Power output of fast and slow skeletal muscles of *mdx* (dystrophic) and control mice after clenbuterol treatment

Gordon S. Lynch*, Richard T. Hinkle and John A. Faulkner

Institute of Gerontology and Departments of Physiology and Biomedical Engineering, The University of Michigan, Ann Arbor, MI 48109-2007, USA

The *mdx* mouse is the most commonly used animal model for Duchenne muscular dystrophy. We tested the null hypothesis that 20 weeks of clenbuterol treatment ($\sim$2 mg kg^{-1} day^{-1}) of *mdx* and control mice would have no effect on the absolute and specific force (P_{o}, kN m^{-2}) and absolute and normalised power output (W kg^{-1}) of extensor digitorum longus (EDL) and soleus muscles. For *mdx* and control mice, clenbuterol treatment produced modest increases in the mass of the two muscles but did not increase absolute or specific force or normalised power output. For absolute power output, only the EDL muscles of *mdx* mice showed a difference following treatment, with the power output of treated mice being 118% that of the untreated mice. The modest effects of clenbuterol treatment on the dynamic properties of skeletal muscle provide little support for any improvement in muscle function for the dystrophic condition . *Experimental Physiology* (2000) **85.3**, 295–299.

Chronic treatment with clenbuterol, a β_2-adrenoceptor agonist, has been proposed as a treatment to reverse the muscle atrophy observed with ageing (Carter *et al.* 1991), denervation (Maltin *et al.* 1987, 1993; Zeman *et al.* 1987; Agbenyega & Wareham, 1990), muscle unloading (Delday & Maltin, 1997) and muscle wasting diseases, such as muscular dystrophy (Maltin *et al.* 1987, 1993; Martineau *et al.* 1992; Zeman *et al.* 1994; Dupont-Versteegden, 1996). It is well established that clenbuterol treatment increases muscle mass in a number of different species (Kim & Sainz, 1992) with an increased protein synthesis and/or decreased protein degradation proposed as the mechanism for the hypertrophy of skeletal muscle (Choo *et al.* 1992; Moore *et al.* 1994).

As a strategy for treating muscular dystrophy, major inconsistencies exist regarding the effect of clenbuterol on the functional properties of skeletal muscles of dystrophic *mdx* mice. Hayes & Williams (1994) and Dupont-Versteegden and colleagues (1995) treated young (3-week-old) *mdx* mice with clenbuterol for 3 and 15 weeks, respectively, and both groups reported increases in absolute maximum isometric force (P_{o}) for soleus muscles of treated mice relative to untreated mice. Only Hayes & Williams (1994) reported a greater specific P_{o} (force normalised to muscle cross-sectional area) for the soleus muscles, although P_{o} was normalised only for muscle mass. In another study, Zeman *et al.* (1994) reported that the P_{o} for EDL muscles of clenbuterol-treated mice was 131 % that for untreated *mdx* mice. This finding is confounded by a value of P_{o} that was only 40 % of the values normally reported for

EDL muscles of mice (Brooks & Faulkner, 1988, 1991; Dupont-Versteegden *et al.* 1995; Lynch *et al.* 1999).

A major function of skeletal muscle is the generation of power output to do work and provide bodily motion (Wilkie, 1960). Data exist on the absolute and normalised power outputs of diaphragm muscles and also recently on limb muscles of *mdx* mice (Lynch *et al.* 1997; Deconinck *et al.* 1998). No data are available on the effect of clenbuterol treatment on power output of limb muscles of *mdx* mice. After 52 weeks of clenbuterol treatment of control mice, Lynch *et al.* (1999) reported increased muscle masses for the EDL and soleus muscles but no differences in absolute or normalised P_{o} or power output of these muscles. Since previous studies have only looked at the effect of clenbuterol on the isometric contractile properties of dystrophic skeletal muscle, the purpose of this study was to investigate the effects of 20 weeks of clenbuterol treatment on the dynamic properties of skeletal muscles from *mdx* and control mice. We tested the null hypothesis that clenbuterol treatment ($\sim$2 mg kg^{-1} day^{-1}) would not affect absolute and normalised force and power output of EDL and soleus muscles of *mdx* and control mice.

METHODS

Animal groups and drug administration

Six-month-old male specific pathogen-free (SPF) *mdx* and control (C57BL/10ScSn mice) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed at The University of Michigan in barrier protected facilities and provided with standard laboratory

* To whom correspondence should be addressed at the Department of Physiology, The University of Melbourne, Victoria, 3010, Australia.

chow and water *ad libitum*. All experiments on the mice were conducted in accordance with the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals* (DHEW Publication No. 85-23 (NIH) Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205, USA). The *mdx* and control mice were separated into either a treated or an untreated group. The mice in the treated group were administered clenbuterol (Sigma Chemical Company, 1.5 to 2 mg kg^{-1} day^{-1}) in their drinking water, every day for the first week. A '3 day on–3 day off' cycle was employed for weeks 2–20 in order to reduce attenuation of the clenbuterol response (Yang & McElligott, 1989). The clenbuterol solution was freshly prepared each week to avoid oxidation and possible reduction in its efficacy. The effectiveness of clenbuterol administration via the drinking water and the dosage are well-established (Zeman *et al.* 1988; Hayes & Williams, 1994; Moore *et al.* 1994; Dupont-Versteegden *et al.* 1995; Lynch *et al.* 1996). The duration of the clenbuterol treatment was 20 weeks. A relatively high dosage of clenbuterol was administered in order to determine the maximum effects of the β_2-agonist on skeletal muscle.

Two days after the completion of the last treatment period, mice were anaesthetised deeply with pentobarbital sodium (70 mg kg^{-1}, I.P.) such that no response occurred to tactile stimuli. The fast-twitch extensor digitorum longus (EDL), and the predominantly slow-twitch soleus muscles were surgically excised from the left hind-limb. The deeply anaesthetised mice were killed by the creation of a pneumothorax.

Contractile properties

Contractile properties of EDL and soleus muscles were measured *in vitro* by techniques that we have described previously (Lynch *et al.* 1999). Briefly, silk suture was tied to each tendon of the isolated muscles. The muscles were placed in an experimental chamber filled with Ringer solution (containing (mM): NaCl 137; NaHCO$_3$ 24; glucose 11; KCl 5; CaCl$_2$ 2; MgSO$_4$ 1; NaH$_2$PO$_4$ 1; and tubo-curarine chloride 0.025; pH 7.4) oxygenated with 95 % O$_2$ and 5 % CO$_2$ and maintained at 25 °C. Muscles were aligned horizontally
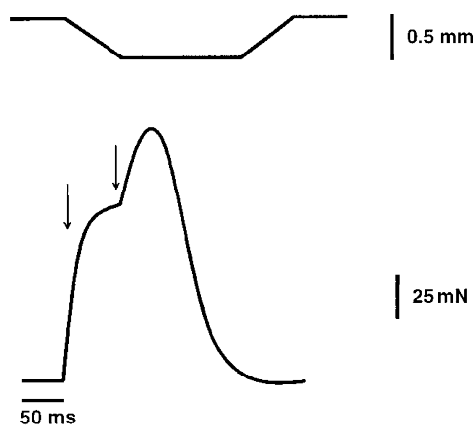

Figure 1

Experimental record of an isovelocity shortening contraction used to calculate power during a single tetanic contraction of an isolated EDL muscle (control, treated group). Upper trace indicates displacement of muscle length by the lever arm of the servomotor. Lower trace shows the corresponding force generated by the muscle. Arrows indicate times at which stimulation began and stopped. Power was calculated using the ramp velocity and average force during the ramp as described in the Methods.

between a lever arm of a position feedback servomotor (model 300H, Cambridge Technology Inc. Watertown, MA, USA) and the stainless steel hook of a force transducer (model BG-50, Kulite Semiconductor Products Inc., Leonia, NJ, USA). The muscles were stimulated directly by an electric field between two platinum plate electrodes placed longitudinally on either side of the muscle. Square wave pulses 0.2 ms in duration were produced by a stimulator (model S88, Grass Instruments) and amplified (model DC-300A Series II, Crown International Inc., Elkhart, IN, USA) to increase and sustain current intensity to a sufficient level to produce a maximum isometric tetanic contraction. Stimulation voltage and muscle length (L_o) were adjusted to obtain maximum isometric twitch force. Optimum fibre length (L_f) was determined by multiplying L_o by fibre length to muscle length ratios determined previously (Brooks & Faulkner, 1988; Lynch *et al.* 1999). The ratios used were 0.44 for the EDL muscle and 0.71 for the soleus muscle. Maximum isometric tetanic force production (P_o) was determined from the plateau of the frequency–force relationship.

Power output was determined by isovelocity shortenings during maximum muscle activation. Initiation of the isovelocity shortening ramp and stimulation of the muscle occurred simultaneously. Stimulation was terminated at the end of the shortening ramp and the muscle was held isometric for 100 ms allowing it to relax before returning to resting length (see Fig. 1). For the EDL and soleus muscles, power output was determined initially from isovelocity shortenings from 105 % L_f to 95 % L_f during maximum muscle activation which allowed the muscle to shorten an equal distance either side of L_o (Brooks *et al.* 1990). When a maximum power measurement was determined, further isovelocity shortenings were made from 100 % L_f to 90 % L_f. Frequently, a higher value for maximum power output was achieved employing this protocol. Maximum power output was determined from whichever of the two protocols yielded the highest value and calculated as the product of average force and velocity of shortening. The average force generated during the shortening ramp was determined by integrating the area under the force curve and dividing by the elapsed time. This method gives slightly lower estimates of muscle power than that obtained following analysis of force (*P*)–velocity (*V*) relationships (Ranatunga, 1998). The velocity of shortening and the frequency of stimulation were adjusted to elicit maximum power output. The optimum shortening velocity (V_{opt}) was defined as the velocity at which the power was maximum (Brooks *et al.* 1990). After the contractile properties were measured, each muscle was trimmed of its tendons and visible connective tissue, blotted on filter paper, and weighed on an analytical balance (Mettler AE-50). The total muscle fibre cross-sectional area (CSA) of the muscles was determined by dividing muscle mass (mg) by the product of L_f and 1.06 mg mm^{-3}, the density of mammalian skeletal muscle (Méndez & Keys, 1960). The values for specific force (kN m^{-2}) were normalised to CSA and power (W) was normalised to muscle mass (W kg^{-1}).

Statistical analysis

Results are presented in the tables as means $\pm$ S.E.M. Using NCSS software (Number Cruncher Statistical System 5.01, Kaysville, UT, USA), differences were assessed using a two-way analysis of variance (ANOVA) for mouse strain (*mdx vs.* control) and treatment (treated *vs.* untreated), with the Student-Newman-Keul's multiple comparison procedure used to identify specific differences when significance was detected. Specific comparisons were made between untreated *mdx* and untreated control mice, treated and untreated control mice and treated and untreated *mdx* mice. The level of significance was set *a priori* at $P < 0.05$.

Table 1. Selected morphological characteristics of adult control (C57BL/10) and *mdx* mice following 20 weeks clenbuterol administration

		Control		*mdx*	
		Untreated ($n = 11$)	Treated ($n = 11$)	Untreated ($n = 11$)	Treated ($n = 11$)
Body mass (g)		38 ± 1	33 ± 1	36 ± 1	37 ± 1
EDL muscle					
	Mass (mg)	12.6 ± 0.3	$13.7 \pm 0.4\,*$	$16.4 \pm 0.4\,\dagger$	17.9 ± 0.6
	CSA (mm^2)	2.0 ± 0.1	$2.2 \pm 0.1\,*$	2.5 ± 0.1	$2.8 \pm 0.1\,*$
	L_f (mm)	5.9 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	6.1 ± 0.1
Soleus muscle					
	Mass (mg)	10.3 ± 0.4	10.7 ± 0.1	$14.6 \pm 0.5\,\dagger$	$16.3 \pm 0.4\,*$
	CSA (mm^2)	1.2 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	$1.8 \pm 0.1\,*$
	L_f (mm)	8.2 ± 0.2	7.8 ± 0.1	8.6 ± 0.1	8.6 ± 0.1

EDL, extensor digitorum longus; CSA, cross-sectional area; L_f, muscle fibre length. Values for muscle mass/body mass (relative mass) are expressed (mg g^{-1} × 1000); * Significant differences ($P < 0.05$) between untreated and treated mice of each group; † significant differences ($P < 0.05$) between control and *mdx* mice.

Table 2. Contractile properties of extensor digitorum longus (EDL) and soleus muscles from control and *mdx* mice following the 20 week clenbuterol administration protocol

		Control		*mdx*	
		Untreated ($n = 11$)	Treated ($n = 11$)	Untreated ($n = 11$)	Treated ($n = 11$)
EDL muscles					
	P_o (mN)	507 ± 13.1	518 ± 19.5	532 ± 14.5	557 ± 18.1
	P_o (kN m^{-2})	253 ± 6.5	236 ± 5.3	$214 \pm 6.7\dagger$	201 ± 6.0
	V_{opt} (L_f s^{-1})	1.59 ± 0.04	1.60 ± 0.04	1.57 ± 0.04	1.67 ± 0.04
	Power (mW)	1.59 ± 0.09	1.59 ± 0.08	1.58 ± 0.04	$1.88 \pm 0.09\,*$
	Power (W kg^{-1})	126 ± 5.6	116 ± 5.8	$96 \pm 4.2\,\dagger$	105 ± 4.5
Soleus muscles					
	P_o (mN)	276 ± 5.6	298 ± 14.1	334 ± 12.8	347 ± 8.7
	P_o (kN m^{-2})	236 ± 9.1	230 ± 12.1	212 ± 12.6	195 ± 10.0
	V_{opt} (L_f s^{-1})	0.67 ± 0.04	0.77 ± 0.05	$0.52 \pm 0.03\,\dagger$	0.59 ± 0.03
	Power (mW)	0.33 ± 0.02	0.35 ± 0.03	$0.41 \pm 0.03\,\dagger$	0.50 ± 0.04
	Power (W kg^{-1})	32 ± 1.7	33 ± 3.1	28 ± 2.2	31 ± 2.4

P_o, tetanic force; V_{opt}, optimum velocity for maximum power output. * Significant differences ($P < 0.05$) between untreated and treated mice of each group; † significant differences ($P < 0.05$) between control and *mdx* mice.

RESULTS

Comparisons of data from untreated *mdx* and untreated control mice

The body masses of the 11-month-old *mdx* and control mice were not different (Table 1). As reported previously (Dupont-Versteegden *et al.* 1995; Faulkner *et al.* 1997), compared with the masses of the EDL and soleus muscles of control mice, those of *mdx* mice were 130 % and 140 %, respectively. Despite the hypertrophy of the muscles in the *mdx* compared with the control mice, the absolute P_o and power of EDL muscles of *mdx* mice were not different from those of control mice (Table 2). In contrast, absolute P_o and power of the soleus muscles of *mdx* mice were 121 % and 124 %, respectively, of the values for control mice (Table 2). For specific P_o and the normalised power, values for EDL muscles from *mdx* mice were 84 % and 76 %, respectively, of the values for the control mice, whereas no differences were observed for soleus muscles.

Effects of clenbuterol administration on body and muscle masses

Following clenbuterol treatment, the body masses of *mdx* and control mice were not different from the untreated mice in their respective groups (Table 1). For control mice, the mass of the EDL muscles of treated compared with untreated mice was 109 %, but the masses of soleus muscles in treated and

untreated mice were not different. For treated compared with untreated *mdx* mice, the mass of soleus muscles was 112 %, whereas the EDL muscle did not show any difference.

Effects of clenbuterol administration on contractile properties

The EDL and soleus muscles of treated and untreated control mice did not differ in any of the contractile measurements (Table 2). For treated compared with untreated *mdx* mice, the only difference in any of the contractile properties of EDL or soleus muscles was the value of 118 % for the absolute power of the EDL muscle (Table 2). Thus, the null hypothesis that clenbuterol administration would not affect the force and power output of skeletal muscles from dystrophic mice was supported, with the single exception of the normalised power of the EDL muscle.

DISCUSSION

Although the potential therapeutic role for clenbuterol in muscular dystrophy has received much attention (Maltin *et al.* 1987, 1993; Martineau *et al.* 1992; Zeman *et al.* 1994; Hayes & Williams 1994; Dupont-Versteegden *et al.* 1995), the majority of these studies have investigated only whether clenbuterol alters the mass and isometric force-producing capacity of isolated muscles. Whether clenbuterol affects the dynamic properties of dystrophic skeletal muscle has not been addressed. Since the ability of skeletal muscles to do work and provide bodily motion is dependent upon the generation of power output, the therapeutic potential of clenbuterol is tested more rigorously by investigating its effects on the power output of dystrophic skeletal muscles. In this study, we found that clenbuterol treatment produced only modest changes in the dynamic properties of fast- and slow-twitch skeletal muscles of the *mdx* mouse.

The continuous cycle of degeneration and regeneration associated with the dystrophic process in *mdx* mice appears to initiate a hypertrophy that is sustained if not augmented during the first 12 months of the life of the mouse (Faulkner *et al.* 1997). This hypertrophy does much to maintain the absolute force and power of the limb muscles of dystrophic mice close to the values for control mice. For mice 6–12 months of age, several different studies have reported the masses of small limb muscles of untreated *mdx* mice to be 125–150 % of the values for control mice (for review see Faulkner *et al.* 1997). Our values for EDL and soleus muscles of 11-month-old untreated *mdx* mice compared with untreated control mice of 130 % to 142 % are in excellent agreement with the published values. For limb muscles of both *mdx* mice and control mice and rats, published data are contradictory regarding the effects of clenbuterol on absolute muscle masses (Agbenyega & Wareham, 1990; Hayes & Williams 1994; Dupont-Versteegden *et al.* 1995; Dodd *et al.* 1996). Based on both the published data and our data on the EDL and soleus muscles of *mdx* and control mice, the effects of clenbuterol continue to be unpredictable and inconclusive for both fast and slow skeletal muscles. Clenbuterol treatment produced for both fast and slow muscles either a modest ~10 % hypertrophy, or no change.

Following both short-term and long-term administration of clenbuterol, a number of investigators have reported a greater absolute P_o for EDL muscles of *mdx* mice (Zeman *et al.* 1994) and soleus muscles of both *mdx* and control mice (Hayes & Williams, 1994; Dupont-Versteegden *et al.* 1995). In the present study, only the EDL muscles of the treated *mdx* mice showed any difference, with absolute power increased to 118 % that of the untreated *mdx* mice. The greater absolute power of the treated compared with the untreated *mdx* mice arose from small insignificant differences in both force and V_{opt}. All the other muscles of clenbuterol-treated *mdx* or control mice showed no difference in absolute force or power or a small loss. Our data provide little support for clenbuterol treatment producing any change in the development of absolute force or power of skeletal muscles.

Some conflicting results have appeared (Hayes & Williams 1994), but most short- and long-term treatments of control rats with clenbuterol have shown no difference in specific P_o of limb muscles (Zeman *et al.* 1988; Dodd *et al.* 1996; Van der Heijden *et al.* 1998; Lynch *et al.* 1999). Similarly, we found no evidence to support any increase in specific force of fast or slow limb muscles following clenbuterol treatment of either *mdx* or control mice. As with the specific force, no differences in normalised power were observed for either limb muscle of treated or untreated *mdx* or control mice. We conclude that clenbuterol was ineffective in improving the specific force or normalised power output of dystrophic skeletal muscles.

AGBENYEGA, E. T. & WAREHAM, A. C. (1990). Effect of clenbuterol on normal and denervated muscle growth and contractility. *Muscle and Nerve* **13**, 199–203.

BROOKS, S. V. & FAULKNER, J. A. (1988). Contractile properties of skeletal muscles from young, adult and aged mice. *Journal of Physiology* **404**, 71–82.

BROOKS, S. V. & FAULKNER, J. A. (1991). Forces and powers of slow and fast skeletal muscles in mice during repeated contractions. *Journal of Physiology* **436**, 701–710.

BROOKS, S. V., FAULKNER, J. A. & McCUBBERY, D. A. (1990). Power outputs of slow and fast skeletal muscles of mice. *Journal of Applied Physiology* **68**, 1282–1285.

CARTER, W. J., DANG, A. Q., FAAS, F. A. & LYNCH, M. E. (1991). Effects of clenbuterol on skeletal muscle mass, body composition, and recovery from surgical stress in senescent rats. *Metabolism* **40**, 855–860.

CHOO, J. J., HORAN, M. A., LITTLE, R. A. & ROTHWELL, N. J. (1992). Anabolic effects of clenbuterol on skeletal muscle are mediated by β_2-adrenoceptor activation. *American Journal of Physiology* **263**, E50–56.

DECONINCK, N., RAFAEL, J. A., BECKERSBLEUKX, G., KAHN, D., DECONINCK, A., DAVIES, K. E. & GILLIS, J. M. (1998). Consequences of the combined deficiency in dystrophin and utrophin on the mechanical properties and myosin composition of some limb and respiratory muscles of the mouse. *Neuromuscular Disorders* **8**, 362–370.

DELDAY, M. I. & MALTIN, C. A. (1997). Clenbuterol increases the expression of myogenin but not MyoD in immobilized rat muscles. *American Journal of Physiology* **273**, E941–944.

DODD, S. L., POWERS, S. K., VRABAS, I., CRISWELL, D., STETSON, S. & HUSSAIN, R. (1996). Effects of clenbuterol on contractile and biochemical properties of skeletal muscle. *Medicine and Science in Sports and Exercise* **28**, 669–676.

DUPONT-VERSTEEGDEN, E. E. (1996). Exercise and clenbuterol as strategies to decrease the progression of muscular dystrophy in *mdx* mice. *Journal of Applied Physiology* **80**, 734–741.

DUPONT-VERSTEEGDEN, E. E., KATZ, M. S. & MCCARTER, R. J. (1995). Beneficial *versus* adverse effects of long-term use of clenbuterol in *mdx* mice. *Muscle and Nerve* **18**, 1447–1459.

FAULKNER, J. A., BROOKS, S. V., DENNIS, R. G. & LYNCH, G. S. (1997). The functional status of dystrophic muscles and functional recovery by skeletal muscles following myoblast transfer. *Basic and Applied Myology* **7**, 257–264.

HAYES, A. & WILLIAMS, D. A. (1994). Long-term clenbuterol administration alters the isometric contractile properties of skeletal muscle from normal and dystrophin-deficient *mdx* mice. *Clinical and Experimental Pharmacology and Physiology* **21**, 757–765.

KIM, Y. S. & SAINZ, R. D. (1992). β-Adrenergic agonists and hypertrophy of skeletal muscles. *Life Sciences* **50**, 397–407.

LYNCH, G. S., HAYES, A., CAMPBELL, S. P. & WILLIAMS, D. A. (1996). Effects of β_2-agonist administration and exercise on contractile activation of skeletal muscle fibers. *Journal of Applied Physiology* **81**, 1610–1618.

LYNCH, G. S., HINKLE, R. T. & FAULKNER, J. A. (1999). Yearlong treatment with clenbuterol increases muscle mass but not force or power output of skeletal muscles of mice. *Clinical and Experimental Pharmacology and Physiology* **26**, 118–120.

LYNCH, G. S., RAFAEL, J. A., HINKLE, R. T., COLE, N. M., CHAMBERLAIN, J. S. & FAULKNER, J. A. (1997). Contractile properties of diaphragm muscle segments from old *mdx* and old transgenic *mdx* mice. *American Journal of Physiology* **272**, C2063–2068.

MALTIN, C. A., DELDAY, M. I., WATSON, J. S., HEYS, D., NEVISON, I. M., RITCHIE, I. K. & GIBSON, P. H. (1993). Clenbuterol, a β-adrenoceptor agonist, increases relative muscle strength in orthopaedic patients. *Clinical Science* **84**, 651–654.

MALTIN, C. A., HAY, S. M., DELDAY, M. I., SMITH, F. G., LOBLEY, G. E. & REEDS, P. J. (1987). Clenbuterol, a beta-agonist induces growth in innervated and denervated rat soleus muscle via apparently different mechanisms. *Bioscience Reports* **7**, 525–532.

MARTINEAU, L., HORAN, M. A., ROTHWELL, N. J. & LITTLE, R. A. (1992). Salbutamol, a β_2-adrenoceptor agonist, increases skeletal muscle strength in young men. *Clinical Science* **83**, 615–621.

MÉNDEZ, J. & KEYS, A. (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184–189.

MOORE, N. G., PEGG, G. G. & SILLENCE, M. N. (1994). Anabolic effects of the β_2-adrenoceptor agonist salmeterol are dependent on route of administration. *American Journal of Physiology* **267**, E475–484.

RANATUNGA, K. W. (1998). Temperature dependence of mechanical power output in mammalian (rat) skeletal muscle. *Experimental Physiology* **83**, 371–376.

VAN DER HEIJDEN, H. F. M., DEKHUIJZEN, P. N. R., FOLGERING, H., GINSEL, L. A. & VAN HERWAARDEN, C. L. A. (1998). Long-term effects of clenbuterol on diaphragm morphology and contractile properties in emphysematous hamsters. *Journal of Applied Physiology* **85**, 215–222.

WILKIE, D. R. (1960). Man as a source of mechanical power. *Ergonomics* **3**, 1–8.

YANG, Y. T. & MCELLIGOTT, M. A. (1989). Multiple actions of β_2-adrenergic agonists on skeletal muscle and adipose tissue. *Biochemical Journal* **261**, 1–10.

ZEMAN, R. J., LUDEMANN, R., EASTON, T. G. & ETLINGER, J. D. (1988). Slow to fast alterations in skeletal muscle fibers caused by clenbuterol, a β_2-receptor agonist. *American Journal of Physiology* **254**, E726–732.

ZEMAN, R. J., LUDEMANN, R. & ETLINGER, J. D. (1987). Clenbuterol, a β_2-agonist, retards atrophy in denervated muscles. *American Journal of Physiology* **252**, E152–155.

ZEMAN, R. J., ZHANG, Y. & ETLINGER, J. D. (1994). Clenbuterol, a β_2-agonist, retards wasting and loss of contractility in irradiated dystrophic *mdx* muscle. *American Journal of Physiology* **267**, C865–868.

Acknowledgements