ABSTRACT: Previous investigations of the effects of clenbuterol have used suprapharmacological doses that induce myocyte death, alter muscle phenotype, and do not approximate the proposed therapeutic dose for humans. Recently, we reported that smaller doses of clenbuterol induce muscle growth without causing myocyte death. In the present study we used histochemical and proteomic techniques to investigate the molecular effects of this dose. Male Wistar rats ($n = 6$, per group) were infused with saline or 10 μg/kg/day clenbuterol via subcutaneously implanted osmotic pumps. After 14 days the animals' plantaris muscles were isolated for histochemical and proteomic analyses. Clenbuterol induced significant muscle growth with concomitant protein accretion and preferential hypertrophy of fast oxidative glycolytic fibers. Clenbuterol reduced the optical density of mitochondrial staining in fast fibers by 20% and the glycogen content of the muscle by 30%. Differential analysis of two-dimensional gels showed that heat shock protein 72 and β-enolase increased, whereas aldolase A, phosphoglycerate mutase, and adenylate kinase decreased. Only heat shock protein 72 has previously been investigated in clenbuterol-treated muscles. The clenbuterol-induced increase in muscle growth was concomitant with qualitative changes in the muscle's proteome that need to be considered when proposing therapeutic uses for this agent.

Muscle Nerve **35:** 217–223, 2007

ANABOLIC EFFECTS OF A NON-MYOTOXIC DOSE OF THE β_2-ADRENERGIC RECEPTOR AGONIST CLENBUTEROL ON RAT PLANTARIS MUSCLE

JATIN G. BURNISTON, PhD,[1] LYNN McLEAN, BSc,[2] ROBERT J. BEYNON, PhD,[2] and DAVID F. GOLDSPINK, DSc[1]

[1] Muscle Physiology and Proteomics Laboratory, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Webster Street, Liverpool L3 2ET, UK
[2] Protein Function Group, Department of Veterinary Preclinical Sciences, University of Liverpool, Liverpool, UK

Accepted 1 September 2006

The β_2-adrenergic receptor (β_2-AR) agonist clenbuterol has a potent anabolic effect on striated muscles.[12,25,27,38] The increased muscle growth is mediated through stimulation of the β_2-AR[9,18] and is associated with an increased rate of protein synthesis[17,30] and decreased rates of calcium-dependent[29] and adenosine triphosphate (ATP)–dependent[8,43] proteolysis. Consequently, clenbuterol and similar β_2-AR agonists have been proposed as a therapeutic intervention to counter muscle wasting concomitant with aging[39] or chronic disease.[1] However, the majority of studies in animals have used large doses (e.g., 1 mg/kg) that also induce myocyte death.[4,6,7] Muscle growth induced by suprapharmacological doses is associated with substantial transformation toward a faster-contracting, less fatigue-resistant phenotype as evidenced by alterations in myosin heavy chains[35] and light chains,[3] myosin ATPase activity,[45] energy metabolism,[38] and contractile characteristics.[12]

It is not entirely clear whether these are inevitable intrinsic effects of β_2-AR stimulation or purely a consequence of using suprapharmacological doses of clenbuterol. For example, although the anabolic effect of clenbuterol is reported to be greater in fast muscles,[5,12,38] clenbuterol-induced anabolism is mediated through the β_2-AR, which are greater in density in slow than fast muscles.[22] Slow-twitch muscles are also more susceptible than fast-twitch muscles to clenbuterol's myotoxicity,[5] and so the shift toward a

Abbreviations: AR, adrenergic receptor; ATP, adenosine triphosphate; CSA, cross-sectional area; FG, fast glycolytic; FOG, fast oxidative glycolytic; HSP 72, heat shock protein 72; MALDI-ToF, matrix-assisted laser desorption ionization–time-of-flight; MHC, myosin heavy chain; NADH-TR, nicotinamide dinucleotide–tetrazolium reductase; PAS, periodic acid–Schiff; PGM, phosphoglycerate mutase; SO, slow oxidative

Key words: 2D electrophoresis; clenbuterol; histochemistry; mass spectrometry; muscle growth; muscle wasting

Correspondence to: J. Burniston; e-mail: j.burniston@ljmu.ac.uk

Published online 20 October 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/mus.20684

faster muscle phenotype might be through the selective deletion of slow myofibers. Compatible with this, clenbuterol has more pronounced deleterious effects on the calcium handling of the sarcoplasmic reticulum of slow compared with fast-twitch fibers,[2] and this aberrant calcium handling may be the mechanism by which clenbuterol induces cell death.[14]

Recently, we reported that the myotoxic effects of clenbuterol can be separated from its anabolic effects by controlling the dose administered. That is, infusion of large doses (100 μg or 1 mg/kg/day) induced muscle growth and caused myofiber death, whereas a lower dose (10 μg/kg/day) induced growth without myocyte death.[5] Here we attempt to identify the subtle effects of this lower dose of clenbuterol using traditional muscle histochemistry and proteomic analysis, involving differential analysis of two-dimensional (2D) gels and mass spectrometry.[13] A significant advantage of this proteomic approach is its inductive nature and inherent ability to identify new effects or processes not previously associated with a particular intervention.[36] Our hypothesis was based on the ideal of a pure anabolic stimulus, that is, a quantitative increase in muscle mass through accretion of protein without qualitative changes in protein expression. We sought to determine whether clenbuterol-induced muscle growth differed from this ideal.

MATERIALS AND METHODS

Animal Husbandry. All experimental procedures complied with the British and U.S. national guidelines. Male Wistar rats were bred in-house in a conventional colony and the environmental conditions were controlled at 20 ± 2°C, 45%–50% humidity, and a 12-h light (0600–1800) and dark cycle. Water and food (containing 18.5% protein) were available ad libitum and the daily consumptions of each individual animal were recorded.

Animals (n = 6 per group) were infused with either clenbuterol 10 μg/kg/day or the saline vehicle only for 14 days, via subcutaneous osmotic pumps implanted under isoflurane anesthesia, as described previously.[5] After the 14-day infusion with either clenbuterol or saline, the animals were killed by cervical dislocation. A segment of the mid-belly from the plantaris was resected and mounted in transverse section and supported with liver before being snap-frozen in supercooled isopentane and stored at −80°C. The contralateral plantaris was frozen in liquid nitrogen and stored at −80°C for biochemical analyses.

Muscle Histology. Serial cryosections (5 μm) were cut from each muscle specimen and stained using myosin ATPase (after pre-incubation in either acid pH 4.35 or alkali pH 10.4 solutions[16]), nicotinamide dinucleotide–tetrazolium reductase (NADH-TR), or periodic acid–Schiff (PAS). Cryosections were viewed (×100 magnification) by light microscopy and were digitized using a 12-bit charge-coupled device (1213C; DVC, Austin, Texas). One hundred myofibers from each muscle were randomly selected and identified as being either slow oxidative (SO), fast oxidative glycolytic (FOG), or fast glycolytic (FG) from myosin ATPase–stained cryosections. Calibrated image analysis software (Lucia; LIM, Hostivar, Czech Republic) was used to measure myofiber cross-sectional area (CSA), and the average mitochondrial density and glycogen content were estimated by measuring the optical density of SO, FOG, or FG fibers (100 each) on NADH-TR or PAS-stained cryosections, respectively.

Protein Biochemistry. Muscles were pulverized in liquid nitrogen and an accurately weighed portion (~100 mg) homogenized on ice in 10 volumes of (in millimoles): 100 NaCl, 50 Tris, 2 ethylene-diamine tetraacetic acid (EDTA), and 0.5 dithiothreitol (pH 7.5), plus complete protease inhibitor (Roche Diagnostics, Lewes, UK). The protein concentration of a 5-μl aliquot of this homogenate was measured using a modified microtiter plate version of the Bradford assay (Sigma; Poole, Dorset, UK). The total protein content of each muscle was then calculated by multiplying protein concentration, homogenate volume, and the fraction of the ground portion relative to total muscle wet weight.

Muscle homogenates were prepared for 2D electrophoresis by centrifugation at 12,000 g and 4°C for 45 min. An aliquot of the supernatant (sarcoplasmic fraction), containing 250 μg of protein, was resuspended in standard rehydration buffer containing 8 mol urea, 2% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, 20 mmol dithiothreitol, and 0.5% (v/v) ampholytes, and loaded onto 13-cm immobilized pH gradient (IPG) strips (pH 3–10; GE Healthcare, Little Chalfont, UK). Isoelectric focusing (total 52,000 volt-hours) was conducted and the IPG strips equilibrated in 50 mmol Tris-HCl (pH 8.8), containing 6 mol urea, 30% (v/v) glycerol, 2% (w/v) sodium dodecylsulfate, and a trace of bromophenol blue. Dithiothreitol (10 mg/ml) was present as a reducing agent in the first equilibration and iodoacetamide (25 mg/ml) in the second. Proteins were electrophoresed through a linear 12% polyacrylamide gel at 20°C; initially at a

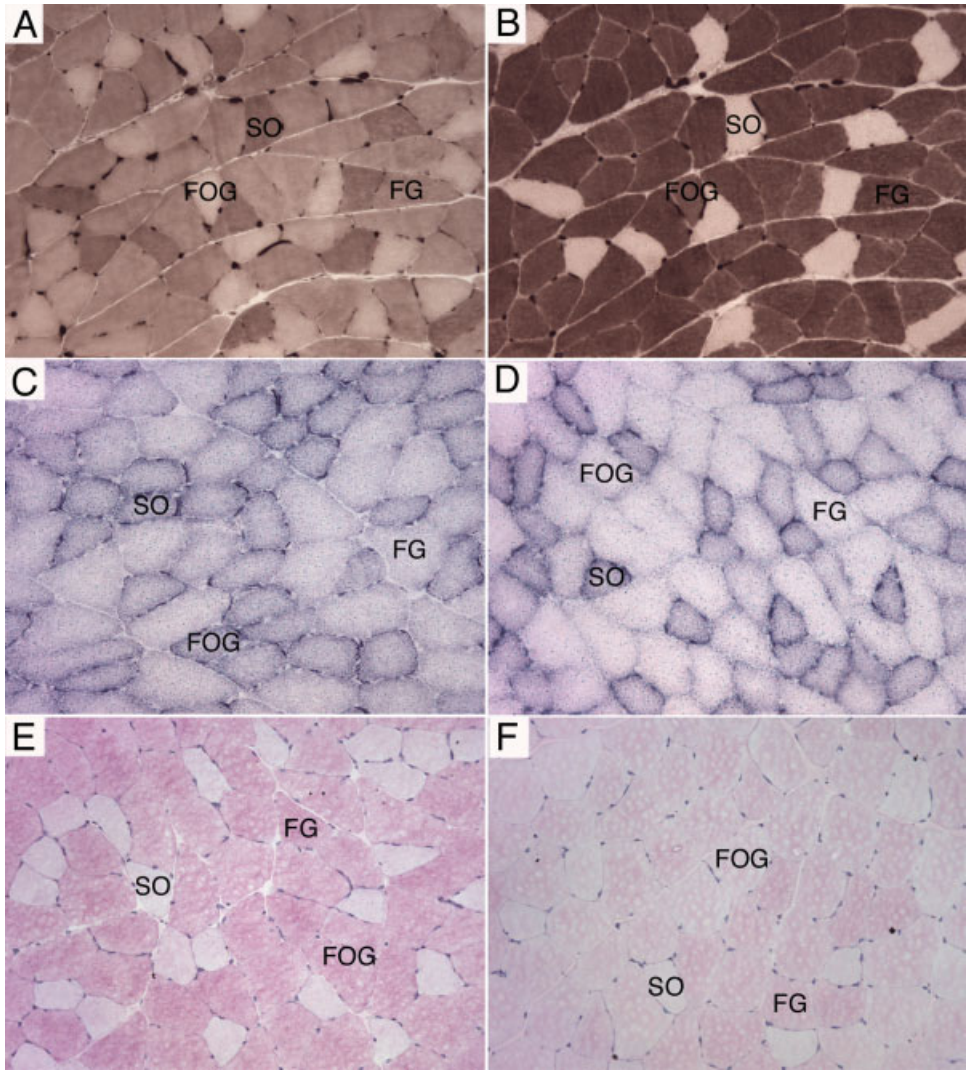

FIGURE 1. Histochemical analysis of muscle adaptation. Serial cryosections of plantaris muscle stained for myosin ATPase using an acid **(A)** or alkali **(B)** pre-incubation and used to assign myofiber types as slow oxidative (SO), fast oxidative glycolytic (FOG), and fast glycolytic (FG). Nicotinamide dinucleotide–tetrazolium reductase **(C, D)** and periodic acid–Schiff **(E, F)** staining demonstrates the marked differences in mitochondrial density and glycogen content, respectively, between saline-treated **(C, E)** and clenbuterol-treated **(D, F)** plantaris muscles. All images are ×200 magnification.

constant current of 15 mA per gel for 30 min and then at 30 mA per gel. Digitized images of colloidal Coomassie-stained gels (Bio-Safe; Bio-Rad, Hercules, California) were analyzed using 2D expression software (Non Linear Dynamics, Newcastle, UK). Spot volumes were expressed relative to the total integrated spot density, and those spots that changed significantly were excised and subjected to in-gel digestion and peptide extraction using a Mass-Prep digestion robot (Micromass, Manchester, UK). Peptides mixed with matrix (α-cyano-4-hydroxcinnamic acid) and spotted onto a target plate were analyzed using matrix-assisted laser desorption ionization–time-of-flight (MALDI-ToF) mass spectrometry (Micromass, Manchester, UK) over the range 1000–3500 thomsons. Proteins were identified from their peptide mass fingerprint by manually searching a locally implemented Mascot server. Search parameters were restricted by taxonomic class (Rodentia) and allowed a single trypsin missed cleavage, carbamidomethyl modification of cysteine, oxidation of methionine, and an m/z error of $\pm$ 250 ppm.

Statistical Analyses. Data are presented as mean $\pm$ SEM. Statistically significant differences between saline- and clenbuterol-treated muscles were determined using Student's two-tailed independent t-test

Table 1. Plantaris muscle characteristics after infusion of saline or clenbuterol.

	SO	FOG	FG
Fiber type proportion (%)			
Saline	12 ± 0.3	28 ± 0.6	60 ± 0.6
Clenbuterol	10 ± 0.9	27 ± 1.1	63 ± 2
Cross-sectional area (μm^2)			
Saline	2751 ± 93	2301 ± 73	4053 ± 71
Clenbuterol	3292 ± 37	2962 ± 81*	4086 ± 64
Calculated total area (arbitrary units)			
Saline	330 ± 13	644 ± 14	2432 ± 44
Clenbuterol	329 ± 33	800 ± 19*	2575 ± 68*
Change in calculated area (%)	−0.3	+24	+6
NADH-TR staining (optical density)			
Saline	0.189 ± 0.005	0.182 ± 0.005	0.134 ± 0.003
Clenbuterol	0.200 ± 0.002	0.136 ± 0.007*	0.122 ± 0.02*
PAS staining (optical density)			
Saline	0.151 ± 0.003	0.259 ± 0.017	0.230 ± 0.012
Clenbuterol	0.127 ± 0.005*	0.160 ± 0.006*	0.153 ± 0.004*

All data are presented as mean ± SEM (n = 6 per group). Calculated total area represents the average cross-sectional area for each fiber type multiplied by fiber type proportion (%). Data were analyzed using Student's two-tailed t-test.
**P < 0.05, significantly different from saline control.*

and differences were considered statistically significant at $P < 0.05$.

RESULTS

At the beginning of the experiment the average body weight of all rats was 285 ± 7 g. This increased by 8% to 311 ± 10 g after infusion with saline and by 13% to 328 ± 6 g with clenbuterol. Clenbuterol significantly ($P < 0.05$) increased the wet weight (271.9 ± 17 mg vs. 317 ± 23 mg) and total protein content (48 ± 3.2 mg vs. 56.1 ± 4.1 mg) of the plantaris. Histochemistry (Fig. 1) was used to investigate any changes in the myofiber profile, mitochondrial density, and glycogen content of the muscles. Clenbuterol increased the CSA of all fiber types (SO, FOG, and FG), but only the hypertrophy of the FOG fibers was statistically significant ($P < 0.05$). When calculated as total area (i.e., percent number of myofibers multiplied by their average CSA) the proportion of muscle composed of SO fibers was identical between saline- and clenbuterol-treated muscles, but the calculated total areas of FOG and FG fibers increased by 24% and 6%, respectively (Table 1). The density of NADH-TR staining, indicative of mitochondrial content, decreased (range 10%–25%) in FOG and FG fibers, whereas the density of PAS staining of muscle glycogen decreased (range 16%–38%) in all three myofiber types (Table 1). Differential analysis of 2D gels matched 87 protein spots, 5 of which were significantly ($P < 0.01$) altered in abundance by clenbuterol (Fig. 2). Peptide ion spectra were collected using MALDI-ToF mass spectrometry and used to identify each protein based on its peptide mass fingerprint (Table 2).

DISCUSSION

This work deepens our knowledge of the effects of clenbuterol on skeletal muscle. By using this lower dose, derived from our previous studies,[4,5] we have avoided the confounding influences of myocyte death[5] and gross perturbations in metabolism[12] that are associated with the use of larger doses of clenbuterol. Proteomic analysis revealed five proteins that were significantly altered in response to clenbuterol 10 μg/kg/day (Table 2). Only one, heat shock protein 72 (HSP 72), has previously been investigated in relation to β_2-agonist administration.[32]

Large doses of clenbuterol significantly increase the abundance of type IIb myosin heavy chain (MHC) in the rat plantaris and other muscles with mixed fiber types,[32,35,40] thereby increasing speed of contraction[12] and providing a useful model to investigate changes in muscle phenotype.[3] In skeletal muscle, HSP 72 is localized to SO myofibers expressing predominantly type I MHC.[31] Interventions, such as functional overload[33] or endurance exercise,[11] that increase the proportion of type I fibers also increase the abundance of HSP 72. In contrast, the administration of large doses of clenbuterol decreases the expression of HSP 72,[32] and this is related to the reduced proportion of type I MHC in the clenbuterol-treated muscles. In this study, the total area of the plantaris occupied by SO fibers

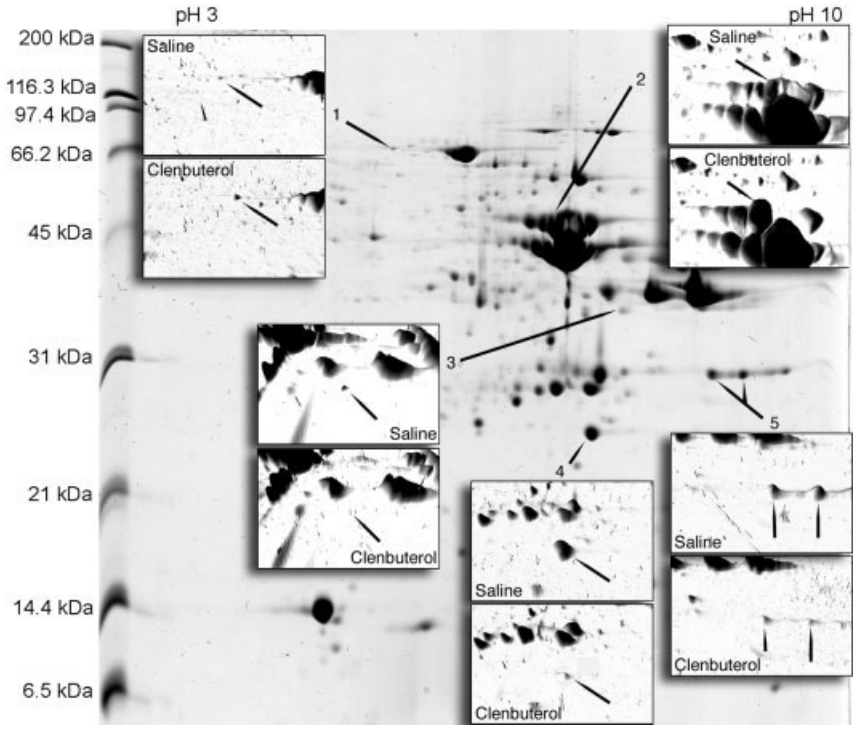

FIGURE 2. Two-dimensional electrophoretic separation of sarcoplasmic proteins. Representative gel image of the soluble protein fraction (250 μg) of plantaris muscle focused on a 13-cm IPG strip (pH 3–10) and electrophoresed through a 16-cm, 12% polyacrylamide gel. Insets show spot densities as three-dimensional images and those protein spots (arrows) significantly different between saline- and clenbuterol-treated muscles. Spot numbers are consistent with Table 2.

(Table 1) was not changed by clenbuterol, but the abundance of HSP 72 was increased (Table 2). Possibly, this increase in HSP 72 is one of the mechanisms by which the muscle is able to resist the cell death induced by larger doses of this agent.[5]

Clenbuterol has been reported to increase the uptake of glucose and the accumulation of glycogen in insulin-resistant skeletal muscles.[34] However, in normal healthy animals, clenbuterol caused a striking decrease in the glycogen content of the plantaris and this was particularly evident in the FOG and FG fibers (Fig. 1 and Table 1). Previously, glycolytic enzymes such as phosphofructokinase have been reported to either increase[35] or decrease[12] after administration of large doses of clenbuterol. In this study we observed opposing changes in the abundance of three glycolytic enzymes; whereas both fructose-bisphosphate aldolase A (aldolase A) and phosphoglycerate mutase (PGM) decreased, β-enolase increased, making it difficult to determine the net

Table 2. Clenbuterol-induced changes in plantaris muscle proteins.

Spot #	Protein	FC (*P*-value)	Accession no.	Coverage	Mowse score	Expect value	pI/MW
1.	Heat shock protein 72	+2.2 ($P = 0.01$)	S31716	18%	88	$3.0\ e^{-05}$	5.43/71,320
2.	β-enolase	+2.5 ($P = 0.00001$)	Q5XIV3	48%	153	$6.1\ e^{-11}$	7.08/47,470
3.	Aldolase A	−2.4 ($P = 0.0003$)	Q63038	43%	187	$4.1\ e^{-15}$	8.39/39,700
4.	Adenylate kinase	−2.7 ($P = 0.006$)	Q5EBC5	45%	84	$4.8\ e^{-05}$	7.66/21,780
5.	Phosphoglycerate mutase	−2.8 ($P = 0.009$)	A33793	29%	91	$1.0\ e^{-04}$	8.85/29,036

Spot numbers correspond to those in Figure 2. Fold changes (FC) are calculated from relative differences in normalized spot density compared with saline-infused controls. Statistical differences in the relative abundance of protein spots were determined using Student's independent t*-test. Accession no. is the protein database entry identified after searching their peptide mass fingerprint against the mass spectrometry protein sequence database using the Mascot search engine. The proportion of the protein sequence that was identified is reported as percent coverage and a Mowse score >63 was statistically significant ($P < 0.05$) in all cases. Expect value is the number of database matches with equal or better scores that are expected to occur by chance alone. The isoelectric point (pI) and molecular weight (MW) are theoretical values calculated from the protein database entry.*

effect of these changes on carbohydrate metabolism. Changes in some glycolytic enzymes may relate to aspects of muscle physiology other than energy metabolism. For example, β-enolase is localized to the perinuclear region,[28] and similar to other glycolytic enzymes, such as lactate dehydrogenase and phosphogylcerate kinase,[37] β-enolase might have an auxiliary role in transcription and DNA replication or repair. The expression of β-enolase is decreased in denervated muscle[41] and in a model of muscle damage and regeneration the abundance of β-enolase decreases immediately after the induction of damage, but is restored during regeneration.[28] Therefore, the clenbuterol-induced increase in the abundance of β-enolase might be more closely associated with its hypertrophic effects, rather than its effects on energy metabolism.

Clenbuterol's lipolytic and anabolic (repartitioning) effects may be desirable as a therapy against metabolic disorders[42] and some of the evidence collected herein supports the therapeutic potential of clenbuterol. In particular, the observed decreases in adenylate kinase and aldolase A (Table 2) oppose the increased concentrations of these proteins measured in skeletal muscles of obese individuals.[19] Similarly, the expression of HSP 72 was lower in the muscle of type II diabetic individuals,[24] but was elevated in the present study (Table 2) after administration of clenbuterol. Nevertheless, several of our observations could also be used to portray a less desirable image of clenbuterol-induced muscle growth. That is, the clenbuterol-induced decrease in aldolase A opposes the increased expression of this enzyme in endurance-trained muscle,[44] phosphoglycerate mutase deficiency is associated with exercise intolerance,[10] and a decreased abundance of adenylate kinase could deleteriously affect the muscle's resistance to fatigue.[21] Such observations seem to correlate well with the decreased exercise capacity commonly reported[15,20,23,25] after administration of larger doses of clenbuterol and suggest that this effect might still be evident after administration of this low non-myotoxic dose.

The dose of clenbuterol used here in rats is thought to be equivalent to the safe therapeutic dose in humans[26] and, as opposed to larger doses, does not induce myocyte death.[5] Contrary to our original hypothesis, the muscle growth induced by this dose was associated with alterations in the muscle's proteome. To some extent, the alterations oppose those observed in insulin-resistant and type II diabetic muscle and support the idea that clenbuterol might be beneficial in such circumstances. However, clenbuterol caused preferential hypertrophy of FOG fibers and reduced the oxidative potential and glycogen content of the muscle, suggesting that the deleterious effects of clenbuterol on muscle function may not have been negated by use of this low non-myotoxic dose.

This research was funded by a British Heart Foundation junior research fellowship award (FS/04/028) to J.G.B.

REFERENCES

1. Argiles J, Almendro V, Busquets S, Lopez-Soriano FJ. The pharmacological treatment of cachexia. Curr Drug Targets 2004;5:265–277.
2. Bakker AJ, Head SI, Wareham AC, Stephenson DG. Effect of clenbuterol on sarcoplasmic reticulum function in single skinned mammalian skeletal muscle fibres. Am J Physiol 1998; 274:C1718–C1726.
3. Bozzo C, Stevens L, Toniolo L, Mounier Y, Reggiani C. Increased phosphorylation of myosin light chain associated with slow-to-fast transition in rat soleus. Am J Physiol 2003;285: C575–C583.
4. Burniston JG, Chester N, Clark WA, Tan L-B, Goldspink DF. Dose-dependent apoptotic and necrotic myocyte death induced by the β_2-adrenergic receptor agonist, clenbuterol. Muscle Nerve 2005;32:767–774.
5. Burniston JG, Clark WA, Tan L-B, Goldspink DF. Dose-dependent separation of the hypertrophic and myotoxic effects of the β_2-adrenergic receptor agonist clenbuterol in rat striated muscles. Muscle Nerve 2006;33:655–663.
6. Burniston JG, Ng Y, Clark WA, Colyer J, Tan L-B, Goldspink DF. Myotoxic effects of clenbuterol in the rat heart and soleus muscle. J Appl Physiol 2002;93:1824–1832.
7. Burniston JG, Tan L-B, Goldspink DF. β_2-adrenergic receptor stimulation in vivo induces apoptosis in the rat heart and soleus muscle. J Appl Physiol 2005;98:1379–1386.
8. Busquets S, Figueras MT, Fuster G, Almendro V, Moore-Carrasco R, Ametiller E, et al. Anticachectic effects of formoterol: a drug for potential treatment of muscle wasting. Cancer Res 2004;64:6725–6731.
9. Choo JJ, Horan MA, Little RA, Rothwell NJ. Anabolic effects of clenbuterol in skeletal muscle are mediated by beta2-adrenoceptor activation. Am J Physiol 1992;263:E50–E56.
10. Cornelio F, Di Donato S. Myopathies due to enzyme deficiencies. J Neurol 1985;232:329–340.
11. Desplanches D, Ecochard L, Sempore B, Mayet-Sornay MH, Favier R. Skeletal muscle HSP72 response to mechanical unloading: influence of endurance training. Acta Physiol Scand 2004;180:387–394.
12. Dodd SL, Powers SK, Vrabas IS, Criswell D, Stetson S, Hussain R. Effects of clenbuterol on the contractile and biochemical properties of skeletal muscle. Med Sci Sport Exerc 1996;28: 669–676.
13. Doherty MK, McLean L, Hayter JR, Pratt JM, Robertson DH, El-Shafei A, et al. The proteome of chicken skeletal muscle: changes in soluble protein expression during growth in a layer strain. Proteomics 2004;4:2082–2093.
14. Duchen MR. Mitochondria and calcium: from cell signalling to cell death. J Physiol (Lond) 2000;529:57–68.
15. Duncan ND, Williams DA, Lynch GS. Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats. Clin Sci 2000;98:339–347.
16. Guth L, Samaha FJ. Procedure for the histochemical demonstration of actomyosin ATPase. Exp Neurol 1970;28:365–367.
17. Hesketh JE, Campbell GP, Lobley GE, Maltin CA, Acamovic F, Palmer RM. Stimulation of actin and myosin synthesis in rat gastrocnemius muscle by clenbuterol; evidence for translational control. Comp Biochem Physiol 1992;102C:23–27.

18. Hinkle RT, Hodge KMB, Cody DB, Sheldon RJ, Kobilka BK, Isfort R. Skeletal muscle hypertrophy and anti-hypertrophic effects of clenbuterol are mediated by the beta2-adrenergic receptor. Muscle Nerve 2002;25:729–734.
19. Hittel DS, Hathout Y, Hoffman EP, Houmard JA. Proteome analysis of skeletal muscle from obese and morbidly obese women. Diabetes 2005;54:1283–1288.
20. Ingalls CP, Barnes WS, Smith SB. Interaction between clenbuterol and run training: effects on exercise performance and MLC isoform content. J Appl Physiol 1996;80:795–801.
21. Janssen E, Dzeja PP, Oerlemans F, Simonetti AW, Heerschap A, de Haan A, et al. Adenylate kinase 1 gene deletion disrupts muscle energetic economy despite metabolic rearrangement. EMBO J 2000;19:6371–6381.
22. Jensen J, Brors O, Dahl HA. Different beta-adrenergic receptor density in different rat skeletal muscle fibre types. Pharmacol Toxicol 1995;76:380–385.
23. Kearns CF, McKeever KH. Clenbuterol diminishes aerobic performance in horses. Med Sci Sports Exerc 2002;34:1976–1985.
24. Kurucz I, Morva A, Vaag A, Eriksson KF, Huang X, Groop L, et al. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. Diabetes 2002;51:1102–1109.
25. Lynch GS, Hayes A, Campbell SP, Williams DA. Effects of beta2-agonist administration and exercise on contractile activation of skeletal muscle fibres. J Appl Physiol 1996;81:1610–1618.
26. Maltin CA, Delday MI, Hay SM, Baillie AG. Denervation increases clenbuterol sensitivity in muscle from young rats. Muscle Nerve 1992;15:188–192.
27. Maltin CA, Hay SM, Delday MI, Lobley GE, Reeds JP. The action of the beta-agonist clenbuterol on protein metabolism in innervated and denervated phasic muscles. Biochem J 1989;261:965–971.
28. Merkulova T, Dehaupas M, Nevers MC, Creminon C, Alameddine H, Keller A. Differential modulation of alpha, beta and gamma enolase isoforms in regenerating mouse skeletal muscle. Eur J Biochem 2000;267:3735–3743.
29. Navegantes C, Resanino NMZ, Migliorini RH, Kettelhut I. Catecholamines inhibit Ca^{+2}-dependent proteolysis in rat skeletal muscle through beta2-adrenoceptors and cAMP. Am J Physiol 2001;281:E449–E454.
30. Navegantes C, Resano NM, Baviera AM, Migliorini RH, Kettelhut IC. Effect of sympathetic denervation on the rate of protein synthesis in rat skeletal muscle. Am J Physiol Endocrinol Metab 2004;286:E642–E647.
31. Ogata T, Oishi Y, Roy RR, Ohmori H. Endogenous expression and developmental changes of HSP72 in rat skeletal muscles. J Appl Physiol 2003;95:1279–1286.
32. Oishi Y, Imoto K, Ogata T, Taniguchi K, Matsumoto H, Fukuoka Y, et al. Calcineurin and heat-shock proteins modulation in clenbuterol-induced hypertrophied rat skeletal muscles. Pflugers Arch 2004;448:114–122.
33. Oishi Y, Ogata T, Ohira Y, Taniguchi K, Roy RR. Calcineurin and heat shock protein 72 in functionally overloaded rat plantaris muscle. Biochem Biophys Res Commun 2005;330:706–713.
34. Pan SJ, Hancock J, Ding Z, Fogt D, Lee M, Ivy JL. Effects of clenbuterol on insulin resistance in conscious obese Zucker rats. Am J Physiol 2001;280:E554–E561.
35. Pellegrino MA, D'Antona G, Bortolotto S, Boschi F, Pastoris O, Bottinelli R, et al. Clenbuterol antagonizes glucocorticoid-induced atrophy and fibre type transformation in mice. Exp Physiol 2004;89:89–100.
36. Piec I, Listrat A, Alliot J, Chambon C, Taylor RG, Bechet D. Differential proteome analysis of aging in rat skeletal muscle. FASEB J 2005;19:1143–1145.
37. Popanda O, Fox G, Thielmann HW. Modulation of DNA polymerases alpha, delta and epsilon by lactate dehydrogenase and 3-phosphoglycerate kinase. Biochim Biophys Acta 1998;1397:102–117.
38. Rajab P, Fox J, Riaz S, Tomlinson D, Ball D, Greenhaff PL. Skeletal muscle myosin heavy chain isoforms and energy metabolism after clenbuterol treatment in the rat. Am J Physiol 2000;279:R1076–R1081.
39. Ryall JG, Plant DR, Gregorevic P, Sillence MN, Lynch GS. Beta 2-agonist administration reverses muscle wasting and improves muscle function in aged rats. J Physiol (Lond) 2004;555:175–188.
40. Stevens L, Ricart-Firinga C, Gohlsch B, Bastide B, Mounier Y, Pette D. Effects of unweighting and clenbuterol on myosin light and heavy chains in fast and slow muscle of rat. Am J Physiol 2000;279:C1558–15663.
41. Tang H, Cheung WM, Ip FC, Ip NY. Identification and characterization of differentially expressed genes in denervated muscle. Mol Cell Neurosci 2000;16:127–140.
42. Torgan CE, Etgen GJ, Kang HY, Ivy JL. Fibre type-specific effects of clenbuterol and exercise training on insulin-resistant muscle. J Appl Physiol 1995;79:163–167.
43. Yimlamai T, Dodd SL, Borst SE, Park S. Clenbuterol induces muscle-specific attenuation of atrophy through effects on the ubiquitin–proteasome pathway. J Appl Physiol 2005;91:71–80.
44. Yoshioka M, Tanaka H, Shono N, Snyder EE, Shindo M, St-Amand J. Serial analysis of gene expression in the skeletal muscle of endurance athletes compared to sedentary men. FASEB J 2003;17:1812–1819.
45. Zeman RJ, Ludemann R, Easton TG, Etlinger JD. Slow to fast alterations in skeletal muscle fibers caused by clenbuterol, a beta 2-receptor agonist. Am J Physiol 1988;254:E726–E732.