intestinal muscle excitability[8] and thus resulting in an improvement in intestinal peristalsis and absorption.

It has been reported previously[5] that, only in the RC12 diet group, clenbuterol induced increases in IGF-1 plasma levels (compared to controls). Several authors have elucidated the relationship between IGF-1 plasma concentrations and intestinal integrity in rats[7,16], with increased intestinal mucosal mass and protein content in IGF-1-treated animals. Similar results were obtained in pigs[2] following oral administration of recombinant human insulin-like growth factor. In the treated, severely-restricted-diet rat group, high plasma levels of IGF-1 could be associated with duodenal changes induced by clenbuterol. Further studies should be undertaken to better investigate β-adrenoceptor-induced action on enterocytes.

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